

Bharat B. Aggarwal • Ajaikumar B. Kunnumakkara
Editors



Molecular Targets and Therapeutic Uses of Spices

Modern Uses for Ancient Medicine



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Dedicated to

**Our Sages, Rishis, Saints, Acharyas,
Scientists, Gurus and Parents whose wisdom
continues to inspire and guide us!**

***"Gururbrahma Gururvishnu Gururdevo Maheshwrah,
Guru Sakshat Parm Brahma Tasme Srigurve Namaha"***

***Yatkaromi Yatashnami Yajjuhomi Dadami Yat
Yatpsyami Kountiya Tatkromi Tavarpanam
(modified from Srimad Bhagwad Gita 9-27)***

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PREFACE

It is believed that Spices is the reason that brought Romans, Jews and Arabs to India. The search for spices was also the impetus for Christopher Columbus's discovery of America and for Vasco de Gama's voyage from Portugal to India, in the 15th century, along what is now called the "Spice Route". The Indonesian island where the nutmeg, cloves, cinnamon, ginger, turmeric and mace were grown is now called "Spice Island". Here, wealthy ladies kept spices in lockets around their necks so they could freshen their breaths, and gentlemen added nutmeg to food and drink. Spices were also used for medicinal purposes, especially in the relief of colic, gout, wounds, and rheumatism. Because of the great demand for spices, their prices soared, and so expeditions were launched to find their source and secure them for Europe. This struggle led to fights between Arabs, Portuguese, Spanish, French, British, and Dutch governments during the 17th and 18th centuries.

This monograph focuses on the medicinal aspects of these spices. Where is the evidence that these spices have medicinal value? Hippocrates remarked almost 25 centuries ago *"Let food be thy medicine and medicine be thy food"*. This aphorism parallels the common American saying "you are what you eat" and the current recommendation from the United States National Institutes of Health to consume as many as "12 servings of fruits and vegetables a day" to prevent common diseases. How spices and their components affect disease and what are their molecular targets, is the collective focus of this book. We intend to demonstrate that, like modern medicine, ancient medicine, including its pharmacopeia, was evidence-based but based on technology different from that of today. We are fortunate that this is so, because products that are safe and yet efficacious

are needed today more than ever before. Overall, we hope that the information provided in this book is useful to scientists, clinicians, herbalogists, naturopaths, and above all the people who use such products. We would like to thank all the contributors who made this book possible and Divya Danda for the cover design. We hope that this book will justify "***Adding Spice to Your Life***".

Bharat B. Aggarwal, Ph.D.
Ajaikumar B. Kunnumakkara, Ph. D.

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ABOUT THE EDITORS

Dr. Bharat B. Aggarwal is a Professor of Medicine, Immunology, Biochemistry, Experimental Therapeutics, and Chief of the Cytokine Research Laboratory at the University of Texas M.D. Anderson Cancer Center, Houston. He currently holds the Ransom Horne, Jr., Endowed Professorship in Cancer Research. He earned his PhD in biochemistry from the University of California, Berkeley, did his postdoctoral fellowship at the University of California Medical Center, San Francisco and then worked for almost ten years with Genentech before moving to Texas. Dr. Aggarwal was the first to isolate TNF-α and TNF-β and identify their receptors.

He has published more than 500 original articles in peer-reviewed journals, currently serving on the editorial boards of more than a dozen journals, edited 12 books and granted 35 patents. He has delivered more than 300 lectures, both nationally and internationally, and has been listed as one of the "World's Most Highly Cited Scientists". He has received numerous awards, most recently the Ranbaxy Award, an Outstanding Scientist Award from the American Association of Indian Scientists in Cancer Research, and a McCormick Science Institute Research Award from the American Society of Nutrition. The primary focus of Dr. Aggarwal's research is the role of inflammatory pathways in tumorigenesis and other diseases and their modulation by natural products including dietary agents, spices, Ayurvedic medicine, and traditional Chinese medicine.

Dr. Ajaikumar B. Kunnumakkara is currently working at the Signal Transduction Section of the Medical Oncology Branch, National Cancer Institute, National Institute of Health, Bethesda in USA. He obtained his PhD in biochemistry from University of Calicut, Kerala (the land of spices where Vasco De Gama first landed), India; did his postdoctoral work at the University of Texas M.D. Anderson Cancer Center in Houston, Texas. Dr. Kunnumakkara has published more than 40 original articles and review papers in peer-reviewed journals, and has authored seven book chapters. The primary focus of his research is to identify safe, efficacious and affordable anti-inflammatory, antitumor and antimetastatic compounds from natural sources and to develop different *in vivo* models for biomedical research.

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Traditional Uses of Spices

Black Pepper
- Gangrene
- Earache
- Diarrhea
- Abdominal tumors
- Constipation
- Sunburn
- Oral abscesses
- Tooth decay
- Liver disorders
- Pyretic
- Epilepsy
- Joint pain
- Lung diseases
- Insomnia
- Insect bites
- Indigestion
- Hernia
- Heart disease

Coriander
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- Digestive disorders
- Respiratory disorders
- Urinary disorders
- Cystitis
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- Cough
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- Hay fever
- Dizziness
- Insomnia
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- Constipation
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- Weight gain
- Edema

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Red Chili
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- Tonsilitis
- Nausea
- Vomiting
- Sore throat

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- Rheumatism
- Body-ache
- Skin diseases
- Stomach disorders
- Intestinal worms
- Fevers
- Hepatic diseases
- Urinary diseases
- Dyspepsia
- Inflammation
- Leukoderma
- Amenorrhea
- Dental diseases
- Ulcers
- Colic inflammation

Rosemary
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- Epilepsy
- Diabetes
- Eczema
- Stomach disorders
- Inflammation
- Dyspepsia
- Dysmenorrhea
- Psychogenic tension
- Rheumatoid arthritis
- Respiratory disorders
- Brain damage
- Hepatotoxicity
- Growth of hair
- Improves memory
- Energy boosting

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1

Traditional Uses of Spices: An Overview

Ajaikumar B. Kunnumakkara, Cemile Koca, Sanjit Dey, Prashasnika Gehlot, Supachi Yodkeeree, Divya Danda, Bokyung Sung and Bharat B. Aggarwal*

From ancient times, spices have played a major role in the lifestyle of people from certain parts of the world. They have served numerous roles through history, including as coloring agents, flavoring agents, preservatives, food additives and medicine. The active phytochemicals derived from these spices have provided the molecular basis for these actions. This chapter reviews the traditional uses of selected spices.

INTRODUCTION

A spice is a dried seed, fruit, root, bark or flower of a plant or a herb used in small quantities for flavor, color or as a preservative. Many of these substances are also used in traditional medicines. Globalization has made these spices easily available, and increasing their popularity. This chapter reviews the traditional uses of selected spices.

BLACK PEPPER

Black pepper (*Piper nigrum* Linn.) is the world's most common spice and known as the "King of Spices." The word "pepper" is derived from the

*Corresponding author.

Sanskrit *pippali*, the word for long pepper, via the Latin *piper*, which was used by the Romans to refer both to pepper and long pepper (as the Romans erroneously believed that both of these spices were derived from the same plant). The English word for pepper is derived from the Old English "pipor." The Latin word is also the source of German *pfeffer*, French *poivre*, Dutch *peper*, and other similar forms. "Pepper" was used in a figurative sense to mean "spirit" or "energy" at least as far back as the 1840s; in the early 20th century, this was shortened to *pep*. Pepper is a perennial vine and a native of South India. In its dried form, the fruit is often referred to as peppercorns. Peppercorns, and the powdered pepper derived from grinding them, may be described as black pepper, white pepper, red/pink pepper, and green pepper. The sole use of black pepper is in the seasoning of food owing to its aroma and pungency. In traditional medicines, this spice is also reported to have digestive power, to improve appetite, and to cure cold, cough, dyspnea, diseases of the throat, intermittent fever, colic, dysentery, worms and piles (Fig. 1).[1] The uses of

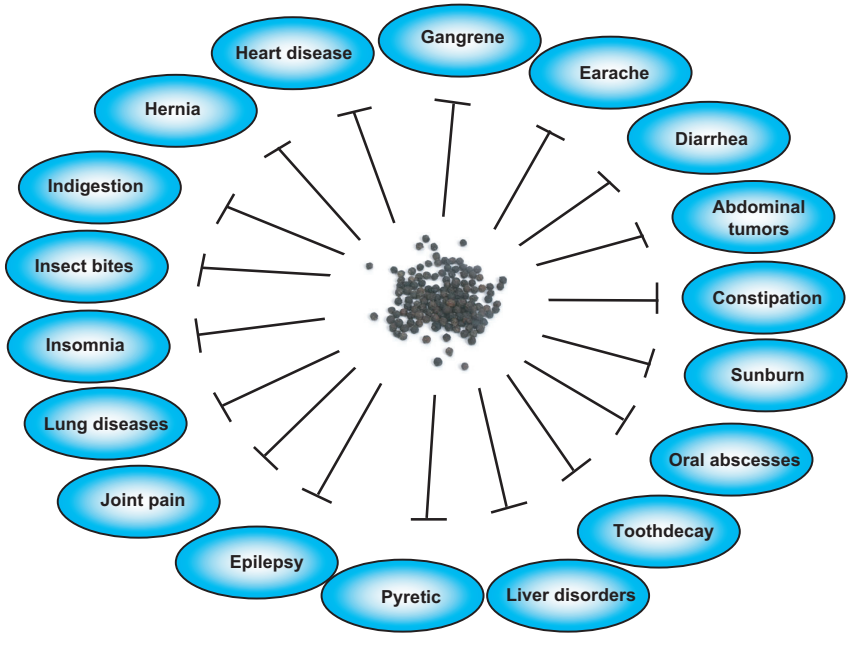

Fig. 1. Traditional uses of black pepper.

black pepper in traditional medicine as an antipyretic and anti-inflammatory are supported by modern science.[2,3] In folk medicine, black pepper is also used against epilepsy and snake bite.[4] The 5th century Syriac Book of Medicines prescribes pepper (or perhaps long pepper) for such illnesses as constipation, diarrhea, earache, gangrene, heart disease, hernia, hoarseness, indigestion, insect bites, insomnia, joint pain, liver problems, lung disease, oral abscesses, sunburn, tooth decay, and toothaches. Pepper root, in the form of ghees, powders, enemas and balms, is a folk remedy for abdominal tumors. Chinese use the spice for urinary calculus. An electuary prepared from the seed is said to help hard tumors, while a salve prepared from the seed is said to help eye indurations and internal tumors.[5]

CARDAMOM

Cardamom consists of two genera of the ginger family Zingiberaceae, namely *Elettaria* and *Amomum*. In South Asia green cardamom is called *elaichi* in Marathi, Hindi and Urdu. It is called *elakkaay* in Telugu and *elam* in Tamil. All these cardamom species are used as cooking spices. Medically, cardamom is used for flatulent indigestion and to stimulate the appetite in people with anorexia (Fig. 2). Moreover, in Ayurvedic medicine it is used as a carminative, diuretic, stomachic and digestive, and for cough, colds and cardiac stimulation. Cardamom has been used in traditional medicine against kidney and urinary disorders,[6] and as a gastrointestinal protective.[7] Cardamom oil has reported anti-inflammatory[8] and antibacterial uses.[9] In India, green cardamom (*A. subulatum*) is broadly used to treat infections of the teeth and gums, to prevent and treat throat trouble, congestion of the lungs and pulmonary tuberculosis, asthma, heart disease, inflammation of the eyelids and digestive disorders. When mixed with neem and camphor, cardamom is used as a nasal preparation to treat colds. An infusion of cardamom can be used as a gargle to relieve sore throats, which has led to its use in cough sweets. Cardamom is also reportedly used as an antidote for both snake and scorpion venom and for food poisoning. In traditional Chinese medicine it is used to treat stomachache, constipation, dysentery, and other digestion problems. Cardamom pods, fried and mixed with mastic and milk, are used for bladder problems. The seeds are popularly believed to be an aphrodisiac.[10]

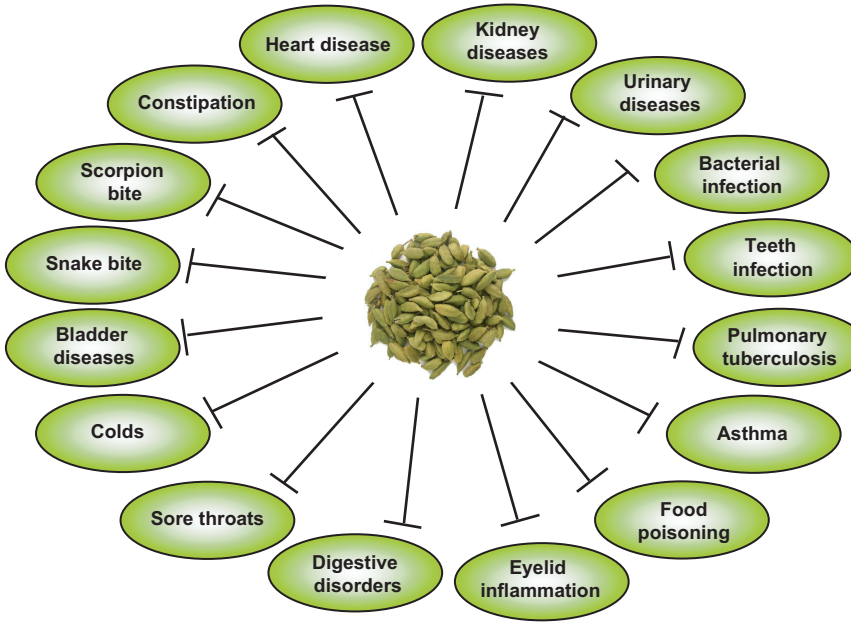

Fig. 2. Traditional uses of cardamom.

CINNAMON

Cinnamon (*Cinnamomum verum* or *C. zeylanicum*) is native to India, Sri Lanka, Bangladesh, and Nepal. The name "cinnamon" comes from Greek *kinnámōmon*, itself ultimately from Phoenician. The botanical name for the spice, *Cinnamomum zeylanicum*, is derived from Sri Lanka's former (colonial) name, Ceylon. In sinhala (Sri Lanka), it is known as *kurundu*, Sanskrit as *tvak* or *dārusitā*, Hindi as *dalchini*, and in Gujarati as *taj*. In Malayalam cinnamon is called *karuva* or *elavarngam*. The dried skin (*karuvappatta*/*elavarngappatta*) of karuva is an important part of spicy curries. This spice is regarded as antipyretic, antiseptic, astringent, balsamic, carminative, diaphoretic, fungicidal, stimulant, and stomachic (Fig. 3). The powdered bark of this spice in water is applied to alleviate headaches and neuralgia. Cinnamon is often combined with ginger to stimulate circulation and digestion. In addition, among people of Kashmiri origin, cinnamon is used to treat infectious diseases. It has been regarded as a folk

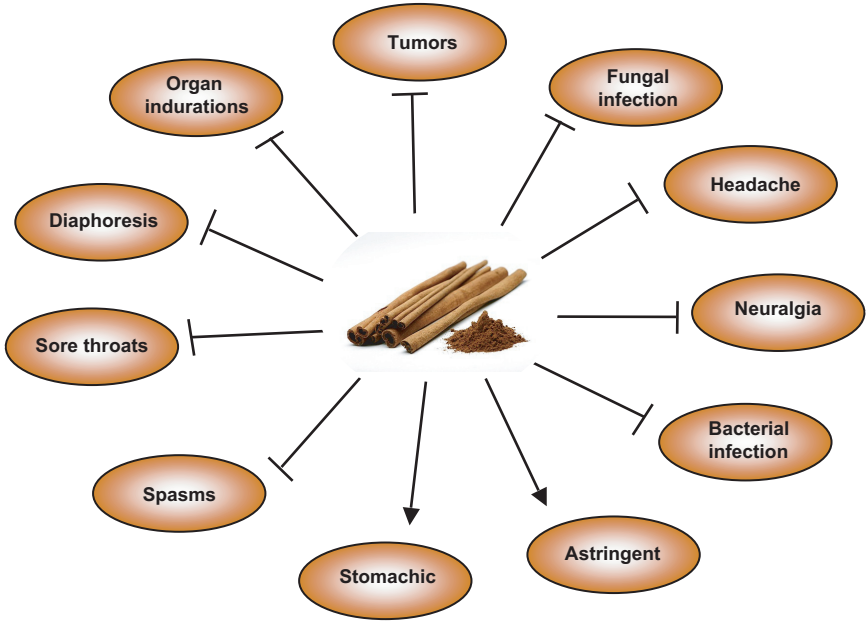

Fig. 3. Traditional uses of cinnamon.

remedy for indurations (of spleen, breast, uterus, liver and stomach) and tumors (especially of the abdomen, liver and sinews).[11–14]

CLOVES

Cloves (*Syzygium aromaticum*, or *Eugenia aromaticum* or *Eugenia caryophyllata*) are the aromatic dried flower buds of a tree in the Myrtaceae family. Cloves are native to Indonesia and are used as a spice in cuisine all over the world. The name derives from the French "*clou*," (meaning "nail") as the buds vaguely resemble small irregular nails in shape. The spice is used in Ayurveda, Chinese medicine and Western herbalism and dentistry, where the essential oil is used as an anodyne (painkiller) for dental emergencies (Fig. 4). It has been reported as analgesic, anesthetic, antibacterial, antiparasitic, antidotal, antioxidant, antiperspirant, antiseptic, carminative, deodorant, digestive, rubefacient, stimulant, stomachic, tonic and vermifugal.[15] Cloves are used as a carminative to increase hydrochloric acid

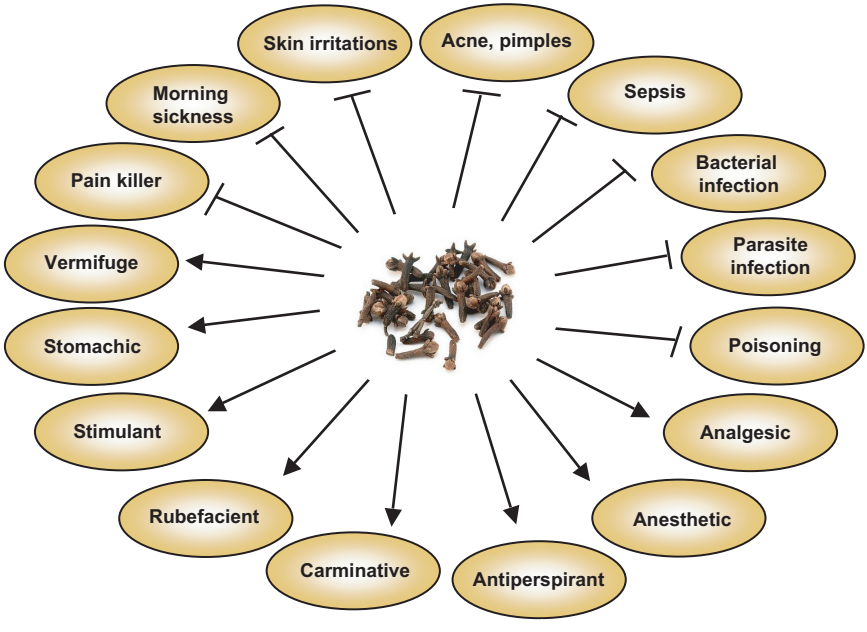

Fig. 4. Traditional uses of cloves.

in the stomach and to improve peristalsis. Cloves are also said to be a natural antihelmintic.[16] The essential oil is used in aromatherapy, especially for digestive problems. Topical application of this spice over the stomach or abdomen will warm the digestive tract. In Chinese medicine cloves are considered acrid, warm and aromatic, entering the kidney, spleen and stomach meridians, and are notable in their ability to warm the middle, direct stomach *qi* (energy flow) downward, treat hiccough and fortify the kidney.[17] Because the herb is so warming, it is contraindicated in any persons with fire symptoms. As such it is used in formulas for impotence or clear vaginal discharge, for morning sickness together with ginseng and patchouli, and for vomiting and diarrhea due to spleen and stomach coldness.[18] Clove oil is used in various skin disorders like acne and pimples, to treat severe burns and skin irritations, and to reduce the sensitiveness of the skin. Cloves are used for the treatment of dog and cat ear problems in British Columbia, Canada. The essential oil extracted from cloves is used as an ointment to relieve pain

and promote healing in herbal medicine. Cloves are also employed as a fragrance in flavoring industries.

CORIANDER

Coriandrum sativum L. Apiaceae (Umbelliferae) (coriander, also known as cilantro, cilantrillo, Arab parsley, Chinese parsley, Mexican parsley, Dhania and Yuen sai), is native to southwestern Asia and regions west to north Africa. The name "coriander" derives from the French *coriandre* through Latin *coriandrum* and in turn from Greek κορίαννον.[19] John Chadwick notes the Mycenaean Greek form of the word, *koriadnon*, "has a pattern curiously similar to the name of Minos' (Minos became a judge of the dead in Hades in Greek mythology) daughter Ariadne," and this explains how the word might have been corrupted later to *koriannon* or *koriandron*.[20] It is an annual herb commonly used in Middle Eastern, Mediterranean, Indian, Latin American, African and Southeast Asian cuisine. Coriander leaves are referred to as cilantro (United States and Canada, from the Spanish name for the plant), *dhania* (Indian subcontinent, and increasingly in Britain), *kindza* (in Georgia), Chinese parsley or Mexican parsley. All parts of the plant are edible, but the fresh leaves and the dried seeds are the most common parts used in cooking.[21] As heat diminishes their flavor quickly, coriander leaves are often used raw or added to the dish right before serving.

In Indian traditional medicine, coriander is used in the disorders of digestive, respiratory and urinary systems as it has diaphoretic, diuretic, carminative and stimulant activities (Fig. 5). The plant is recommended for relief of anxiety and insomnia in Iranian folk medicine,[22] and it is a common plant included in the Mexican diet, usually consumed uncooked, the oil being used as an antimicrobial agent and as a natural fragrance.[23] It is also recommended for urethritis, cystitis, urinary tract infection, urticaria, rash, burns, sore throat, vomiting, indigestion, nosebleed, cough, allergies, hay fever, dizziness and amebic dysentery.[24] Locally known as "Maadnouss" in Morocco, coriander has been documented as a traditional treatment for diabetes, indigestion, flatulence, insomnia, renal disorders and loss of appetite, and as a diuretic.[25]

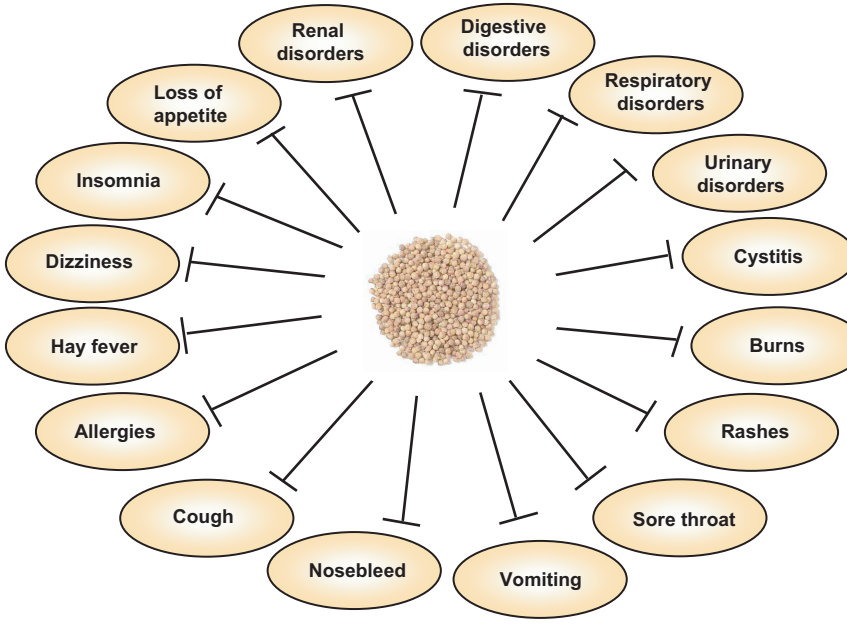

Fig. 5. Traditional uses of corriander.

FENUGREEK

Fenugreek (*Trigonella foenum-graecum*) is commonly known as *maithray* (Bangla, Gujarati), *methi* or *mithi* (Hindi, Nepali, Marathi, Urdu and Sanskrit), *menthyada soppu* (Kannada), *ventayam* (Tamil), *menthulu* (Telugu), *hilbeh* (Arabic), *ulluva* (Malayalam) and *shambalîleh* (Persian). The name "fenugreek" or *foenum-graecum* is from Latin for "Greek hay." In traditional medicines it is used as an aphrodisiac, astringent, demulcent, carminative, stomachic, diuretic, emmenagogue, emollient, expectorant, lactogogue, restorative, and tonic (Fig. 6).[26] Fenugreek is used for a variety of health conditions, including digestive problems, bronchitis, tuberculosis, fevers, sore throats, wounds, arthritis, abscesses, swollen glands, skin irritations, diabetes, loss of appetite, ulcers and menopausal symptoms, as well as in the treatment of cancer. An infusion of the leaves is used as a gargle for recurrent mouth ulcers. As an emollient it is used in poultices for boils, cysts and other complaints. It is used to reduce blood sugar level and to

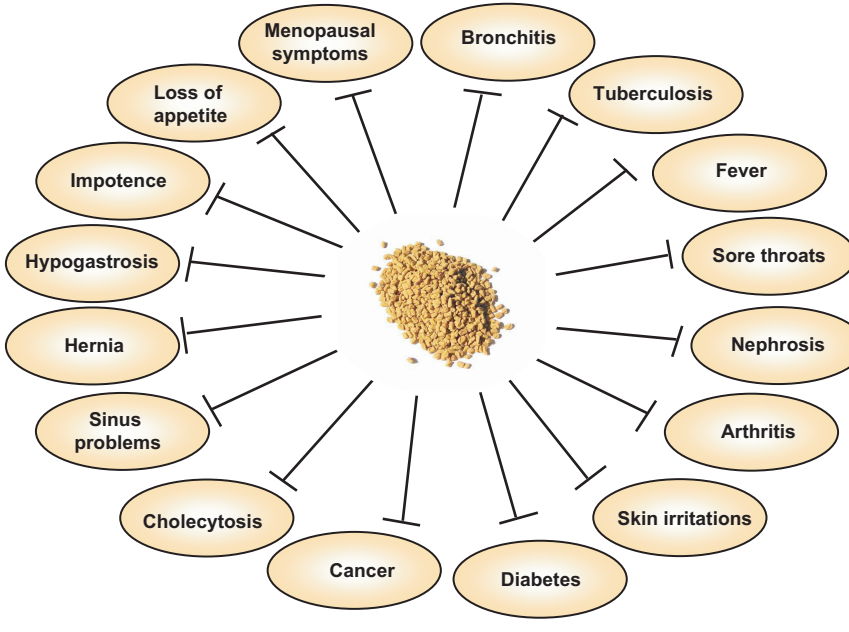

Fig. 6. Traditional uses of fenugreek.

lower blood pressure. Fenugreek has been demonstrated to relieve congestion, reduce inflammation and fight infection. Fenugreek is used for treating sinus and lung congestion, and loosens and removes excess mucus and phlegm. The Chinese use the seed for abdominal pain, chilblains, cholecytosis, fever, hernia, impotence, hypogastrosis, nephrosis, and rheumatism.[26]

GARLIC

Garlic (*Allium sativum* L.) is a species in the onion family, Alliaceae. One of the oldest dietary vegetables, it has been used as early as 3000 BC for the treatment of intestinal disorders and is now known for its fibrinolytic activity and its possible role in lowering blood cholesterol.[27] Dietary patterns in the Mediterranean characterized by high consumption of fruits and vegetables, especially garlic, are believed to be beneficial to the regional patterns of atherosclerotic disease (Fig. 7).[28] The spice has also been used in folk medicine for the treatment of diabetes[29] and inflammation.[30] A well-known

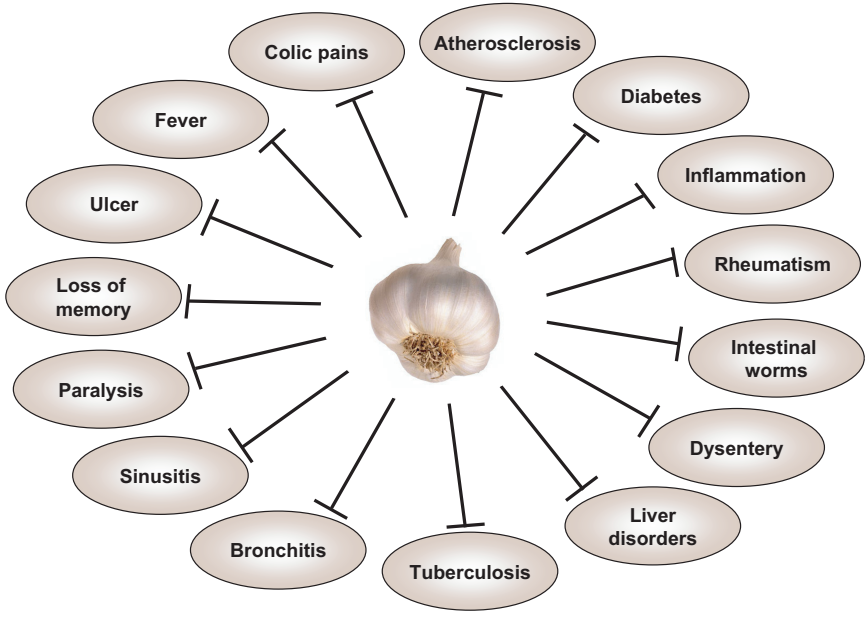

Fig. 7. Traditional uses of garlic.

remedy for local pain is to crush garlic bulbs, apply the crushed garlic to the site of pain and then put a bandage over it. This practice is done by "naturopathic physicians" worldwide and as part of traditional "Arabic Medicine" in the Middle East.[27] In Nepal, East Asia and the Middle East it has been used to treat all manner of illnesses including fevers, diabetes, rheumatism, intestinal worms, colic, flatulence, dysentery, liver disorders, tuberculosis, facial paralysis, high blood pressure and bronchitis. In Ayurvedic and Siddha medicine garlic juice has been used to alleviate sinus problems. In Unani medicine, an extract prepared from the dried bulb is inhaled to promote abortion or taken to regulate menstruation. Unani physicians also use garlic to treat paralysis, forgetfulness, tremor, colic pains, internal ulcers and fevers.

GINGER

Ginger (*Zingiber officinale*) is commonly used as a cooking spice throughout the world. It is also known as *zanjabil* (Arabic), *aadu*

(gujarati), *shunti* (Kannada), *allam* (Telugu), *inji* (Tamil and Malayalam), *alay* (Marathi), *aduwa* (Nepali), and *adrak* (Hindi and Urdu). The rhizome of ginger has long been used in Ayurvedic and traditional Chinese medicine to treat a wide range of ailments including gastrointestinal disorders, mainly nausea and vomiting associated with motion sickness and pregnancy, abdominal spasm, as well as respiratory and rheumatic disorders (Fig. 8). As a home remedy, ginger is widely used for dyspepsia, flatulence, abdominal discomfort and nausea. It has been recommended by herbalists for use as a carminative (an agent that reduces flatulence and expels gas from the intestines), diaphoretic (an agent that produces or increases perspiration), antispasmodic, expectorant, peripheral circulatory stimulant, and astringent (an agent that causes shrinkage of mucous membranes or exposed tissues and that is often used internally to check discharge of blood serum or mucous secretions). Ginger has a reputation for its

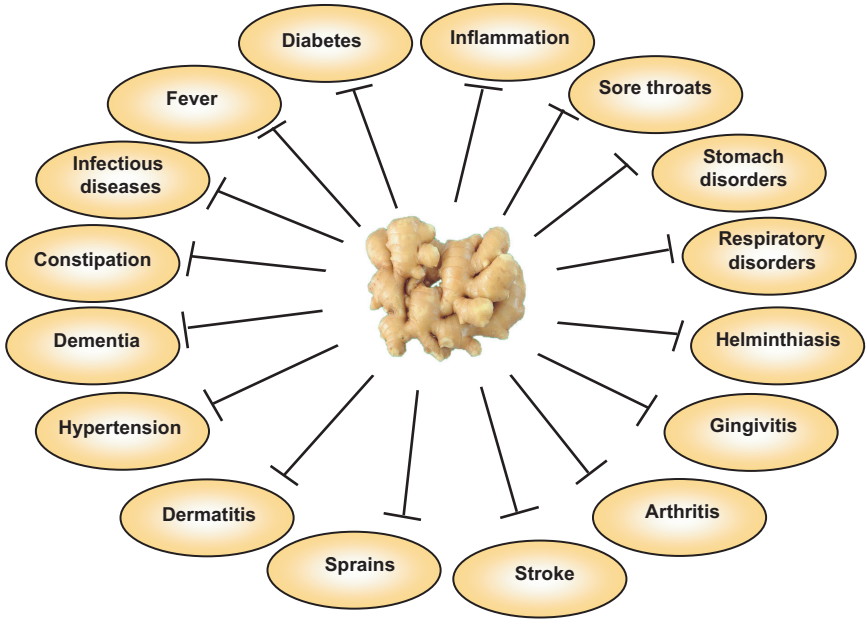

Fig. 8. Traditional uses of ginger.

anti-inflammatory properties. In traditional medicine, ginger has been used to treat a wide array of ailments including sore throats, stomachaches, diarrhea, toothache, gingivitis, arthritis (inflammation of the joints), bronchitis (an acute inflammation of the air passages within the lungs), muscle pains, sprains, constipation dermatitis, hypertension, dementia, fever, infectious diseases, helminthiasis, stroke, constipation, diabetes and asthmatic respiratory disorders.[31–38]

KALONJI

Kalonji (*Nigella sativa*) is an annual flowering plant, native to southwest Asia. The scientific name is a derivative of Latin *niger* meaning "black." In English, *Nigella sativa* seed is variously called black cumin, fennel flower, nutmeg flower, Roman coriander, blackseed, black caraway, or black onion seed. In English-speaking countries with large immigrant populations, it is also known as *kalonji* (Hindi), *kezah* (Hebrew), *chernushka* (Russian), *çörek otu* (Turkish), *habbat albarakah* (Arabic "seed of blessing") or *siyah daneh* (Persian). It is regarded as an aromatic, carminative, diaphoretic, digestive, diuretic, emmenagogue, excitant, lactagogue, laxative, expectorant, antipyretic, antihelminthic, resolvent, stimulant, sudorific, parasiticide, stomachic, tonic, and vermifuge (Fig. 9). The herb may be more important to Muslims than to Christians and Jews. Prophet Muhammad (SAW) once stated that the black seed can heal every disease — except death.[39] In Ayurvedic medicine, it is used as purgative adjunct. In Unani, it is considered an abortifacient and a diuretic and is used for ascites, coughs, eye-sores, hydrophobia, jaundice, paralysis, piles and tertian fever. The Lebanese take the seed extract for liver ailments. In Indonesia, the seeds are added to astringent medicines for abdominal disorders. In Malaya, the seeds are poulticed to treat abscesses, headaches, nasal ulcers, orchitis, and rheumatism. Arabian women use the seeds as a galactagogue.[39] Kalonji seeds and oil, alone or in combination with other drugs, are highly effective in alopecia, vitiligo and other skin ailments. Continuous use of kalonji is effective in mad dog bites. It is useful in paralysis, facial palsy, migraine, amnesia and palpitation. Its powder if taken with water is effective in treating hemorrhoids. If Kalonji seeds are boiled in vinegar and this solution is applied to the gums and teeth, it can reduce inflammation of

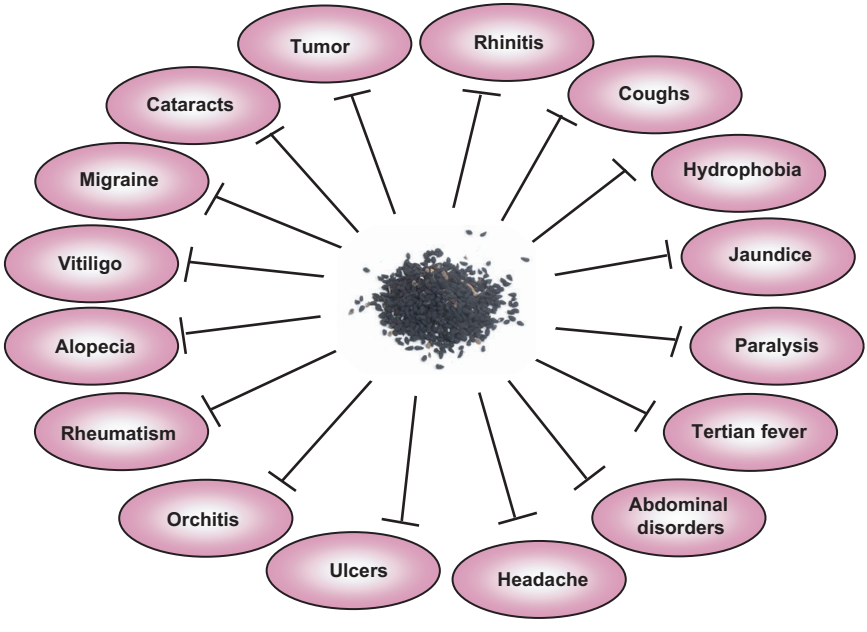

Fig. 9. Traditional uses of kalonji.

the gums and also relieve pain. It has been reported that in a fine powder form it is effective if applied in early stages of cataract. Black seed oil has been a women's beauty secret since ancient times. Black cumin and its oil have been used to purge parasites and worms, detoxify, ameliorate amebic dysentery, shigellosis, abscesses, old tumors, ulcers of the mouth, and rhinitis. For external use, the seed is ground into a powder and mixed with sesame oil, and can be used to treat abscesses, hemorrhoids and orchitis. Finally, the powdered seed has been used to remove lice from the hair.[40,41]

KOKUM

The genus *Garcinia* of the Clusiaceae family includes around 200 species, of which *Garcinia indica* is the most common. *Garcinia indica* is also known as *Brindonia indica*, *Stalagmitis purpurea*, *Garcinia purpurea*, *Garcinia microstigma*, *Stalagmitis indica*, *Garcinia celebica*, and *Oxycarpus indica*. *Garcinia indica*, primarily of Indian origin, is known

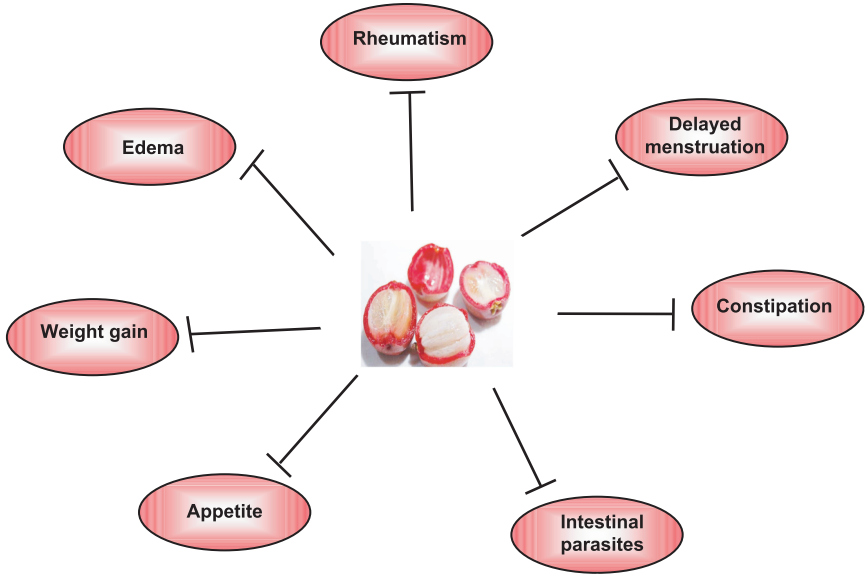

Fig. 10. Traditional uses of kokum.

by many names: *bindin*, *biran*, *bhirand*, *bhinda*, *kokum*, *katambi*, *panarpuli*, *ratamba*, and *amsol*. In the English language, it is commonly known as mangosteen, wild mangosteen, or red mango. The extract and rind of *Garcinia cambogia* is used as a curry condiment in India. In traditional medicine, such as Ayurveda, kokum is prescribed for edema, rheumatism, delayed menstruation, constipation and other bowel complaints, and intestinal parasites (Fig. 10). The extract of *Garcinia cambogia* is used as an herbal appetite suppressant and weight-loss supplement.

MINT

Mentha (mint) is a genus of about 25 species (and many hundreds of varieties) of flowering plants in the family Lamiaceae (mint family).[42] The word "mint" descends from the Latin word *menthe*, which is rooted in the Greek word *minthe*, mentioned in Greek mythology as Minthe, a nymph who was transformed into a mint plant.[43] There are different

types of mint including *Mentha aquatica* — water mint or marsh mint; *Mentha arvensis* — corn mint, wild mint, Japanese peppermint, field mint or pudina; *Mentha asiatica* — asian mint; *Mentha australis* — Australian mint; *Mentha citrata* — bergamot mint; *Mentha crispata* — wrinkled-leaf mint; *Mentha diemenica* — slender mint; *Mentha laxiflora* — forest mint; *Mentha longifolia* or *Mentha sylvestris* — horse mint; *Mentha piperita* — peppermint; *Mentha requienii* — Corsican mint; *Mentha sachalinensis* — Garden mint; *Mentha spicata* — *M. cordifolia*, spearmint, curly mint; *Mentha suaveolens* — apple mint, pineapple mint, and *Mentha vagans* — gray mint. Mint leaves are used in teas, beverages, jellies, syrups, candies, and ice creams. In Middle Eastern cuisine mint is used in lamb dishes. In British cuisine, mint sauce is popular with lamb. Mint is a necessary ingredient in Touareg tea, a popular tea in northern African and Arab countries. The plant is commonly used as a herbal agent in the treatment of loss of appetite, common cold, bronchitis, sinusitis, fever, nausea and vomiting, and indigestion (Fig. 11).[44] Peppermint plants have been used as a

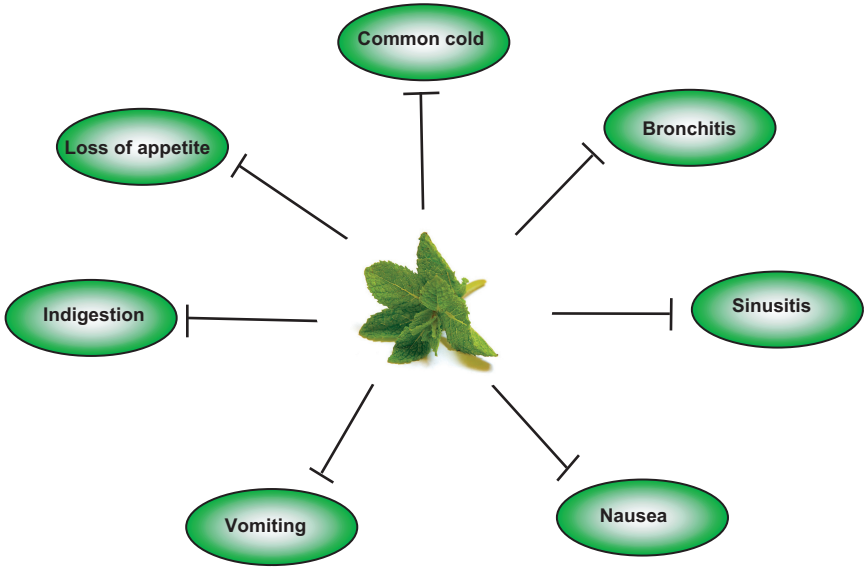

Fig. 11. Traditional uses of mint.

herbal medicine for the same conditions, and others.[45] *Mentha arvensis* is known to possess abortifacient properties in folk medicine (Casey and Satyavati) and is commonly used as a folk remedy for pregnancy termination.[46]

RED CHILI

Red chili, belonging to the plant genus *Capsicum*, is among the most heavily consumed spices throughout the world. The name, which is spelled *chili*, *chile*, or *chilli*, comes from *Nahuatl chīlli* via the Spanish word *chile*. Red chili has been used as an alternative medicine for the treatment of inflammation, diabetes, low back pain and also in homeopathy medicine to treat acute tonsillitis.[47–50] Moreover, capsicum plaster, which contains powdered capsicum and capsicum tincture, has been used in Korean hand acupuncture to reduce postoperative nausea, vomiting and sore throat (Fig. 12).[51,52]

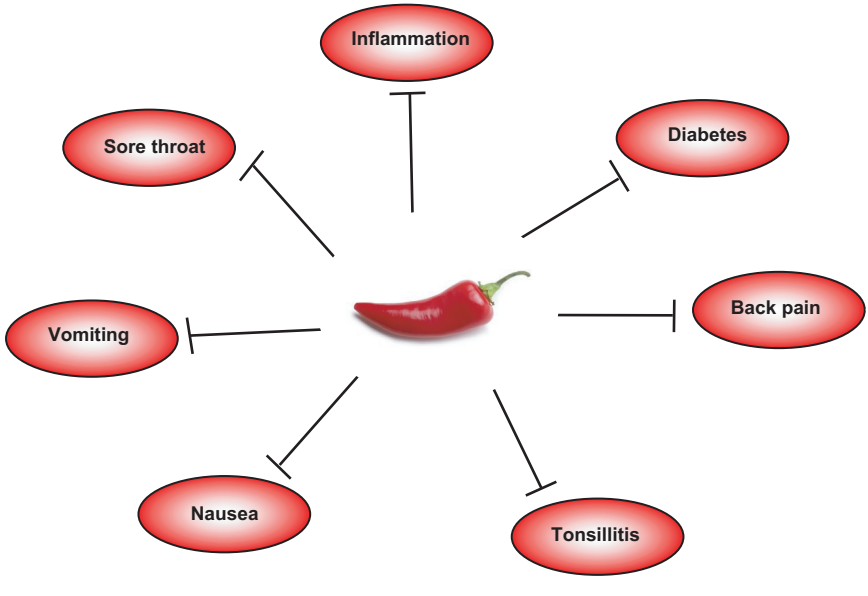

Fig. 12. Traditional uses of red chili.

ROSEMARY

Rosemary (*Rosmarinus officinalis*) is native to the Mediterranean region. The name "rosemary" derives from the Latin name *rosmarinus*, which literally means "dew of the sea." In traditional European medicine, rosemary was used as a tonic, a stimulant, and a carminative to treat flatulence, as well as a diuretic, cholagogue (an agent which promotes the discharge of bile from the system), hepatoprotective, antirheumatic, expectorant, and mild analgesic (Fig. 13). Rosemary has a number of therapeutic applications in folk medicines to treat a wide range of diseases such as headaches, epilepsy, poor circulation, diabetes mellitus, respiratory disorders, eczema, stomach problems and inflammatory diseases, and to stimulate growth of hair. It has been recommended for its positive effects on human fertility. It works as a digestion aid for the treatment of dyspepsia and mild gastrointestinal upsets, and it has been used in renal colic and dysmenorrhea because of its antispasmodic effects. Its aroma is used against coughs

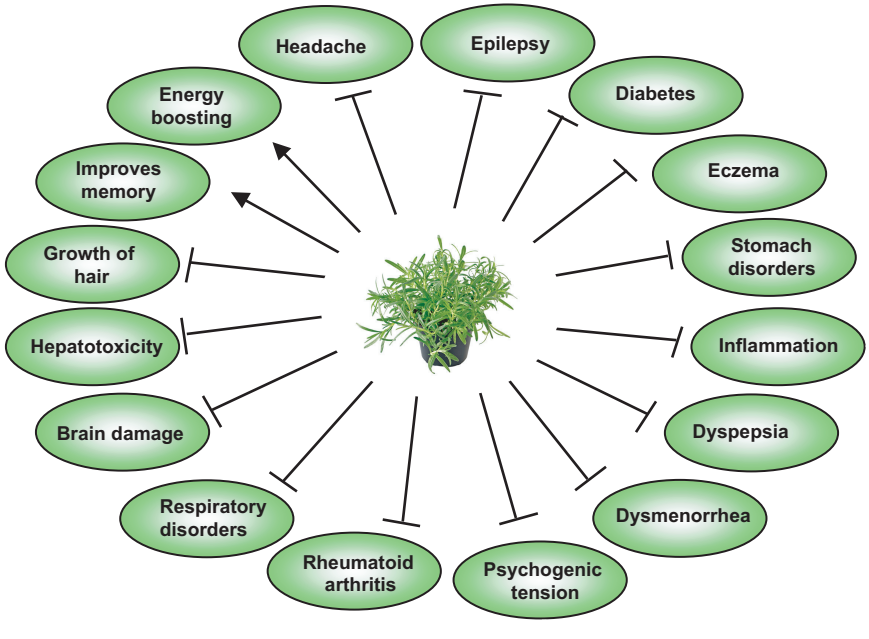

Fig. 13. Traditional uses of rosemary.

and colds. In traditional European medicine, it was believed that the eating of the rosemary flower comforts the brain, the heart and the stomach. It is used to improve memory and concentration, and to boost energy. The leaves of the plant are commonly used as a spice and as a source of antioxidant compounds employed in food conservation; the essential oil is used as a food additive.

The ancient Greeks and Romans used it for improving memory and rejuvenating the spirit. Greek scholars wore garlands of rosemary during examinations in order to improve their memory and concentration.[53] In India, rosemary leaf is used as a component in Ayurvedic and Unani medicines for flatulent dyspepsia associated with psychogenic tension and migraine headaches.[54,55] In Germany, rosemary leaf is licensed as a standard medicinal tea for internal and external use. Rosemary is taken internally as a carminative or stomachic component of gastrointestinal medicines in aqueous infusions, alcoholic fluid extracts, tinctures, and medicinal wine. The aqueous infusion and essential oil are also used in external preparations (e.g. a bath additive, embrocation, liniment or ointment), for rheumatic diseases and circulatory problems.[56,57] In the United States, rosemary is a component of dietary supplement products, in aqueous infusion, alcoholic fluid extract and tincture dosage forms. In both the United States and Germany, the leaf is used in balneotherapy and the essential oil is used in aromatherapy.

TURMERIC

Turmeric is a yellow colored spice derived from the rhizome of the plant *Curcuma longa* and has been used as traditional medicine from ancient times in China and India.[58] It is also known as *kunyit* (Indonesian and Malay), *besar* (Nepali) and *haldi* or *pasupu* in some Asian countries. In Assamese it is called *halodhi*. In medieval Europe, turmeric became known as Indian saffron, since it is widely used as an alternative to the far more expensive saffron spice. The yellow powder from the rhizome of turmeric has been used in Asian cookery, medicine, cosmetics, and fabric coloring for the last 2000 years.[58] As a traditional remedy, turmeric has also been quite extensively used for centuries to treat various disorders such as rheumatism, body ache, skin problems (e.g. wounds, burns and

Fig. 14. Traditional uses of turmeric.

acne), intestinal worms, diarrhea, intermittent fevers, hepatic diseases, urinary discharges, dyspepsia, inflammations, constipation, leukoderma, amenorrhea, dental diseases, digestive disorders such as dyspepsia and acidity, indigestion, flatulence, ulcers, and colic inflammatory disorders such as arthritis, colitis and hepatitis (Fig. 14).[59,60] Moreover, turmeric is a major constituent of Xiaoyao-san, a traditional Chinese medicine that has been used to effectively manage stress and depression-related disorders in China.[61] In Nepal, the rhizome of turmeric is a household remedy. The powder of dried rhizome is considered to be stimulating, carminative, purifying, anti-inflammatory, and anthelmintic.[62]

CONCLUSION

Spices have been shown to be indispensable for daily human health. Besides adding flavor and taste to dishes, they help prevent and alleviate various health problems. Over the last few years several bioactive

compounds have been isolated from spices, providing a scientific basis for the use of spices in our diet.

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2

Black Pepper (*Piper nigrum*) and Its Bioactive Compound, Piperine

Krishnapura Srinivasan

Black pepper (*Piper nigrum*), an Indian native spice, has been widely used in human diet for several thousands of years. It is valued for its characteristic sharp and stinging qualities attributed to the alkaloid *piperine*. While it is used primarily as a food adjunct, black pepper is also used as a food preservative and as an essential component in traditional medicines in India and China. Since the discovery of black pepper's active ingredient, piperine, the use of black pepper has caught the interest of modern medical researchers. Many physiological effects of black pepper, its extracts or its bioactive compound, piperine, have been reported in recent decades. By stimulating the digestive enzymes of the pancreas, piperine enhances digestive capacity and significantly reduces gastrointestinal food transit time. Piperine has been documented to enhance the bioavailability of a number of therapeutic drugs as well as phytochemicals through its inhibitory influence on enzymatic drug biotransforming reactions in liver and intestine. It strongly inhibits hepatic and intestinal aryl hydrocarbon hydroxylase and glucuronyl transferase. Most of the clinical studies on piperine have focused on its effect on drug metabolism. Piperine's bioavailability enhancing property is also partly attributed to increased absorption as a result of its effect on the ultrastructure of the intestinal brush border. Piperine has been demonstrated in *in vitro* studies to protect against oxidative damage by

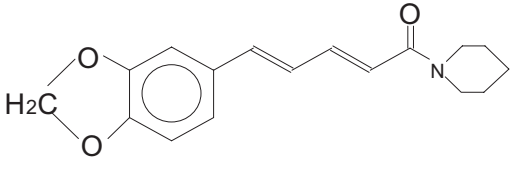
inhibiting or quenching reactive oxygen species. Black pepper or piperine treatment has also been evidenced to lower lipid peroxidation *in vivo* and beneficially influence antioxidant status in a number of experimental situations of oxidative stress. Piperine has also been found to possess anti-mutagenic and anti-tumor influences. Clinical studies are limited, but several have reported the beneficial therapeutic effects of black pepper in the treatment of smoking cessation and dysphagia.

INTRODUCTION

Black pepper (*Piper nigrum*) is one of the most widely used among spices, valued for its characteristic sharp and stinging qualities. It belongs to the family Piperaceae, cultivated for its fruit (berries) that are usually dried and used as a spice and seasoning (Figs. 1a and 1b). Black pepper is native to Southern India and is extensively cultivated in this tropical

(a) Black pepper

(b) Pepper plant



(c) Chemical structure of piperine

Fig. 1. Photographs of (a) the spice, and (b) the spice plant. (c) The chemical structure of piperine.

region. The word "pepper" is derived from the Sanskrit "Pippali", meaning long pepper. Black pepper ("Maricha" in Sanskrit) is known by other names in the local dialect as "Milagu" (Tamil), "Kuru Mulagu" (Malayalam), "Miriyam" (Telugu), "Miriya Konu" (Konkani), and "Kari Menasu" (Kannada). The fruit, also known as peppercorn, is dark red when fully mature, and a small black wrinkled drupe 5 mm in diameter when dried. Black pepper is produced from the green unripe berries of the pepper plant by briefly cooking in hot water. The heat ruptures cell walls in the fruit, activating the browning enzymes during drying. Cooked berries are dried in the sun for several days, during which the fruit around the seed shrinks and darkens into a thin, wrinkled black layer.[1]

White pepper, which is commonly found in Western countries, also comes from the same plant: it consists of the seed only with the outer fruit removed. This is usually accomplished by soaking fully ripe pepper berries in water for about a week, during which the flesh of the fruit softens and decomposes; rubbing off the skin results in the naked seed, which is then dried. Ground black pepper, usually referred to simply as "pepper," may be commonly found on nearly every dinner table, often alongside table salt, in some parts of the world. Dried ground pepper is one of the most common spices in European cuisine and its descendants in other parts of the world.[1]

TRADITIONAL USES

Black pepper has been used as a spice in India since prehistoric times: it has been known to Indian cooking since at least 2000 BC.[2] Peppercorns were a much prized trade good, often referred to as "black gold." Black pepper, along with other spices from India and the Far East, changed the course of world history. Black pepper has been known in China since the 2nd century BC. The pepper trade was first dominated by China, who imported black pepper in mass quantities during the 14th to 16th centuries. Pepper was introduced into Sumatra at the beginning of the 15th century, where pepper cultivation and mass production grew exponentially. It is also recorded that the preciousness of these spices led to European efforts to find a sea route to India and consequently to the European colonial occupation of that country, as well as to the

European discovery and colonization of the Americas. Black pepper is referred to as "King of Spices" and represents one of India's major trade commodities.[3]

It is also known that black pepper was once used as a food preservative. Although it is difficult to believe that in the Middle Ages pepper was used as a preservative for meat, sure enough piperine (Fig. 1c), the compound that gives pepper its spiciness, has some antimicrobial properties, but not at the concentrations present when pepper is used as a spice. However, pepper and other spices probably did play a role in improving the taste of long-preserved meats. Moreover, in the Middle Ages, pepper was a luxury item, affordable only to the wealthy. Having been an item exclusively for the rich, pepper started to become more of an everyday seasoning among those of more average means.

PEPPER AS ANCIENT MEDICINE

Black pepper is historically used not only in human diets but also in traditional medicines and home remedies.[4] The use of black pepper in medicine in India dates back thousands of years. Long pepper (*Piper longum*), being stronger, was often the preferred medication although both were used. Black pepper figures in remedies in Ayurveda, Siddha and Unani medicine in India. The 5th century Syriac Book of Medicines prescribes black pepper (and long pepper) for such illnesses as constipation, diarrhea, earache, gangrene, heart disease, hernia, hoarseness, indigestion, insect bites, insomnia, joint pain, liver problems, lung disease, oral abscesses, sunburn, tooth decay, and toothaches. Black pepper was relied upon to treat specific conditions such as diarrhea and fevers, but it appears that its extensive generalized use was to enhance the effects of many herbal remedies.[4]

Ayurvedic physicians have been prescribing long pepper and black pepper (both of which are now known to contain piperine) for thousands of years, a practice which may have enhanced the pharmacological actions of other compounds in traditional herbal medicines. It is a vital ingredient of many remedies in the traditional Ayurvedic system of medicine in India. Black pepper is a component of "Trikatu" (three acrids) along with long pepper (*Piper longum*) and ginger (*Zingiber officinale*)

in equal proportions. *Trikatu* is widely used in combination with other Ayurvedic medications according to the ancient *Ayurvedic Materia Medica* (600–300 BC). Very few compound prescriptions are free from these three acrids.[5] *Trikatu* aims to correct the imbalance of the three "doshas" (psychophysical components of the human body) that can lead to disease.[6] Black pepper is specifically cited in Ayurveda to internally treat fevers, gastric and abdominal disorders, and urinary problems.[6] Medicinal external treatments with black pepper include treatments for rheumatism, neuralgia, and boils.[6] *Piper nigrum* is also used to treat alopecia.[5] Possible uses of black pepper in Indian folk medicine include the treatment of respiratory diseases, dysentery, pyrexia, and insomnia.[6] Black pepper is part of an herbal folk remedy relied upon by mothers to treat their children's diarrhea.[7] This wide-ranging use of black pepper in India is unprecedented in other medical systems and areas of the world.

Once black pepper reached China, it was incorporated into traditional Chinese medicine. Pepper is cited for its digestive stimulant action — to make food enter the large intestine channels to "warm the middle, disperse cold, drive the food downward while dispelling phlegm, wind-cold, and relieving diarrhea."[8] This is caused by stomach cold, characterized by vomiting, diarrhea, and abdominal pain. Black pepper has been used in China as a folk remedy for epilepsy. A popular Chinese folk remedy for epilepsy calls for a dried powder consisting of one radish and 99 peppercorns.[9] Black pepper is also used as contraceptive in Assam (A north-eastern state in India) folk medicine.

CHEMICAL CONSTITUENTS

The spiciness of black pepper, which is characterized by its distinct sharp and stinging qualities, is due to the alkaloid compound piperine, found both in the outer fruit and in the seed (Fig. 1c).[1] Refined piperine is about 1% as hot as the capsaicin of red chili pepper. The outer fruit layer left on black pepper also contains important odor-contributing terpenes including pinene, sabinene, limonene, caryophyllene, and linalool, which give citrusy, woody, and floral notes. Pepper contains small amounts of safrole, a mildly carcinogenic compound. The bioactive and pungent ingredient of

black pepper was identified as piperine and isolated in 1820 by the Dutch chemist Hans Christian Orstedt.[1]

Many beneficial physiological effects of black pepper, its extracts or its major active principle, piperine, have been reported in recent decades and have been reviewed.[10]

DIVERSE EXPERIMENTALLY VALIDATED BENEFICIAL PHYSIOLOGICAL EFFECTS OF BLACK PEPPER AND PIPERINE

Influence on the Gastrointestinal System (Table 1)

Digestive stimulant action

It is a general perception that aromatic and pungent spices, by imparting flavor and appealing taste to foodstuffs, enhance salivary and gastric secretions. Glatzel, studying the effect of spices on the secretion and composition of saliva in human subjects, observed that black pepper and other spices enhance the secretion of saliva and the activity of salivary amylase.[11] The digestive stimulant action of spices is exerted through: (i) a beneficial stimulation of the liver to produce and secrete bile rich in bile acids, which play a very important role in fat digestion and absorption, or (ii) a beneficial stimulation of the activities of enzymes of pancreas and intestine that participate in digestion.[12] Black pepper and its active principle, piperine, examined for their effect on bile secretion as a result of both a continued intake through the diet for a period of time and as a one-time exposure orally in experimental rats, did not show any beneficial stimulatory influence on bile acid production by the liver and its secretion into bile.[13] On the other hand, oral administration of piperine as a single dose significantly increased bile acid secretion. The influence of dietary intake of piperine (at levels corresponding to about five times the average dietary intake of black pepper by the Indian population) on the pancreatic digestive enzymes and the terminal digestive enzymes of the small intestinal mucosa have been examined in experimental rats.[14,15] Significantly increased activities of pancreatic lipase, amylase, chymotrypsin and trypsin were observed as a result of dietary intake of piperine in these experimental rats.[14] Such beneficial

Table 1. Influence of black pepper and piperine on the gastrointestinal system.

System	Remarks	Reference
Digestive stimulant action		
Rats	a) Stimulation of digestive enzymes of pancreas by dietary piperine.	14
	b) Stimulation of digestive enzymes of intestine by dietary piperine.	15
	c) Oral administration of piperine increases biliary bile acid secretion.	13
Influence on intestinal motility and food transit time		
Humans	a) Increased orocecal transit time after black pepper consumption.	24
Rats	a) Gastrointestinal food transit time shortened by dietary piperine.	27
	b) Piperine inhibits gastric emptying of solids/liquids.	25
Mice	a) Piperine inhibits gastrointestinal transit.	25
	b) Piperine dose-dependently delays gastrointestinal motility.	26
Effect on gastric mucosa		
Humans	a) Black pepper causes increased gastric parietal and pepsin secretion and increased gastric cell exfoliation in humans.	16
Rats	a) Black pepper increases gastric acid secretion in anesthetized rats.	18
	b) Piperine increases gastric acid secretion.	19
	c) Piperine has protective action against stress-induced gastric ulcer.	17
Mice	a) Piperine has protective action against stress-induced gastric ulcer.	17
Antidiarrheal property		
Mice	a) Piperine inhibits diarrhea produced by castor oil, arachidonic acid and magnesium sulfate.	20
	b) Piperine reduces castor oil-induced intestinal fluid accumulation in intestine.	21
Influence on absorptive function		
Rats	a) Piperine stimulates γ-glutamyl transpeptidase activity and enhanced uptake of amino acids in isolated epithelial cells of rat jejunum.	22
	b) Piperine modulates membrane dynamics and permeation characteristics, increasing absorptive surface and induction of synthesis of proteins associated with cytoskeletal function.	23

influence of this spice on the activity of these enzymes was not evident when administered as a single oral dose. Piperine also prominently enhanced the activity of intestinal lipase and amylase in animals given single oral doses of piperine.[15]

Effect on gastric mucosa

Clinical studies: Pungent spices have long been implicated as a cause of gastric mucosal injury; their long-term effect on the gastric mucosa is still less known. In a single-dose study, the effects of black pepper on the gastric mucosa were assessed using double-blind intragastric administration of the spice (1.5 g) to healthy human volunteers, with aspirin (655 mg) as positive control.[16] Black pepper caused significant increases in parietal secretion, pepsin secretion, and potassium loss. Gastric cell exfoliation (as reflected in DNA loss in gastric contents) was increased after black pepper administration and mucosal microbleeding was also seen. These effects of black pepper on gastric mucosa were similar to aspirin.

Animal studies: On the other hand, the protective action of piperine against experimental gastric ulcer has been evidenced in rats and mice wherein the gastric mucosa damage was induced by stress, indometacin, HCl, and pyloric ligation.[17] Piperine at 25, 50, 100 mg/kg i.g. protected animals from gastric ulceration in a dose-dependent manner. Piperine inhibited the volume of gastric juice, gastric acidity, and pepsin activity. Black pepper has been reported to significantly increase gastric acid secretion in anesthetized rats.[18] Piperine has been shown to produce a dose-dependent (20–142 mg/kg) increase in gastric acid secretion in albino rats.[19] Involvement of cholinergic receptors in the observed piperine-induced increase in gastric acid secretion has been ruled out as the effect of piperine was significantly antagonized by cimetidine (1 mg/kg) but not by atropine (1 mg/kg). There is, however, an indication that increased acidity induced by piperine could be due to stimulation of histamine H2 receptors by this spice compound.

Antidiarrheal property

Animal studies: Peppers are added in traditional antidiarrheal formulations of different herbs. In a study undertaken in experimental mice, the antidiarrheal activity of piperine against diarrhea produced by castor oil, $MgSO_4$ and arachidonic acid has been evidenced at 8 and 32 mg/kg p.o.

doses.[20] Piperine's inhibition of castor oil-induced enteropooling suggests its inhibitory effect on prostaglandins. Piperine (2.5–20 mg/kg i.p.) dose-dependently reduced castor oil-induced intestinal fluid accumulation in experimental mice.[21] It was further understood that piperine reduces castor oil-induced fluid secretion with a mechanism involving capsaicin-sensitive neurons but not capsazepine-sensitive vanilloid receptors.

Influence on absorptive function

Animal studies: The effect of piperine on the absorptive function of the intestine has been studied in *in vitro* experiments, showing that piperine (25–100 μM) significantly stimulated γ-glutamyl transpeptidase (γ-GT) activity and enhanced the uptake of amino acids in freshly isolated epithelial cells of rat jejunum.[22] The kinetic behavior of γ-GT towards substrate and acceptor was altered in the presence of piperine, suggesting that piperine may interact with the lipid environment to produce effects leading to increased permeability of the intestinal cells. It is hypothesized that piperine's bioavailability-enhancing property may be partly attributed to increased absorption.[23] Piperine also caused an increase in intestinal brush border membrane fluidity and stimulated leucine amino peptidase and glycyl-glycine dipeptidase activity due to the alteration in enzyme kinetics. This suggests that piperine could modulate the membrane dynamics due to its apolar nature by interacting with surrounding lipids and hydrophobic portions in the protein vicinity, which may decrease the tendency of membrane lipids to act as stearic constraints to enzyme proteins and thus modify enzyme conformation. Ultrastructural studies with piperine showed an increase in microvilli length with a prominent increase in free ribosomes and ribosomes on the endoplasmic reticulum in enterocytes, suggesting that synthesis or turnover of cytoskeletal components or membrane proteins may be involved in the observed effect. Thus, piperine may induce alterations in membrane dynamics and permeation characteristics, along with induction of the synthesis of proteins associated with cytoskeletal function, resulting in an increase in the absorptive surface, thus assisting efficient permeation through the epithelial barrier.

Influence on gastrointestinal motility and food transit time

Clinical studies: In a study of the effect on small intestinal peristalsis evaluated by measuring orocecal transit time utilizing the lactulose hydrogen breath test in healthy subjects, an increase in orocecal transit time was observed after black pepper (1.5 g) consumption.[24]

Animal studies: Piperine has been found to inhibit gastric emptying (GE) of solids/liquids in rats and gastro-intestinal transit (GT) in mice in a dose- and time-dependent manner.[25] It significantly inhibited GE of solids and GT at the doses extrapolated from humans (1 mg/kg and 1.3 mg/kg p.o. in rats and mice, respectively). One week oral treatment of 1 mg/kg and 1.3 mg/kg in rats and mice, respectively, did not produce a significant change in activity as compared to single-dose administration. The GE inhibitory activity of piperine is independent of gastric acid and pepsin secretion. Piperine, which activates vanilloid receptors (0.5–20 mg/kg i.p.) dose-dependently, delayed gastrointestinal motility in mice.[26] The inhibitory effect of piperine (10 mg/kg) was strongly attenuated in capsaicin-treated (75 mg/kg in total, s.c.) mice. The study indicated that the vanilloid ligand piperine can reduce upper gastrointestinal motility. The effect of piperine involves capsaicin-sensitive neurones but not vanilloid receptors.

The gastrointestinal food transit time in experimental rats has been shown to be significantly shortened by dietary piperine.[27] The reduction in food transit time produced by dietary piperine roughly correlated with its beneficial influence either on digestive enzymes or on bile secretion.[12] Thus, dietary piperine, which enhanced the activity of digestive enzymes, also markedly reduced the food transit time at the same level of consumption. This reduction in food transit time could probably be attributed to acceleration in the overall digestive process as a result of increased availability of digestive enzymes.

Inhibitory Influence of Piperine on Drug Metabolizing Enzyme System (Table 2)

In the context of piperine having been reported to enhance drug bioavailability, Atal *et al.* studied the interaction of piperine with drug

Table 2. Influence of piperine on the drug metabolizing enzyme system.

System	Remarks	Reference
In vitro	a) Inhibition of aryl hydroxylation, N-demethylation, O-deethylation and glucuronidation *in vitro* by piperine.	28
	b) Decreased UDP-glucuronic acid concentration and rate of glucuronidation in isolated epithelial cells of guinea pig small intestine by piperine.	29
	c) Inhibition of aryl hydroxylase and O-deethylase activities by piperine *in vitro* in pulmonary microsomes.	32
	d) Suppression of aryl hydroxylation in cell culture is mediated by direct inter-action of piperine with cytochrome P450 and not by downregulation of its gene expression.	34
	e) Piperine decreases the activities of liver microsomal aryl hydroxylase, N-demethylase and UDP-glucuronosyl transferase and cytochrome P450.	36
Rats	a) Lower aryl hydroxylase and UDP-glucuronyl transferase activities, prolonged hexobarbital sleeping time in piperine treated rats.	28
	b) Inhibition of aryl hydroxylase and O-deethylase activities by piperine *in vivo* in pulmonary microsomes.	32
	c) Decreased activities of hepatic microsomal cytochrome P450, N-demethylase, aryl hydroxylase by intragastric/ intraperitoneal piperine.	30
	d) Inhibition of UDP-glucose dehydrogenase and UDP-glucuronyl transferase in liver and intestine by piperine.	33
	e) Lowered activity of N-demethylase, UDP-glucuronosyl transferase and NADPH-cytochrome-C reductase as a result of piperine feeding.	36
Guinea pigs	a) Inhibition of UDP-glucose dehydrogenase and UDP-glucuronyl transferase in liver and intestine by piperine.	33

biotransforming reactions in hepatic tissue *in vitro* and *in vivo*.[28] Piperine inhibited hydroxylation of aryl hydrocarbon, N-demethylation of ethylmorphine, O-deethylation of 7-ethoxycoumarin and glucuronidation of 3-hydroxybenzo (α) pyrene (3-OH-BP) in rat liver *in vitro* in a dose-dependent manner. Piperine caused noncompetitive inhibition of hepatic

microsomal aryl hydrocarbon hydroxylase (AHH) from untreated and 3-methylcholanthrene-treated rats with a K_i of 30 μM. Similarly, the kinetics of inhibition of ethylmorphine-N-demethylase from control rat liver exhibited noncompetitive inhibition with a K_m of 0.8 mM and K_i of 35 μM. These studies demonstrate that piperine is a nonspecific inhibitor of drug metabolism which shows little discrimination between different cytochrome P450 forms. Oral administration of piperine in rats strongly inhibited the hepatic AHH and UDP-glucuronyl transferase activities, the inhibition of AHH being observed within 1 hr and restored to normal by 6 hrs. Pretreatment with piperine prolonged hexobarbital sleeping time and zoxazolamine paralysis time in mice. These results demonstrate that piperine is a potent inhibitor of drug metabolism. The basis of inhibition of glucuronidation by piperine has been explored by examining the rate of glucuronidation of 3-OH-BP and UDP-glucuronic acid (UDPGA) content in the intact isolated epithelial cells of the guinea-pig small intestine.[29] It was found that glucuronidation of 3-OH-BP was dependent on duration of incubation, cellular protein and endogenous UDPGA concentration. Piperine caused a concentration-related decrease in UDPGA content and the rate of glucuronidation in these cells. Piperine also caused noncompetitive inhibition of hepatic microsomal UDP-glucuronyltransferase with a K_i of 70 μM. The study demonstrated that piperine modifies the rate of glucuronidation by lowering the endogeneous UDPGA content and also by inhibiting the transferase activity.

Although an increase in hepatic microsomal cytochrome P450 and cytochrome b_5, NADPH-cytochrome *c* reductase, benzphetamine N-demethylase, aminopyrine N-demethylase and aniline hydroxylase was observed 24 hrs following intra-gastric administration of piperine (100 mg/kg) in adult Sprague–Dawley rats, a higher intra-gastric dose (800 mg/kg) or i.p. (100 mg/kg) dose of piperine produced a significant decrease in the levels of cytochrome P450, benzphetamine N-demethylase, aminopyrine N-demethylase and aniline hydroxylase 24 hrs after treatment.[30] An i.p. administration of rats with piperine (100 mg/kg) produced a significant decrease in hepatic cytochrome P450 and activities of benzphetamine N-demethylase, aminopyrine N-demethylase and aniline hydroxylase 1 hr after the treatment.[31] Twenty-four hours later, these parameters along with cytochrome b_5 and NADPH-cytochrome *c* reductase

remained depressed in piperine-treated rats. This suggested that the effect of piperine on hepatic mixed-function oxidases is monophasic.

Piperine caused concentration-related non-competitive inhibition *in vitro* (50% at 100 μM) of AHH and 7-ethoxycoumarin deethylase activities in lung microsomes of rats and guinea pigs.[32] *In vivo*, piperine given at a dose of 25 mg/kg body weight to rats caused a maximal inhibition at 1 hr of both the enzymes, while only AHH returned to the normal value within 4 hrs. Similarly, upon daily treatment of piperine (15 mg/kg body weight) to rats for 7 days, deethylase activity was consistently inhibited, while AHH showed faster recovery. Piperine thus appeared to cause differential inhibition of two forms of cytochrome P450 and thus would accordingly affect the steady-state level of those drugs metabolized by these pulmonary forms of cytochrome P450.

Piperine caused a concentration-related strong non-competitive inhibition of UDP-glucose dehydrogenase (UDP-GDH) (50% at 10 μM) reversibly and equipotently in rat and guinea pig liver and intestine.[33] However, the UDPGA contents were decreased less effectively by piperine in isolated rat hepatocytes compared with enterocytes of guinea pig small intestine. Piperine at 50 μM caused a marginal decrease of UDPGA in hepatocytes when the rate of glucuronidation of 3-OH-BP decreased by about 40%. Piperine did not affect the rate of glucuronidation of 4-OH-biphenyl in rat liver, whereas that of 3-OH-BP was impaired significantly. In guinea pig small intestine, both these activities were inhibited significantly, requiring less than 25 μM piperine to produce a more than 50% inhibition of UDP-glucuronyl transferase. The results suggest that piperine is a potent inhibitor of UDP-GDH and it exerts stronger effects on intestinal glucuronidation than in rat liver.

By studying the modulation of B(α)p metabolism and regulation of cytochrome CYP1A1 gene expression by piperine in 5L cells in culture, it has been observed that piperine mediated inhibition of AHH activity, and that the consequent suppression of the procarcinogen activation is the result of direct interaction of piperine with cytochrome P4501A1-protein and not because of down regulation of its gene expression.[34] Piperine was evaluated for beneficial effects in Alzheimer's disease by studying the potential for herb-drug interactions involving cytochrome P450,

UDP-glucuronosyl transferase, and sulfotransferase enzymes. Piperine was a relatively selective noncompetitive inhibitor of CYP3A (IC_{50} of 5.5 μM, K_i of 5.4 μM) with less effect on other enzymes evaluated (IC_{50} > 29 μM).[35] Piperine inhibited recombinant CYP3A4 much more potently (more than five fold) than CYP3A5.

The effect of dietary supplementation of piperine (0.02%) on the activities of the liver drug-metabolizing enzyme system has been examined in rats.[36] Piperine significantly stimulated the activity of aryl hydroxylase. The activity of N-demethylase, UDP-glucuronosyl transferase and NADPH-cytochrome *c* reductase activity was significantly lowered as a result of piperine feeding, while the levels of hepatic microsomal cytochrome P450 and cytochrome b_5 were not influenced by piperine. Piperine also significantly decreased the activities of liver microsomal AHH, N-demethylase and UDP-glucuronosyl transferase *in vitro* at a 1×10^{-6} mol/L level in the assay medium. Piperine also brought about a significant decrease in liver microsomal cytochrome P450 when included at 1×10^{-6} mol/L.

The modifying potential of black pepper on the hepatic biotransformation system has been assessed in mice fed on a diet containing 0.5%, 1% and 2% black pepper for 10 and 20 days.[37] Data revealed a significant and dose-dependent increase in glutathione S-transferase and sulfhydryl content in the experimental groups on the 1% and 2% black pepper diets. Elevated levels of cytochrome b_5 and cytochrome P450 were also significant and dose dependent. As a potential inducer of the detoxication system, the possible chemopreventive role of black pepper in chemical carcinogenesis was suggested.

Piperine Enhances the Bioavailability of Drugs and Phytochemicals (Table 3)

Clinical studies: Piperine, the alkaloid constituent of both black and long pepper, is now established as a bioavailability enhancer of various structurally and therapeutically diverse drugs and other substances. The potential of piperine to increase the bioavailability of drugs in humans is of great clinical significance. Most of the clinical trials done on black pepper have shown that piperine increases levels of certain medications: phenytoin (an epileptic treatment), propranolol (used for hypertension and

Table 3. Modulation of bioavailability of drugs, phytochemicals, and carcinogens by black pepper and piperine.

System	Remarks	Reference
Humans	a) Increased bioavailability of vasicine and sparteine as a result of *Piper longum*/piperine treatment.	39
	b) Enhanced systemic availability of propranolol and theophylline as a result of piperine treatment.	40
	c) Increased serum concentration of curcumin by concomitant administration of piperine.	41
	d) Increased plasma levels of coenzyme Q_{10} by coadministration of piperine.	42
	e) Increased plasma concentration of phenytoin when coadministered along with piperine.	43
	f) Increased plasma concentration of antiretroviral agent nevirapine when coadministered along with piperine.	44
Rats	a) Decreased metabolic activation of fungal toxin aflatoxin B_1 and hence its increased accumulation in plasma.	48
	b) Enhanced bioavailability of β-lactam antibiotics amoxicillin trihydrate cefotaxime by coadministration of piperine.	47
Mice	a) Delayed elimination of anti-epileptic drug phenytoin by treatment of piperine.	46
	b) Increased plasma levels and delayed excretion of epigallocatechin-3-gallate from green tea as a result of intragastric cotreatment with piperine.	45

stage fright), rifampicin (a tuberculosis medication), theophylline (lung medication), and even coenzyme Q_{10}. This observed effect is due to the inhibitory interaction of piperine with cytochrome P450 enzymes of the liver and gastrointestinal tract that are also involved in drug metabolism: CYP1A2, CYP1A1, CYP2D6, CYP3A4; P-glycoprotein (P-gp) is also affected.[38] Since piperine inhibits both P-glycoprotein and CYP3A4 expressed in enterocytes and hepatocytes, it contributes to a major extent to first-pass elimination of many drugs.[38]

The scientific basis of the use of the *trikatu* group of acrids (long pepper, black pepper and ginger) in a large number of prescriptions in the

indigenous Ayurvedic system of medicine in India has been evaluated by Atal *et al.*[39] The observed increase of over 200% in the blood levels of the test drug vasicine by *Piper longum* and of the blood levels of the test drug sparteine by over 100% under the influence of piperine in a clinical study suggested that these acrids have the capacity to increase the bioavailability of certain drugs. The authors concluded that the *trikatu* group of drugs increases bioavailability of drugs either by promoting rapid absorption from the gastrointestinal tract or by protecting the drug from being metabolized in its first passage through the liver after being absorbed, or by a combination of these two mechanisms. The effect of piperine on the bioavailability and pharmacokinetics of propranolol and theophylline has been examined in a crossover study wherein subjects received a single oral dose of propranolol (40 mg) or theophylline (150 mg) alone or in combination with piperine (20 mg/day for 7 days).[40] An enhanced systemic availability of oral propranolol and theophylline was evidenced as a result of piperine treatment.

A pharmacokinetic study has examined the effect of piperine, a known inhibitor of hepatic and intestinal glucuronidation on the bioavailability of curcumin, the bioactive ingredient of the spice turmeric administered with piperine in healthy human volunteers.[41] The human study was done in a cross-over design with two weeks separating two clinical testing sessions. After a dose of 2 g of curcumin taken without piperine, serum levels were either undetectable or very low. Concomitant administration of piperine (20 mg) produced 2000% higher concentrations from 0.25 to 1 hr post-drug. The study showed that, in the dosages used, piperine enhances the serum concentration, extent of absorption and bioavailability of curcumin in humans. This assumes importance in the context of the diverse medicinal properties of *Curcuma longa*. Black pepper extract consisting of 98% piperine has been evidenced to increase plasma levels of orally supplemented coenzyme Q_{10} in a clinical study using a double-blind design.[42] The relative bioavailability of 90 mg and 120 mg of coenzyme Q_{10} administered in a single dose or for 14 and 21 days with placebo or with 5 mg of piperine was determined by comparing measured changes in plasma concentration. Supplementation of 120 mg coenzyme Q_{10} with piperine for 21 days produced a significant, approximately 30% greater AUC than with coenzyme Q_{10} plus placebo.

Piperine has been reported to enhance the oral bioavailability of phenytoin in human volunteers. The effect of a single dose of piperine in patients with uncontrolled epilepsy on the steady-state pharmacokinetics of phenytoin has been examined.[43] Piperine (20 mg administered along with phenytoin) increased significantly the mean plasma concentration of phenytoin at most of the time points in patients receiving either 150 mg or 200 mg twice daily doses of phenytoin. There was a significant increase in AUC, C_{max} and K_a.

Nevirapine is a potent non-nucleoside inhibitor of HIV-1 reverse transcriptase and is indicated for use in combination with other antiretroviral agents for the treatment of HIV-1 infection. In a cross-over, placebo-controlled study conducted in eight healthy adult males, subjects received piperine 20 mg or placebo for 6 days, and on day 7, nevirapine 200 mg plus piperine 20 mg or nevirapine plus placebo in a crossover fashion.[44] Mean maximum plasma concentration, the area under the plasma concentration-time curve, from 0 to 144 hrs post-dose were increased significantly when co-administered with piperine. This evidence for enhanced bioavailability of nevirapine when administered with piperine suggests a possible clinical advantage arising from the bioenhancement capabilities of piperine in the treatment of HIV infection.

Animal studies: It has been observed that intragastric cotreatment with dietary piperine enhances the bioavailability of epigallocatechin-3-gallate (EGCG; demonstrated to have chemopreventive activity) from green tea in mice.[45] Coadministration of 164 μmol/kg EGCG and 70 μmol/kg piperine to male mice increased the plasma C_{max} and area under the curve (AUC) by 1.3-fold compared to mice treated with EGCG only. Piperine appeared to increase EGCG bioavailability by inhibiting glucuronidation and gastrointestinal transit. A similar effect of piperine in altering the pharmacokinetics of phenytoin, an anti-epileptic drug, was reported from a study on mice.[46] Pretreatment of piperine significantly delayed the elimination of phenytoin. Coadministration of piperine enhanced the bioavailability of β-lactam antibiotics, amoxycillin trihydrate and cefotaxime significantly in rats.[47] The improved bioavailability is reflected in various pharmacokinetic parameters, *viz.* t_{max}, C_{max}, half-life and AUC, of these antibiotics and was attributed to the effect of piperine on microsomal metabolizing enzymes.

When curcumin was given alone at 2 g/kg to rats, moderate serum concentrations were achieved over a period of 4 hrs.[41] Concomitant administration of piperine (20 mg/kg) increased the serum concentration of curcumin for a short period of 1–2 hrs post-drug. Time to maximum was significantly increased while plasma half-life and clearance significantly decreased, and the bioavailability was increased by 154%.

The effect of piperine on the metabolic activation and distribution of aflatoxin B_1 (AFB_1) in rats has been studied.[48] Rats pretreated with piperine accumulated considerable AFB_1 in plasma and in the tissues examined as compared to the controls. Piperine had no influence on hepatic AFB_1-DNA binding *in vivo*, which could possibly be due to the null effect of piperine on liver cytosolic glutathione transferase activity. Piperine-treated rat liver microsomes demonstrated a tendency to enhance AFB_1 binding to calf thymus DNA *in vivo*. Piperine markedly inhibited liver microsome-catalyzed AFB_1 binding to calf thymus DNA *in vitro*, in a dose-dependent manner.

Antioxidant Effect of Piperine (Table 4)

In vitro studies: Oxygen radical injury and lipid peroxidation have been suggested as major causes of atherosclerosis, cancer and the aging process. Piperine has been demonstrated in *in vitro* experiments to protect against oxidative damage by quenching free radicals and reactive oxygen species and inhibiting lipid peroxidation.[49] Piperine is reported to have marginal inhibitory effects on ascorbate/Fe^{2+}-induced lipid peroxidation in rat liver microsomes even at high concentrations (600 μM) when compared to the beneficial inhibition of lipid peroxidation by antioxidants vitamin E, t-butylhydroxytoluene and t-butylhydroxyanisole.[50] Both water and ethanol extract of black pepper exhibited strong total antioxidant activity, and significant inhibition of peroxidation of linoleic acid emulsion.[51] Piperine is shown to be an effective antioxidant and offer protection against oxidation of human low density lipoprotein (LDL) as evaluated by copper ion-induced lipid peroxidation of human LDL by measuring the formation of thiobarbituric acid reactive substance and relative electrophoretic mobility of LDL on agarose gel.[52] The aqueous

Table 4. Antioxidant, antimutagenic and cancer preventive effects of piperine.

System	Remarks	Reference
Antioxidant influence of black pepper and piperine		
In vitro	a) Inhibition/quenching of super oxides and hydroxyl radicals by piperine; inhibition of lipid peroxidation.	49
	b) Marginal inhibitory effect of piperine on ascorbate-Fe^{++}-induced lipid peroxidation in rat liver microsome.	50
	c) Water and ethanol extract of black pepper exhibits strong total anti-oxidant activity and inhibits peroxidation of linoleic acid emulsion.	51
	d) Piperine protects Cu^{++}-induced lipid peroxidation of human LDL.	52
	e) Black pepper aqueous extract and piperine inhibit human PMNL 5-lipoxygenase.	53
Rats	a) Piperine treatment protects against oxidative stress induced in intestinal lumen by carcinogens.	55
Streptozotocin-diabetic rats	a) i.p. administration of piperine for 2 wks partially protects against diabetes-induced oxidative stress.	54
High-fat fed rats	a) Dietary black pepper/piperine reduces high-fat diet-induced oxidative stress by lowering lipid peroxidation, restoring activities of anti-oxidant enzymes and GSH.	57
Mice	a) Piperine treatment decreases mitochondrial lipid peroxidation and augmented antioxidant defense system during benzo(α)pyrene-induced lung carcinogenesis.	56
Antimutagenic and tumor inhibitory effects		
In vitro and cell lines	a) Black pepper is effective in reducing mutational events induced by procarcinogen ethylcarbamate in *Drosophila*.	58
	b) Piperine markedly reduces the AFB_1-induced formation of micro-nuclei in H4IIE cells in a concentration-dependent manner.	60
	c) Piperine counteracts CYP450 2B1 mediated toxicity of AFB_1 in Chinese hamster cells and therefore has chemopreventive effects against procarcinogens activated by CYP450 2B1.	61

(*Continued*)

Table 4. (*Continued*)

System	Remarks	Reference
Rats	a) Piperine administration effectively reduces cyclophosphamide-induced chromosomal aberrations in bone marrow cells.	62
	b) Dietary black pepper was evidenced to suppress colon carcinogensis induced by the procarcinogen 1,2-dimethylhydrazine.	71
Mice	a) Tumor inhibitory activity of black pepper in mice implanted with Ehrlich ascites tumor.	64
	b) Piperine inhibits tumor development in mice induced with Dalton's lymphoma cells and increases the lifespan of afflicted mice.	59
	c) Antimetastatic activity of piperine on lung metastasis induced by melanoma cells.	65
	d) Chemopreventive effect of piperine on benzo(α)pyrene-induced experimental lung cancer.	66, 69, 70

extract of black pepper as well as piperine have been examined for their effect on human PMNL 5-lipoxygenase (5-LO), the key enzyme involved in biosynthesis of leukotrienes.[53] The formation of 5-LO product 5-HETE was significantly inhibited in a concentration-dependent manner with IC_{50} values of 0.13 mg for aqueous extracts of pepper and 60 μM for piperine. Thus, piperine from black pepper might exert an antioxidant physiological role by modulating the 5-LO pathway.

Animal studies: Piperine treatment (10 mg/kg/day i.p. for 14 days) has been assessed for protection against diabetes-induced oxidative stress in streptozotocin-induced diabetic rats.[54] Treatment with piperine reversed the diabetic effects on glutathione concentration in brain, on renal glutathione peroxidase and superoxide dismutase activities, and on cardiac glutathione reductase activity and lipid peroxidation, but did not reverse the effects of diabetes on hepatic antioxidant status. Thus, subacute treatment with piperine for 14 days is only partially effective as an antioxidant in diabetes. The ability of piperine to reduce the oxidative changes induced by chemical carcinogens (7,12-dimethylbenzanthracene,

dimethylaminomethylazobenzene and 3-methylcholanthrene) has been investigated in a rat intestinal model.[55] A protective role of piperine against the oxidative alterations by these carcinogens was indicated by the observed inhibition of TBARS, a significant increase in the glutathione levels and restoration in γ-GT and Na^+, K^+-ATPase activity in intestinal mucosa. The impact of piperine on alterations of the mitochondrial antioxidant system and lipid peroxidation in benzo(α)pyrene (B(α)p) induced experimental lung carcinogenesis has been investigated in mice.[56] Oral supplementation of piperine (50 mg/kg body weight) effectively suppressed lung carcinogenesis by B(α)p as revealed by a decrease in the extent of mitochondrial lipid peroxidation and concomitant increase in the activities of enzymatic antioxidants and nonenzymatic antioxidant levels when compared to lung carcinogenesis bearing animals. This suggests that piperine may extend its chemo-preventive effect by modulating lipid peroxidation and augmenting the antioxidant defense system.

The effect of supplementation of black pepper (0.25 g or 0.5 g/kg body weight) or piperine (0.02 g/kg body weight) for a period of 10 wks on tissue lipid peroxidation, enzymic and non-enzymic antioxidants has been examined in rats fed a high-fat diet (20% coconut oil and 2% cholesterol) and it was observed that these can reduce high-fat diet-induced oxidative stress.[57] Simultaneous supplementation with black pepper or piperine lowered TBARS and conjugated diene levels and maintained antioxidant enzymes and glutathione levels in the liver, heart, kidney, intestine and aorta near to those of control rats.

Antimutagenic and Tumor Inhibitory Effects (Table 4)

Cell line studies: Black pepper has been shown to be effective in reducing the mutational events induced by the promutagen ethyl carbamate in *Drosophila melanogaster*.[58] Suppression of metabolic activation or interaction with the active groups of mutagens could be mechanisms by which this spice exerts its antimutagenic action. While studying piperine for its immuno-modulatory and antitumor activity, piperine was found to be cytotoxic towards Dalton's lymphoma ascites (DLA) and Ehrlich ascites carcinoma (EAC) cells at 250 μg/ml.[59] Piperine was also found to produce cytotoxicity towards L929 cells in culture at a concentration of

50 µg/ml. Administration of piperine (1.14 mg/animal) could inhibit solid tumor development in mice induced with DLA cells and increase the lifespan of mice bearing Ehrlich ascites carcinoma tumors to 59%.

The effect of piperine on the cytotoxicity and genotoxicity of aflatoxin B_1 (AFB_1) has been studied in rat hepatoma cells H4IIEC3/G-(H4IIE) using cellular growth and formation of micronuclei as endpoints.[60] AFB_1 inhibited the growth of H4IIE cells with an ED_{50} of 15 nM. Piperine markedly reduced the toxicity of the mycotoxin. Piperine reduced the AFB_1-induced formation of micronuclei in a concentration-dependent manner. The results suggest that piperine is capable of counteracting AFB_1 toxicity by suppressing cytochrome P450 mediated bioactivation of the mycotoxin. The potential of piperine to inhibit the activity of cytochrome P450 2B1 and protect against AFB_1 has been investigated in r2B1 cells (Chinese hamster cells) engineered for the expression of rat CYP450 2B1.[61] Piperine inhibited 7-methoxycoumarin demethylase in preparations of r2B1 cells with an IC_{50} of 10 µM. Piperine at 60 µM completely counteracted cytotoxicity and formation of micronuclei by 10 µM AFB_1 and reduced the toxic effects of 20 µM AFB_1 by more than 50%. The results suggest that (i) piperine is a potent inhibitor of rat CYP450 2B1 activity, (ii) AFB_1 is activated by r2B1 cells to cytotoxic and genotoxic metabolites, and (iii) piperine counteracts CYP450 2B1 mediated toxicity of AFB_1 in the cells and might, therefore, offer a potent chemopreventive effect against procarcinogens activated by CYP450 2B1.

Animal studies: The antimutagenic effect of piperine has been studied particularly with respect to its influence on chromosomes in rat bone marrow cells.[62] Male Wistar rats orally administered piperine (100, 400 and 800 mg/kg body weight) were challenged with cyclophosphamide (i.p. 50 mg/kg body weight), sacrificed 24 hrs thereafter and bone marrow samples were collected. Piperine at a dose of 100 mg/kg body weight gave a statistically significant reduction in cyclophosphamide-induced chromosomal aberrations, suggesting that piperine may have antimutagenic potential. Black pepper extracts have been demonstrated to possess tumor inhibitory activity.[63] The tumor reducing activity of orally administered extracts of black pepper was studied in mice transplanted i.p. with Ehrlich

ascites tumor.[64] Lifespan was increased in these mice by 65%, indicating the potential use of the spice as anti-cancer agents as well as anti-tumor promoters. The antimetastatic activity of piperine has been demonstrated by the inhibition of lung metastasis induced by B16F-10 melanoma cells in C57BL/6 mice.[65] Simultaneous administration of the compound with tumor induction produced a significant reduction in tumor nodule formation. The elevated levels of serum sialic acid and serum γ-GT activity in the untreated animals were significantly reduced in the animals treated with piperine.

The cytoprotective effect of piperine on B(α)p-induced experimental lung cancer has been investigated in mice and it was observed that piperine may extend its chemopreventive effect by modulating lipid peroxidation and augmenting the antioxidant defense system.[66] Oral administration of piperine (100 mg/kg body weight) effectively suppressed lung cancer initiated with B(α)p as revealed by the decrease in the extent of lipid peroxidation with concomitant increase in the activities of enzymatic antioxidants and nonenzymatic antioxidant levels when compared to lung cancer bearing animals.

The protective role of piperine was examined during experimental lung carcinogenesis with reference to its effect on DNA damage and the detoxification enzyme system.[67] The activities of detoxifying enzymes such as glutathione transferase, quinone reductase and UDP-glucuronosyl transferase were found to be decreased while the hydrogen peroxide level was increased in the lung cancer bearing animals. Supplementation of piperine (50 mg/kg) enhanced these detoxification enzymes and reduced DNA damage. These results explain the understanding of association between the anti-peroxidative effect of piperine and ultimately the capability of piperine to prevent cancer. A significant suppression in the micronuclei formation induced by B(α)p and cyclophosphamide following oral administration of piperine at doses of 25, 50 and 75 mg/kg in mice has been reported.[68]

Piperine has been evidenced to show chemopreventive effects when administered orally on lung cancer bearing animals.[69] The beneficial effect of piperine is primarily exerted during the initiation phase and post-initiation stage of B(α)p-induced lung carcinogenesis via beneficial modulation of lipid peroxidation and membrane-bound ATPase enzymes. The ability of piperine to prevent lung carcinogenesis induced by B(α)p in mice

and its effects on cell proliferation has been studied.[70] Administration of piperine significantly decreased the levels of lipid peroxidation, protein carbonyls, nucleic acid content and polyamine synthesis that were found to be increased in lung cancer bearing animals. Piperine could effectively inhibit B(α)p-induced lung carcinogenesis in albino mice by offering protection from protein damage and also by suppressing cell proliferation. Dietary black pepper (0.5% in the diet for 15 wks) has been evidenced to suppress colon carcinogensis induced by the procarcinogen 1,2-dimethylhydrazine (15 s.c. injections of 20 mg/kg at weekly intervals) in rats.[71]

Other Physiological Effects (Table 5)

Animal studies

Deleterious effect of piperine on the reproductive system: Black pepper is used as a contraceptive in folk medicine. The reproductive toxicity of piperine has been studied in albino mice with respect to the effect on estrous cycle, mating behavior, toxicity to male germ cells, fertilization, implantation and growth of pups.[72] Piperine (10 and 20 mg/kg body weight) increased the period of the diestrous phase resulting in decreased mating performance and fertility. Post-partum litter growth was not affected by the piperine treatment and sperm shape abnormalities were not induced at doses up to 75 mg/kg. Considerable anti-implantation activity was recorded after 5 days post-mating oral treatment with piperine. These results show that piperine interferes with several crucial reproductive events in a mammalian model. The effect of piperine on the fertilization of eggs with sperm has been investigated in female hamsters intragastrically treated with piperine at doses of 50 or 100 mg/kg body weight from day 1 through day 4 of the estrous cycle.[73] During piperine treatment, these females were superovulated and artificially inseminated (AI) with spermatozoa from untreated male hamsters at 12 hrs after hCG injection. Administration of piperine to the superovulated animals markedly enhanced the percent fertilization at 9 hrs after AI.

Piperine administered to mature male albino rats at 10 mg/kg body weight p.o. for 30 days caused a significant reduction in the weights of

Table 5. Other biological effects of black pepper and piperine.

System	Remarks	Reference
Effect on reproductive system		
In vitro	a) Piperine decreases fertilizing ability of hamster sperm and degree of polyspermia *in vitro*.	75
Rats	b) Continued oral intake of piperine produces reduction in weights of testis, fall in sperm concentration, and decrease in intra-testicular testosterone.	74
Mice	c) Oral intake of piperine decreases fertility due to interference with crucial reproductive events in albino mice.	72
Anti-inflammatory activity		
Rats	a) Anti-inflammatory activity of piperine in experimental models: carrageenan-induced rat paw edema, cotton pellet granuloma, croton oil-induced granuloma pouch.	77
Hepatoprotective activity		
Mice	a) Piperine exerted protection against t-butyl hydroperoxide and carbon tetra-chloride in hepatotoxicity by reducing lipid peroxidation.	79
Melanocyte stimulation		
In vitro	a) Growth stimulatory activity of black pepper extract in cultured melanocytes.	83
Neuropharmacological activity		
Rats	a) Piperine administered animals possess antidepressant-like activity and experience a cognitive enhancing effect.	80
	b) Antidepressant-like effects of chronically administered piperine depend on the augmentation of the neurotransmitter synthesis.	81
Anticonvulsant effects		
Humans	a) Piperine treatment reduces the number of seizures in epileptic children.	9
Amelioration of dysphagia		
Humans	a) Inhalation of black pepper essential oil has remarkable effects on swallowing dysfunction in patients suffering from dysphagia.	87

testis and accessory sex organs.[74] Histological studies revealed that piperine caused severe damage to the seminiferous tubule, a decrease in seminiferous tubular and Leydig cell nuclear diameter, and desquamation of spermatocytes and spermatids. The effect of piperine on the fertilizing ability of hamster sperm has been investigated *in vitro*.[75] Addition of 0.18–1.05 mM piperine reduced both the percentage of eggs fertilized and the degree of polyspermia in a dose-dependent manner. The effect of piperine on the epididymal antioxidant system of adult male rats has been studied. Rats orally administered piperine at doses of 1, 10 and 100 mg/kg body weight each day for 30 consecutive days showed a decrease in the activity of antioxidant enzymes and sialic acid levels in the epididymis and thereby increased reactive oxygen species levels that could damage the epididymal environment and sperm function.[76]

Anti-inflammatory activity: The anti-inflammatory activity of piperine has been reported in rats employing different experimental models like carrageenan-induced rat paw edema, cotton pellet granuloma, and croton oil-induced granuloma pouch.[77] Piperine acted significantly on early acute changes in inflammatory processes and chronic granulative changes. The pungent principles of dietary spices including piperine have been reported to induce a warming action via adrenal catecholamine secretion.[78]

Hepatoprotective activity: Piperine has been evaluated for its antihepatotoxic potential in order to validate its use in traditional therapeutic formulations.[79] It exerted a significant protection against *t*-butyl hydroperoxide and carbon tetrachloride induced hepatotoxicity by reducing lipid peroxidation, leakage of enzymes alanine aminotransferase and alkaline phosphatase, and by preventing the depletion of glutathione and total thiols in the intoxicated mice.

Neuropharmacological activity: To understand the effect of piperine on the central nervous system, the neuropharmacological activity of piperine administered Wistar rats (5, 10 and 20 mg/kg body weight once daily) were determined after single, 1, 2, 3 and 4 wks of treatment.[80] Piperine at all dosages examined in this study possessed antidepressant-like

activity and cognitive enhancing effects at all treatment durations, suggesting that piperine could be a potential functional food to improve brain function. The antidepressant-like effects of piperine and its derivative antiepilepsirine were investigated in two depressive models: the forced swimming test and the tail suspension test.[81] To further explore the mechanisms underlying their antidepressant-like activities, the brain monoamine levels and monoamine oxidase A and B activities were also determined. The results indicated that after 2 wks of chronic administration, these compounds at doses of 10–20 mg/kg significantly reduced the duration of immobility in both models. The study demonstrated that the antidepressant-like effects of piperine and antiepilepsirine might depend on the augmentation of the neurotransmitter synthesis or the reduction of the neurotransmitter reuptake. The antidepressant properties of piperine were supposed to be mediated via the regulation of serotonergic system.

In vitro studies

Melanocyte stimulation: Melanocyte proliferation stimulants are of interest as potential treatments for the depigmentary skin disorder vitiligo. *P. nigrum* contains several amides with an ability to stimulate melanocyte proliferation. It has been suggested that the methylenedioxyphenyl function is essential for melanocyte stimulatory activity.[82] Black pepper water extract and piperine promote melanocyte proliferation *in vitro*. Black pepper extract was found to possess growth-stimulatory activity in cultured melanocytes.[83] Its aqueous extract at 0.1 mg/ml was observed to cause nearly 300% stimulation of the growth of a cultured mouse melanocyte line, in 8 days. Hence, it is inferred that piperine is a potential repigmenting agent for the treatment of vitiligo. This finding supports the traditional use of *P. nigrum* extracts in vitiligo and provides new lead compounds for drug development for this disease.

The *in vitro* effects of piperine on three bioenergetic reactions, namely, oxidative phosphorylation, ATPase activity and calcium transport by isolated rat liver mitochondria, have been investigated.[84] The study suggested that piperine inhibits mitochondrial oxidative phosphorylation at the level of the respiratory chain. Piperine did not inhibit the mitochondrial ATPase

activity induced by dinitrophenol and was found to diminish calcium uptake. The influence of piperine on the enzymes and bioenergetic functions in isolated rat liver mitochondria and hepatocytes has been studied, and it was observed that piperine produces concentration-related, site-specific effects on mitochondrial bioenergetics and enzymes of energy metabolism.[85]

Clinical trials: Piper longum and *Piper nigrum* are conventionally used as immuno-enhancers in the Indian system of traditional medicine. The underlying mechanism, however, remains unknown.

Pepper has been used in China as a folk remedy for epilepsy. Piperine has been identified by researchers as having anticonvulsant effects in animal models, and antiepilepsirine, a derivative of piperine, has been used in China to treat epilepsy since 1975. A recent clinical trial on epileptic children tested antiepilepsirine (10 mg/kg body weight; two or three times a day) in a randomized, placebo-controlled, cross-over, double-blind trial decreased the number of seizures in the majority of subjects.[9]

Black pepper's irritant action on the respiratory tract has been harnessed to ease smoking withdrawal. Inhalation of black pepper essential oil was shown to stimulate sensory signals that promoted greater smoking cessation by decreasing withdrawal symptoms more than breathing in air or mint/menthol.[86] A clinical study has also evidenced the remarkable effects of black pepper aromatherapy (inhalation of black pepper oil) on dysphagia, or the difficulty to swallow, in the elderly who are at risk of developing pneumonia, the beneficial effect being mediated by an increase in serum levels of substance P (a neuropeptide).[87]

MOLECULAR TARGETS

The principal bioactive constituent of both black pepper (*Piper nigrum*) and long pepper (*Piper longum*), the ingredients of *Trikatu*, which in turn is a constituent of many medications in the ancient systems of medicine, has now been established as piperine. The mode of action of this alkaloid in various medicinal effects is undoubtedly its bioavailability enhancing influence on various structurally and therapeutically diverse drugs. Piperine's potential to increase the bioavailability of drugs when pretreated

or coadministered is of great clinical significance. Clinical trials have established that piperine increases circulatory levels of drugs such as phenytoin (an epileptic treatment), propranolol (used for hypertension), rifampicin (a tuberculosis medication), theophylline (lung medication), and curcumin (a spice compound having cancer preventive and suppressive potential, besides several other medicinal effects). This observed drug bioavailability enhancing effect is due to the inhibitory interaction of piperine with cytochrome P450 enzymes of the liver and small intestine that are involved in drug metabolism: CYP1A2, CYP1A1, CYP2D6, CYP3A4 and P-glycoprotein.[38] Since piperine inhibits both P-glycoprotein and CYP3A4 expressed in intestinal enterocytes and hepatocytes, it contributes to a major extent to first-pass elimination of many drugs.[38]

Piperine displays antipyretic, analgesic and anti-inflammatory activities. In the process of identifying non-steroidal anti-inflammatory molecules from natural sources, it has been demonstrated that piperine inhibits adhesion of neutrophils to the endothelial monolayer.[88] The inhibition of adhesion of neutrophils to the endothelial monolayer by piperine is due to its ability to block the tumor necrosis factor-alpha (TNF-α) induced expression of cell adhesion molecules, i.e. ICAM-1 (intercellular adhesion molecule-1), VCAM-1 (vascular cell adhesion molecule-1) and E-selectin. As nuclear factor-kappaB (NF-κB) is known to control the transcriptional regulation of cell adhesion molecules, the effect of piperine on NF-κB in the cytoplasm and in the nucleus of endothelial cells was measured. It was observed that pretreatment of endothelial cells with piperine blocks the nuclear translocation and activation of NF-κB by blocking the phosphorylation and degradation of its inhibitory protein, I-kBα. Piperine blocks the phosphorylation and degradation of I-kBα by attenuating TNF-α induced IkB kinase activity. These results suggest a possible mechanism of the anti-inflammatory activity of piperine.

A current area of basic research is the activity of piperine as a TRPV1 vanilloid agonist, more powerful than the capsaicin found in chili peppers, to treat gastrointestinal disorders such as irritable bowel syndrome and diarrhea, as well as chronic breast pain and urinary incontinence.[89] Since piperine has been used to stimulate the gastrointestinal tract, it could be helpful for conditions such as diarrhea and irritable bowel syndrome, which are not easily managed by standard care.

ABSORPTION AND METABOLISM OF PIPERINE

Animal studies: When piperine was administered to male albino rats at a dose of 170 mg/kg by gavage or 85 mg/kg i.p., about 97% was absorbed irrespective of the mode of dosing.[90] Three percent of the administered dose was excreted as piperine in the feces, while it was not detectable in urine. When everted sacs of rat intestines were incubated with 100–1000 µg of piperine, about 44-63% of the added piperine disappeared from the mucosal side.[90,91] Absorption of piperine, which was maximum at 800 µg per 10 ml, was about 63%. The absolute amounts of piperine absorbed in this *in vitro* system exceeded the amounts of other structurally closer spice compounds such as curcumin.[90] The absorbed piperine could be traced in both the serosal fluid and in the intestinal tissue, indicating that piperine did not undergo any metabolic change during the process of absorption. When piperine was associated with mixed micelles, its *in vitro* intestinal absorption was relatively higher. Piperine absorption in the everted intestinal sac significantly increased when the same was present in micelles.[91]

Examination of the passage of piperine through the gut indicated that the highest concentration in stomach and small intestine was attained at about 6 hrs. Only traces of piperine were detected in serum, kidney and spleen from 30 mins to 24 hrs. About 1–2.5% of the intraperitoneally administered piperine was detected in the liver during 0.5–6 hrs after administration as compared with 0.1–0.25% of the orally administered dose. The increased excretion of conjugated uronic acids, conjugated sulphates and phenols indicated that scission of the methylenedioxy group of piperine, glucuronidation and sulphation appear to be the major steps in the disposition of piperine in the rat. After oral administration of piperine (170 mg/kg) to rats, the metabolites in urine (0–96 hrs) were identified to be piperonylic acid, piperonyl alcohol, and piperonal and vanillic acid in the free form, whereas only piperic acid was detected in bile (0–6 hrs).[92] The kidney appears to be the major excretion route for piperine metabolites in rats as no metabolite could be detected in feces. In a recent investigation,[93] to further study the reported differences in its metabolism in rats and humans, a new major urinary metabolite was detected in rat urine and plasma using HPLC and characterized as 5-(3,4-methylenedioxy phenyl)-2,4-pentadienoic acid-N-(3-yl propionic acid)-amide. This metabolite has a unique structure in that it

retains the methylenedioxy ring and conjugated double bonds while the piperidine ring is modified to form the propionic acid group.

The absorption dynamics of piperine in intestine on oral absorption has been studied.[94] Using intestinal everted sacs and cycloheximide treatment and exclusion of Na^+ salts from incubating medium as variables, absorption half-life, absorption rate, absorption clearance and apparent permeability coefficient were computed. The data suggested that piperine is absorbed very fast across the intestinal barrier, possibly acting as an apolar molecule and forming an apolar complex with drugs and solutes. It may modulate membrane dynamics due to its easy partitioning, thus helping in efficient permeability across the barrier.

Being essentially water insoluble, piperine is presumed to be assisted by serum albumin for its transport in blood after its intestinal absorption. The binding of piperine to serum albumin has been examined by employing steady-state and time-resolved fluorescence techniques.[95] The binding constant for the interaction of piperine with human serum albumin, which was invariant with temperature in the range of 17–47°C, was found to be $0.5 \times 10^5\ M^{-1}$, having stoichiometry of 1:1. Steady-state and time-resolved fluorescence measurements suggested the binding of piperine to the subdomain-IB of serum albumin. These observations are significant in understanding the transport of piperine in blood under physiological conditions.

CONCLUSIONS

Black pepper or its bioactive compound piperine, the ingredients used in a number of ancient and folk medicines, has now been demonstrated by a number of independent investigators to possess diverse beneficial physiological effects (Fig. 2). The most far-reaching attribute of piperine is its inhibitory influence on the hepatic, pulmonary and intestinal drug metabolizing systems. It strongly inhibits a particular cytochrome P450 and hence phase-I reactions mediated by the same, especially aromatic hydroxylation. It also strongly retards glucuronidation reactions of phase-II. As a result of interference with crucial drug metabolizing reactions in the liver, piperine enhances the bioavailability of therapeutic drugs, i.e. it increases their plasma half-life and delays their

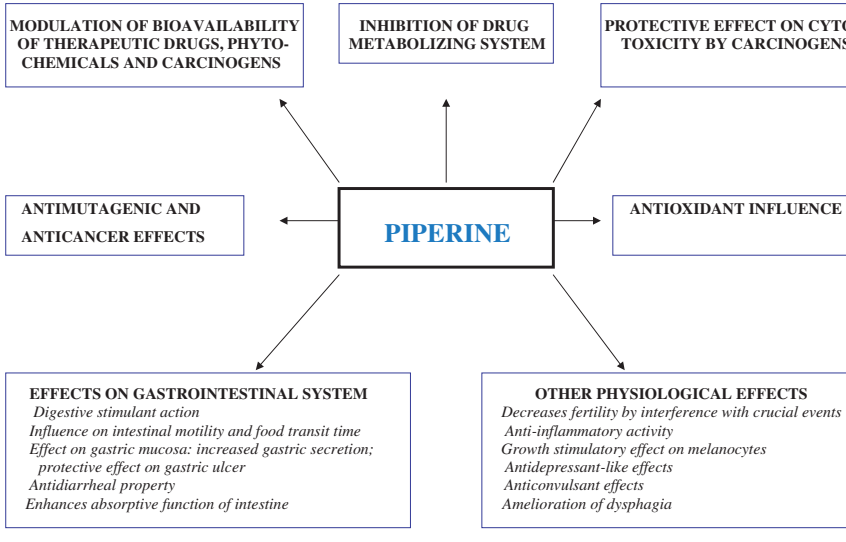


Fig. 2. The diverse physiological effects of piperine.

excretion. This particular inhibitory effect of piperine on drug metabolism and hence on drug bioavailability may be harnessed for increasing therapeutic effects. Most of the clinical studies on piperine have focused on its effect on drug metabolism. The gastrointestinal system is affected by black pepper and piperine in many ways. Both black pepper and piperine have been evidenced to have antidiarrheal properties and a definite effect on intestinal motility and on the ultrastructure of intestinal microvilli improving the absorbability of nutrients. Piperine has been evidenced to protect against oxidative damage by inhibiting or quenching free radicals as well as lower lipid peroxidation and beneficially influence cellular antioxidant status in different situations of oxidative stress. Piperine also possesses cytoprotective effects by retarding the activation of certain procarcinogens by the drug metabolizing system. The antimutagenic and anti-tumor properties of piperine have been evidenced in a few animal and cell-line studies. Among other physiological effects piperine exerts, its potential antifertility influence on the reproductive system has been clearly established in *in vitro* and animal systems.

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3

Cardamom (*Elettaria cardamomum*) and Its Active Constituent, 1,8-cineole

Archana Sengupta and Shamee Bhattacharjee

Green cardamom (*Elettaria cardamomum*) is an aromatic spice cultivated mainly in southern India, Sri Lanka, Southeast Asia, Guatemala, the Malabar coast and in Ceylon. It is a native crop of India. Also known as lesser or true cardamom, the spice is very important in world trade. Cardamom belongs to the Zingiberaceae family. It has well-established culinary value and is used in a wide range of sweets and confectionery. Cardamom is an important ingredient of "garam masala," a combination spice for many vegetarian and non-vegetarian dishes. Cardamom is a rich source of the compound 1,8-cineole, which is present in most oils used by aromatherapists to treat various ailments and relieve tension. The oil extracted from cardamom seeds is a unique gift of nature, containing combinations of terpene, esters, flavonoids and other compounds. In traditional medicine, cardamom seeds are used for the treatment of a variety of ailments including acute respiratory disorders, stomach complaints, bad breath, sore throat, colds, fever, bronchitis, gallbladder problems, flatulence, and colic. Cineole, the major active component of cardamom oil, is a potent antiseptic that kills the bacteria in bad breath and treats other infections and is also known to have expectorant activity for clearing breathing passages. However, not

enough scientific evidence has been presented to back up these claims. In this chapter we have aimed to consolidate some of the important scientific information that is available on the therapeutic potential of cardamom and its active constituent, 1,8-cineole, in a bid to explore the potential therapeutic and pharmacological properties of this wonderful aromatic spice.

INTRODUCTION

Cardamom, often referred to as the "Queen of Spices" because of its very pleasant aroma and taste, is the third most expensive spice in the world, after saffron and vanilla.[1] It is the true or lesser cardamom, *Elettaria cardamomum* Maton var. *miniscula* Burkill, that is the widely cultivated variety and important in world trade.[2] The plant is a large perennial, rhizomatous herb belonging to the Zingiberaceae family. It is a native plant of the moist evergreen forests of the Western Ghats of Southern India, Sri Lanka and parts of Southeast Asia, where it occurs in the wild. It has been introduced to other parts of Asia and is widely grown for its aromatic seeds. A mature cardamom plant may measure about 2–4 m in height (Fig. 1a). It is a shallow rooted plant. The leaves are dark green, long and sword-shaped (Fig. 1b). The underside is paler and pubescent. Flowers are born on panicles, which emerge directly from the swollen base of the aerial shoot, and are bisexual. The part of the plant that is of commercial interest is its dried ripe fruit (capsules of the cardamom plant). The capsules are globose, ovoid or narrowly ellipsoid to elongate in shape, trilocular, and contain 15–20 seeds (Fig. 1d). On maturity, seeds turn dark brown to black. Capsules are pale to dark green in color.

Cardamom belongs to the Genus *Elletaria* and species *cardamomum* (Maton). The generic name is derived from the Tamil root Elletari, meaning cardamom seeds. The genus consists of six species. The different cardamom species and varieties are used mainly as cooking spices and as medicines. In the Middle East and Turkey, green cardamom powder is used as a spice for sweet dishes as well as traditional flavoring in coffee and tea. It is also used to some extent in dish recipes. In Arabic, cardamom is called *al-Hayl*; in Persian, it is called *hel*. In South Asia, green cardamom is often used in traditional Indian sweets and in tea. Cardamom is

(a)

(b)

Fig. 1. **(a)** A view of cardamom plantation. **(b)** A closer view of cardamom plant (*source*: aidanbrooksspices.blogspot.com) **(c)** Cardamom plant with cardamom young berries (right bottom corner showing enlarged view) at the bottom of the plant (*source*: www.spgr-spices.com). **(d)** Green cardamom pods (*source*: www.uni-graz.at).

(c)

(d)

Fig. 1. (*Continued*)

sometimes used in garam masala for curries. It also occasionally used as garnish on basmati rice and other dishes. In Hindi, Urdu, and Gujarati, cardamom is called *elaichi*, and "yelakki" in Kannada and other South Indian languages. It is called Elakka in Malayalam, which is the language of Kerala, an Indian province that accounts for the production of most of the country's cardamom.

Only *E. cardamomum* occurs in India and this is the only economically important species.[3–5] From time immemorial, India has been known as the home of cardamom. It is grown extensively in parts of the monsoon forests of the Western Ghats in southern India at elevations of 800–1,300 m. This area has come to be known as the Cardamom Hills and includes part of the southern Western Ghats located in southeast Kerala and southwest Tamil Nadu in south India. The Western Ghats, Periyar Sub-Cluster including the Cardamom Hills, is under consideration by the UNESCO World Heritage Committee for selection as a World Heritage Site.[6]

Indian cardamom has a history as old as human civilization. Cardamom is used as a spice for sweet dishes as well as traditional flavoring in coffee and tea. It is sometimes used in garam masala for curries. Small cardamom (*Elettaria cardamomum* Maton) was mentioned in approximately 3000 BC in Sanskrit texts in India.[7] It is known as *Ela* in Sanskrit. Taitreya Samhita, which belongs to the later Vedic period (3000 BC), contains mention of cardamom among the ingredients poured in the sacrificial fire on the occasion of the marriage ceremony.[8] The ancient Indial Ayurvedic texts, Charak Samhita and Susrutha Samhita, written in the post epic period (1400–600 BC) also mention cardamom on many occasions.

Cardamom has been commercially cultivated in the Western Ghats for 150 years, and India has had a virtual trade monopoly until recently. At present, the largest producers of true cardamom are Guatemala and India, and smaller producers include Tanzania, Sri Lanka, Papua New Guinea, El Salvador, Laos, and Vietnam. Cheaper substitutes for real cardamom (*Amomum* spp. and *Aframomum* spp.) are grown and used in some Asian countries.[8]

India and Saudi Arabia consume more than half of the world's total cardamom. In Arab countries and India, cardamom is a common flavoring ingredient in coffee and tea. In Scandinavia, as well as in Germany and Russia, it is used to flavor cakes, pastries and sausages. It is popular in Indian and south Asian cooking and used to make spice blends, such as curries and *garam masala*. Chewing cardamom after a meal is recommended to aid digestion and to clean the teeth. In Eastern medicinal practices it is used for curing such ailments as influenza, infections, asthma, bronchitis, cardiac disorders, diarrhea, nausea and cataracts, and for strengthening the nervous system. It is also said to have a cooling effect

in hot climates. The ancient Greeks and Romans used its delicate aroma to make perfume.[7] Cardamom seeds are widely used for flavoring purposes in food and as a carminative.

Research on cardamom was initiated after India's independence. A full-fledged research institute, the Indian Cardamom Research Institute (ICRI), was established in 1978 by the Spice Board, functioning under the Ministry of Commerce. In 1974 the Cardamom Research and Central Plantation Research Institute had started functioning at Appangala in Coorg district, Karnataka. This center was later (1986) amalgamated with the National Research Centre for Spices (currently the Indian Institute of Spice Research). The main objective of this research center has been to increase cardamom yield by cultivating viral disease-resistant varieties or by developing high production technology for the area. To date there have been very few scientific studies on cardamom seeds or its constituents that provide definitive evidence for its medicinal, pharmacological and therapeutic properties. However, research in this area is gradually gaining impetus all over the world in view of cardamom's traditional history and in order to explore the real benefits of including this spice in our diet.

CHEMICAL COMPOSITION OF CARDAMOM

The chemical composition of cardamom has attracted the interest of several research groups over the last two decades. Several volatile as well as non-volatile compounds of cardamom have already been identified.

The chemical composition of cardamom varies considerably with variety, region and age of the product. The content of volatile oil in the seeds is strongly dependent on storage conditions, but may be as high as 8%. The volatile oil contains about 1.5% α-pinene, 0.2% β-pinene, 2.8% sabinene, 1.6% myrcene, 0.2% α-phellandrene, 11.6% limonene, 36.3% 1,8-cineole, 0.7% γ-terpinene, 0.5% terpinolene, 3% linalool, 2.5% linalyl acetate, 0.9% terpinen 4-01, 2.6% α-terpineol, 31.3% α-terpinyl acetate, 0.3% citronellol, 0.5% nerd, 0.5% geraniol, 0.2% methyl eugenol and 2.7% trans-nerolidol.[9] The basic cardamom aroma is produced by a combination of the major components, 1,8-cineole and α-terpinyl acetate.[10]

Another group of researchers have detected 25 compounds comprising 97% of the total composition of cardamom oil. The main components

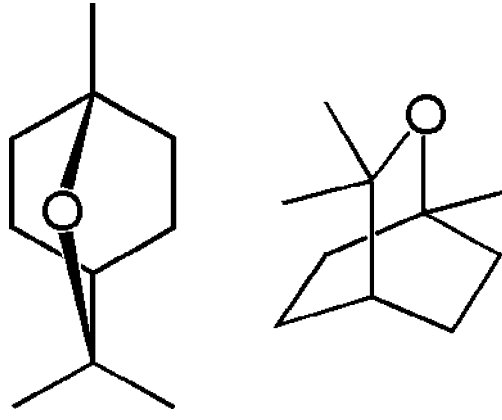

Fig. 2. Chemical structure of 1,8-cineole.

were identified as 1,8-cineole (30.7%) and α-terpinyl acetate (30.6%).[11] In several other studies 1,8-cineole and α-terpinyl acetate were reported as the main constituents.[12–14] Based on these data it can be concluded that the major component of cardamom oil are 1,8-cineole and α-terpinyl acetate.

1,8-cineole (cineole), an oxygenated monoterpene (Fig. 2), is also present in many other plant essential oils such as eucalyptus[15,16] and bay leaves[17] and is thought to be responsible for contributing to their aroma. It is used for medicinal, flavor and fragrance purposes and has significant biological activity. Owing to its pharmacological activity and its being the major compound in cardamom oil, it can be hypothesized that 1,8-cineole might be the major contributing factor in the supposed therapeutic potential of cardamom. These therapeutic properties of cardamom oil include antiseptic, antispasmodic, anti-carcinogenic, carminative, digestive, diuretic, expectorant and stimulant properties, as well as its stomachic property, which might be attributed to anti-inflammatory, anti-oxidant and anti-mutagenic activities. The scientific evidence in favor of these effects of cardamom and its major active compound 1,8-cineole are outlined here.

IN VITRO STUDIES

Chemical analysis of several foodstuffs comprising of cereals, pulses, nuts, oilseeds, vegetables, fruits and beverages was carried out to determine the

antioxidant phenolics and flavonoids in commonly consumed Indian foods. Cardamom was found to contain medium levels (50–100 mg) of flavonoids.[18]

In one study it was found that eugenol, a compound present in many spices such as cloves and cardamom, significantly inhibited tobacco-induced mutagenicity at concentrations of 0.5 and 1 mg/plate. Eugenol also inhibited the nitrosation of methylurea in a dose-dependent manner.[19]

Essential oils from common spices such as nutmeg, ginger, cardamom, celery, xanthoxylum, black pepper, cumin and coriander were found to inhibit DNA adduct formation by aflatoxin B1 *in vitro* very significantly and in a dose-dependent manner.[20]

Specific induction of apoptosis by 1,8-cineole was observed in human leukemia Molt 4B and HL-60 cells. The fragmentations of DNA by cineole to oligonucleosomal sized fragments that is a characteristic of apoptosis were concentration- and time-dependent in Molt 4B and HL-60 cells.[21]

The inhibitory activity of cardamom extract has been studied on human platelets. Platelet aggregation and lipid peroxidation were evaluated with platelet rich plasma (PRP) and platelet membranes, respectively, obtained from the blood of healthy volunteers. Human platelets were subjected to stimulation with a variety of agonists and a dose-dependent inhibitory effect on platelet aggregation was observed with cardamom. Moreover, an increase in concentration of cardamom also decreased malondialdehyde formation significantly.[22]

One investigation has reported 1,8-cineole to be a strong inhibitor of cytokines that might be suitable for long-term treatment of airway inflammation in bronchial asthma and other steroid-sensitive disorders.[23] Another study was designed to investigate the effect of 1,8-cineole (Soledum) on arachidonic acid (AA) metabolism in blood monocytes of patients with bronchial asthma. It was found that 1,8-cineole inhibited LTB4 and PGE2, both pathways of AA-metabolism.[24]

Extract of the cardamom seed, which was prepared using diethyl ether, had a strong inhibitory activity on some pathogens such as *M. smegmatis*, *K. pneumoniae*, *S. aureus*, *E. coli*, *E. faecalis*, *M. luteus* and *C. albicans*.[25]

In another study it was observed that the addition of cardamom, cinnamon and clove powders or their volatile oils to cookies had a

distinctive effect of extending shelf life by suppressing the growth of microorganisms.[26]

IN VIVO STUDIES

Cardamom (*Elettaria cardamomum*) is traditionally used in various gastrointestinal, cardiovascular and neuronal disorders. One study was carried out to rationalize cardamom use in constipation, colic, diarrhea, hypertension and as a diuretic. Cardamom crude extract caused an atropine-sensitive stimulatory effect in isolated guinea-pig ileum at 3–10 mg/ml. At 3–100 mg/kg, it also induced a drop in the arterial blood pressure of anesthetized rats. Cardamom crude extract (1–10 mg/kg) produced diuresis in rats, accompanied by a saluretic effect. It enhanced pentobarbital-induced sleeping time in mice. These results indicate that cardamom exhibits gut excitatory and inhibitory effects.[27] As the inhibition of contractile overactivity of the ileum is the basis for the treatment of some gastrointestinal disorders such as colic, cardamom oil may have clinical benefits for the treatment of these conditions.

GASTROPROTECTIVE EFFECTS

The gastroprotective effects of cardamom and its main aroma constituent *viz.* 1,8-cineole was established in the following studies. A crude methanolic extract (TM), essential oil (EO), petroleum ether soluble (PS) and insoluble (PI) fractions of methanolic extract were studied in rats at doses of 100–500, 12.5–50, 12.5–150 and 450 mg/kg, respectively, for their ability to inhibit the gastric lesions induced by aspirin and ethanol. All fractions (TM, EO, PS, PI) significantly inhibited gastric lesions.[28]

In a separate study the gastroprotective effect of 1,8-cineole (cineole) on ethanol-induced gastric mucosal damage in rats was investigated. 1,8-cineole (50–200 mg/kg), given orally 1 hr before administration of 1 ml of pure ethanol significantly attenuated the ethanol-induced gastric injury.[29]

Results from yet another investigation confirm the potential value of 1,8-cineole as a dietary flavoring agent in the prevention of gastrointestinal ulceration. A marked reduction in gross damage scores and wet

weights (mg/cm) of colonic segments were evident in animals pretreated but not post-treated with 1,8-cineole following trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats.[30]

Hepatoprotective Effect

Another study was conducted to investigate the effects of 1,8-cineole on the D-galactosamine/lipopolysaccharide (GalN/LPS)-induced shock model of liver injury in mice. Pretreatment with 1,8-cineole (400 mg/kg, p.o.) 60 mins before GalN/LPS, offered complete protection (100%) against the lethal shock. Hepatic necrosis induced by GalN/LPS was also greatly reduced by 1,8-cineole treatment.[31]

Cardiovascular Effects

The cardiovascular effects of 1,8-cineole were investigated in normotensive rats by Lahlou *et al.*,[32] providing the first physiological evidence that i.v. treatment with 1,8-cineole in either anesthetized or conscious rats elicits hypotension, which seems related to an active vascular relaxation.[32]

Anti-Inflammatory Effect

One study observed a marked anti-inflammatory activity of the oil extracted from commercial *Elettaria cardamomum* seeds in doses of 175 and 280 ml/kg against acute carrageenan-induced plantar edema in male albino rats.[33] This anti-inflammatory activity of cardamom is supported by other studies conducted using 1,8-cineole. This active monoterpene component in cardamom oil displays an inhibitory effect on some types of experimental inflammation in rats, e.g. paw edema induced by carrageenan and cotton pellet-induced granuloma. This result when taken together with the other reports that describe the inhibitory effects of cineole on the formation of prostaglandins and cytokines by stimulated monocytes *in vitro* may provide additional evidence for its potential beneficial use in therapy as an anti-inflammatory agent.[34]

Analgesic and Anti-Spasmodic Effect

Investigation of analgesic activity has proved that a dose of 233 µl/kg of cardamom oil produced 50% protection against the writhing (stretching syndrome) induced by intraperitoneal administration of a 0.02% solution of *p*-benzoquinone in mice. In addition, antispasmodic activity has been determined on a rabbit intestine preparation using acetylcholine as agonist.[33]

Anti-Oxidative Effect

Peroxynitrite, a potent cytotoxic agent, can damage a variety of biomolecules such as proteins, lipids and DNA, and is considered to be one of the major pathological causes of several diseases. Methanolic extracts of eight culinary spices including cardamom were selected in order to search for potential protectors against the actions of peroxynitrite. All of the tested spices exerted some level of protective ability against peroxynitrite-mediated biomolecular damage as determined by these extracts' ability to attenuate the formation of, respectively, nitrotyrosine in albumin, thiobarbiturate acid-reactive substances (TBARS) in liposome and strand breakages for plasmid DNA.[35]

In another study conducted by Santos *et al*., post-treatment with cineole was found to significantly reduce myeloperoxidase activity and caused repletion of glutathione in the colonic segments following induction of colitis by trinitrobenzene sulfonic acid (TNBS) in rats.[30]

The essential oils from cardamom, when fed by gavage in Swiss albino mice, was found to significantly elevate the level of the carcinogen metabolizing enzyme glutathione S-transferase (GST) as well as that of the acid-soluble sulfhydryl.[36]

This finding is in good agreement with another finding by Bhattacharjee *et al*.[37] in which it was reported that aqueous suspension of cardamom was responsible for inducing hepatic and colonic GST activities in azoxymethane (AOM) treated Swiss albino mice.

Anti-Carcinogenic Effect

Bhattacharjee *et al*. have investigated the anti-carcinogenic effect of cardamom and it was observed that aqueous suspension of cardamom

significantly inhibited aberrant crypt foci (ACF) formation in AOM induced colon carcinogenesis in Swiss albino mice.[38] ACF are recognized as early preneoplastic lesions of the colon.[39] In the study conducted by Bhattacharjee *et al*. cell proliferation in the colon was found to decrease and apoptosis was found to increase after cardamom treatment. It is well known that the balance between cell proliferation and apoptosis is important in the genesis of colon carcinoma[40]; this study indicates a good correlation between the ability of the spice to prevent AOM induced ACF through reduction in cell proliferation and enhanced apoptosis.

Sengupta *et al*. has also explored the chemopreventive efficacy of cardamom against DMBA induced and croton oil promoted skin carcinogenesis in Swiss albino mice and has observed a marked decrease in papilloma count after cardamom treatment (unpublished data).

Effect of Cardamom as an Ingredient of Garam Masala

As we know that cardamom is an essential ingredient in the Indian spice mixture *garam masala*, many studies are being carried out to explore the beneficial health effects, if any, of garam masala. In one investigation, when pregnant mice were given 10 mg and 30 mg of garam masala per day from Days 13–19 of gestation in addition to DMBA (5 mg/day) on Days 15–17 of gestation, the multiple-site tumor incidence declined significantly in the F1 progeny.[41] In another study Singh and Rao assessed the chemopreventive role of garam masala through modulatory impact on the hepatic levels of detoxification enzymes like glutathione S-transferase (GST), cytochrome b5 (cyt. b5) and cytochrome P450 (cyt. P450), and acid soluble sulfhydryl ($^{-}$SH) content in Swiss albino mice fed garam masala in their diet.[42]

MOLECULAR TARGETS

The potential of cardamom and its active constituent 1,8-cineole to exhibit such a wide variety of pharmacological properties must be through modulation of specific molecular targets. However, to date only a few studies have been conducted to identify these targets.

Many investigations have proven that cardamom shows anti-inflammatory activity *in vitro* and *in vivo*. Although not fully understood, several action mechanisms are proposed to explain *in vivo* anti-inflammatory action. One recent study has shown that cardamom expresses its anti-inflammatory activity at least in part by modulation of pro-inflammatory protein expression such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS).[39] An inhibition of these enzymes by cardamom reduces the production of arachidonic acid (AA), prostaglandins (PG), leukotrienes (LT), and NO, crucial mediators of inflammation. Thus, the inhibition of these enzymes exerted by cardamom is definitely one of the important cellular mechanisms of anti-inflammation. Tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) are pro-inflammatory cytokines produced by macrophages/monocytes during acute inflammation. These are important mediators of intestinal inflammation in inflammatory bowel disease.[43,44] Results from experiments characterize 1,8-cineole as a strong inhibitor of TNF-α and IL-1β.[31,45] As TNF-α is responsible for a diverse range of signaling events within cells, leading to necrosis or apoptosis,[46] inhibition of this molecule may account for a wide range of pharmacological effects exhibited by cadamom and/or 1,8-cineole, including its role in controlling airway mucus hypersecretion, conferring protection against liver injury, and so on.

As already mentioned cardamom has traditionally been used for curing ailments such as asthma, bronchitis and diarrhea. It has also been used as an analgesic and as an antispasmodic. It can be tentatively said from experimental evidence that by blocking a single target molecule cardamom performs all these activities. Cardamom oil was found to exhibit its inhibitory effect against the contractile response elicited by the neurotransmitter acetylcholine on rabbit intestine preparation. Acetylcholine (ACh) is the most common neurotransmitter at the parasympathetic nerve ending to induce smooth muscle contractions. In the gastrointestinal tract, ACh is released from the primary excitatory motor neurons and mediates an immediate smooth muscle contraction.[47,48] Cholinergic signaling is mediated by the muscarinic ACh receptor expressed on the surface of the smooth muscle cells. As the antispasmodic activity of cardamom oil was investigated using acetylcholine as an agonist, this suggests that cardamom exerts its effect by muscarinic receptor blockade.[33]

By blocking muscarinic receptors on airway smooth muscle on submucosal gland cells, anticholinergics have also proved to be of particular value in the treatment of chronic obstructive pulmonary disorder (COPD).[49] Parasympathetic nerves provide the dominant autonomic innervation of the airways. Release of acetylcholine from parasympathetic nerves activates postjunctional muscarinic receptors present on airway smooth muscle, submucosal glands and blood vessels, causing bronchoconstriction, mucus secretion and vasodilatation, respectively. The increasing evidence of the role of 1,8-cineole in controlling airway mucus hypersecretion might be by virtue of muscarinic receptor blockage.

CLINICAL STUDY

So far little has been reported on the pharmacological and toxicological properties of cardamom seeds or their volatile oil. In a recent study toxicity of this spice was investigated on Swiss albino mice. Daily, mice were treated orally with 0.003 mg and 0.3 mg over 7 days. The results from this experiment showed that *E. cardamomum* induces toxicity at 0.3 mg/g mouse.[50] However, to date no human studies support dosing recommendations. As a result, there has been a dearth of clinical studies using cardamom seeds or its oil. However, a few clinical and per-clinical studies have been conducted using its active monoterpene ingredient, 1,8-cineole, as reported in the following.

(i) A study was conducted to evaluate the anti-inflammatory efficacy of 1,8-cineole by determining its prednisolone equivalent potency in patients with severe asthma. Thirty-two patients with steroid-dependent bronchial asthma were enrolled in a double-blind, placebo-controlled trial. After determining the effective oral steroid dosage during a 2-month run-in phase, subjects were randomly allocated to receive either 200 mg 1,8-cineole t. i.d. or placebo in small gut soluble capsules for 12 weeks. Reductions in daily prednisolone dosage of 36% with active treatment (range 2.5–10 mg, mean: 3.75 mg) versus a decrease of only 7% (2.5–5 mg, mean: 0.91 mg) in the placebo group ($P = 0.006$) were tolerated. Twelve of 16 cineole versus 4 out of 16 placebo patients achieved a reduction of oral steroids ($P = 0.012$).

It was concluded that long-term systemic therapy with 1,8-cineole has a significant steroid-saving effect in steroid-dependent asthma.[51]

(ii) To provide further evidence for the role of 1,8-cineole in controlling airway mucus hypersecretion, a preclinical study was designed to test the potential anti-inflammatory efficacy of 1,8-cineole (eucalyptol) in inhibiting polyclonal stimulated cytokine production by human unselected lymphocytes and LPS-stimulated monocytes. Cytokine production was determined following 20 hrs of incubation cells with 1,8-cineole simultaneously with the stimuli in culture supernatants by enzyme immunoassay. Therapeutic concentrations of 1,8-cineole (1.5 μg/ml = 10^{-5} M) significantly inhibited cytokine production in both lymphocytes and monocytes.[52]

(iii) A prospective, randomized, double-blinded, placebo-controlled study was designed to compare the efficacy and safety of cineole capsules with placebo capsules in 152 patients with acute rhinosinusitis (76 patients in each treatment group). The dosage of the active ingredient was two 100-mg capsules of cineole three times daily. Initially, the mean symptoms-sum-score was 15.6 in both treatment groups. The mean values for the symptoms-sum-scores in the cineole group were 6.9 +/– 2.9 after 4 days and 3.0 +/– 2.8 after 7 days, and in the placebo group, 12.2 +/– 2.5 after 4 days and 9.2 +/– 3.0 after 7 days. Thus, the study showed that in patients with acute nonpurulent rhinosinusitis timely treatment with cineole is effective and safe before antibiotics.[53]

(iv) Very recently, in a similar prospective, randomized, double-blinded, controlled study, the efficacy and safety of two different treatment options with the herbal medicines cineole and a combination of five different components were compared for acute viral rhinosinusitis. One hundred and fifty patients with acute and viral rhinosinusitis (75 patients in each treatment group) were enrolled. The primary endpoint was the amelioration of the symptoms-sum-score, which includes all the relevant characteristics for rhinosinusitis such as headache on bending, frontal headache, sensitivity of pressure points of the trigeminal nerve, impairment of general condition, nasal obstruction, rhino-secretion, secretion quantity, secretion viscosity and fever, in a treatment period of 7 days. A clinically relevant and statistically significant mean reduction of the symptoms-sum-score

was observed after 4 and 7 days in both treatment groups. However, treatment with cineole was found to be more effective in comparison to the alternative herbal preparation with five different components.[54]

CONCLUSION

Spices as defined by the US Food and Drug Administration are "aromatic vegetable substances, in the whole, broken, or ground form, whose significant function in food is seasoning rather than nutrition." The flavors are provided by the essential oils and oleoresins present in spices. Besides being used as flavor enhancers, spices have also been used since antiquity to preserve food due to the presence of antioxidant phytochemicals. By virtue of these powerful phytochemicals, herbs and spices are known to exhibit an array of biochemical and pharmacological activities including antioxidant and anti-inflammatory properties. For several thousands of years, cultures from around the world have used naturally occurring dietary components, which have been discovered to be biologically active. These plant-derived chemicals have generated considerable interest recently for their potential to combat various human diseases. This renewed interest in natural medicines today is mainly due to the fact that many chronic diseases including cancer, inflammation and atherosclerosis still remain difficult to cure. As such, attempts are being made to identify naturally occurring chemicals which may lead to new strategies for the prevention and treatment of such diseases. Cardamom is a widely used flavoring agent for sweets and coffee and a standard ingredient in curry. Its medicinal use dates back to ancient times. Herbalists recommended it to improve digestion and relieve flatulence. It is also popular in Ayurvedic medicine. The herb has been used for bronchitis, colds and cough, and is recommended as an appetite stimulant in anorexic patients.

Recent scientific findings have been encouraging, uniformly showing that cardamom and its most active ingredient, cineole, possess a multitude of beneficial effects in the treatment of gastrointestinal disorders, cancers, cardiovascular disease, and inflammation. Despite these advances, no clinical trials have tested these effects, and its therapeutic benefit remains largely unproven. Further study is warranted to evaluate the metabolism, pharmacokinetics and toxicity of cardamom and its active component, and

human data are needed before it can be recommended to treat any medical conditions.

However, with the available experimental data, many research groups around the world, involving both private and public funding, are focused on the use of this plant-derived chemical in humans. Given the current body of evidence, cardamom, as it has for thousands of years in ancient Asian culture, may provide us with a safe, effective therapy for many disease processes which plague society.

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4

Molecular Targets and Health Benefits of Cinnamon

Kiran Panickar, Heping Cao, Bolin Qin and Richard A. Anderson*

Common cinnamon (*Cinnamomum verum*, *C. zeylanicum*) and cassia (*C. aromaticum*) have a long history of uses as spices, flavoring agents, preservatives, and pharmacological agents. Recent studies also demonstrate that compounds found in cinnamon improve the function of insulin, function as antioxidants, anti-inflammatory agents, and may be neuroprotective. Human studies involving control subjects, subjects with the metabolic syndrome, type 2 DM, and polycystic ovary syndrome show beneficial effects of whole cinnamon and aqueous extracts of cinnamon on glucose, insulin, insulin sensitivity, lipids, antioxidant status, lean body mass and gastric emptying. *In vitro* and animal studies demonstrate that aqueous extracts of cinnamon, high in type A polyphenols, increase insulin receptor efficiency by increasing tyrosine phosphorylation and decreasing phosphatase activity, leading to increased insulin sensitivity; increase the amounts of insulin receptor, glucose transporter and anti-inflammatory tristetraprolin proteins; increase glycogen synthase activity and glycogen accumulation; regulate inflammatory responses and exert neuroprotective effects against amyloid beta or oxygen-glucose deprivation induced neural damage. Thus cinnamon, and its components, may be important in the alleviation

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of chronic diseases associated with insulin resistance such as type 2 diabetes, inflammatory and cardiovascular diseases and neuroprotective effects on stroke and Alzheimer's disease.

INTRODUCTION: OTHER NAMES AND TRADITIONAL USES

Cinnamon (*Cinnamomum verum*) is a small evergreen tree 10–15 m tall with greenish flowers (Fig. 1) and has been known since antiquity. It was mentioned in Chinese texts as long as 4,000 years ago and imported to Egypt as early as 2000 BC. Common cinnamon (*Cinnamomum verum*, *C. zeylanicum*) and cassia (*C. aromaticum*) have a long history of uses as spices, flavoring agents, preservatives, and pharmacological agents. A review of the safety and efficacy of cinnamon on antioxidant activity, *Heliobacter pylori* infection, activation of olfactory cortex and brain, oral candidiasis in HIV, and chronic salmonellosis has been published.[1] In addition, there are several recent studies examining the effects of cinnamon on glucose, insulin and lipid metabolism, and these will be the focus of this review.

Fig. 1. Cinnamon flower and leaves.

In 1990, we reported that compounds found in cinnamon have insulin-potentiating properties and may be involved in the alleviation of the signs and symptoms of diabetes and cardiovascular diseases related to insulin resistance.[2] Furthermore, comparing 49 herb, spice and medicinal extracts for insulin-like or insulin-potentiating activity in an *in vitro* model,[3] aqueous extracts of cinnamon potentiated insulin activity more than 20-fold, higher than any other compound tested at comparable dilutions. The effects of adding more of the aqueous extract of cinnamon are often similar to adding more insulin. This is extremely important from a human health standpoint since it results in increased insulin sensitivity and less insulin is required to have larger insulin effects. This chapter will discuss primarily these insulin related effects and is an expansion and update of a previous review.[4]

CHEMICAL CONSTITUENTS

The main constituent of cinnamon bark is cinnamon oil, which contains mainly cinnamic acid, cinnamaldehyde, and cinnamic alcohol.[5] The components of cinnamon are listed in Table 1. Cinnamaldehyde is the most prevalent compound in cinnamon with concentrations ranging from 6,000 to 30,000 ppm and has anesthetic, antibacterial, anti-inflammatory, antiulcer, and antiviral actions. It is also a cyclooxygenase-2 (COX-2) inhibitor and has hypotensive and tranquilizer effects.[5] In addition, cinnamaldehyde reduces blood glucose and lipids in rats made chemically diabetic, increases circulating insulin, decreases HbA1c (glycated hemoglobin), and restores the activities of plasma enzymes including aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and alkaline and acid phosphatases.[6]

However, cinnamaldehye functions as a prooxidant, and when given orally to rats at 73.5 mg/kg body weight for 90 days, leads to increased levels of thiobarbituric acid-reactive substances.[7] In contrast, as discussed later, the aqueous extracts of cinnamon function as antioxidants in experimental animals and humans.

Cinnamic acid and its derivatives have been reported to possess a variety of pharmacological properties including antioxidant and antihyperglycemic effects.[5] 3,4-Di(OH)-cinnamate and 3,4-di(OH)-hydroxycinnamate lowered cholesterol and triglycerides in rats as well as

Table 1. Chemical constituents of cinnamon.

Chemicals in: *Cinnamomum verum* J. PRESL (Lauraceae) — Ceylon Cinnamon, Cinnamon
Chemicals
1,8-cineole, 165–800
2-phenylethyl benzoate
2-vinylphenol, 3–12
3-phenylpropyl acetate, 13–52
acetoeugenol, 16–64
alpha-pinene, 20–236
alpha-terpineol, 40–264
alpha-ylangene, 31–124
ascorbic acid, 309
barium, 60
benzyl alcohol
benzyl benzoate, 66–400
beta-carotene, 1–2
beta-pinene bark, 14–76
borneol bark, 2–8
bornyl acetate bark, 10–20
boron bark, 7–15
bromine bark, 10
calcium bark, 5,329–6,000
camphene bark, 18–72
caryophyllene bark, 135–1,316
chlorine bark, 300
chromium bark, 2–10
cinnamaldehyde bark, 6,000–30,000
cinnamyl acetate bark, 510–2,040
cinnamyl alcohol bark, 26–104
cinnzeylanine bark
cinnzeylanol bark
cis-ocimene bark, 3–12
cobalt bark, 0.6
copper bark, 4.9–9
coumarin bark
cumene bark, 66–264
cuminaldehyde bark, 4–100

(*Continued*)

Table 1. (*Continued*)

Chemicals in: *Cinnamomum verum* J. PRESL (Lauraceae) — Ceylon Cinnamon, Cinnamon
delta-3-carene bark, 3–12
dihydrofumigatin bark
eugenol bark, 220–3,520
farnesol bark, 3–12
fat bark, 14,000
fiber bark, 270,000
furfural bark, 3–12
furfurol bark
gamma-terpinene bark, 3–12
gamma-terpineol bark
gamma-ylangene bark
geranial bark
geraniol bark, 6–24
geranyl acetate bark
gum bark
humulene bark, 20–124
hydrocinnamaldehyde bark, 40–160
iodine bark, 3
iron bark, 60–421
isocaryophyllene bark
isoeugenol bark, 2–8
lead bark
limonene bark, 46–184
linalol bark, 230–956
linalyl acetate bark
manganese bark, 66–140
methyl eugenol bark
methyl vinyl ketone bark
mucilage bark, 20,000–37,000
myrcene bark, 5–20
niacin bark, 8
nickel bark, 1.1
nonylaldehyde bark
p-cymene bark, 55–448
pelargonaldehyde bark
phellandrene bark, 63–252

(*Continued*)

Table 1. (*Continued*)

Chemicals in: *Cinnamomum verum* J. PRESL (Lauraceae) — Ceylon Cinnamon, Cinnamon
phenylethyl alcohol bark, 41–164
phenylethyl acetate bark, 7–28
phosphorus bark, 674–1,100
piperitone plant, 7–28
potassium bark, 5,525–6,000
protein bark, 35,000–43,000
riboflavin bark, 1
rubidium bark, 20
sabinene bark, 2–8
sodium bark, 287
strontium bark, 80
sulfur bark, 1,900
tannin
terpinen-4-ol bark, 36–144
terpinolene bark, 11–44
thiamin bark, 1
titanium bark, 40
trans-cinnamic acid
trans-linalol oxide bark, 5–20
zinc bark, 11.4–20

Values are in parts per million.
Source: Dr. Duke's *Phytochemical and Ethnobotanical Databases*. Online Database 30 July 2008 (http://sun.ars-grin.gov:8080/npgspub/xsql/duke/plantdisp.xsql?taxon=269).

hepatic HMG-CoA reductase.[8] In addition, these compounds displayed antioxidant activity with lowered thiobarbituric acid-reactive substances and elevated glutathione peroxidase activity.[8] 2-alkoxydihydrocinnamates function as peroxisome proliferator-activated receptor (PPAR) agonists and decrease blood glucose and triglycerides in a genetic diabetic animal model.[9] These compounds function similar to glitazone drugs used in the treatment of diabetes, which are also potent inhibitors of PPAR-gamma. The naphthalenemethyl ester derivative of dihydroxycinnamic acid lowered blood glucose to near normal in chemically and genetically diabetic mice,

and increased glucose transport by increasing translocation of GLUT4.[10] P-methoxycinnamic acid reduced the blood glucose of chemically diabetic rats and normalized hepatic glucose-6-phosphatase, hepatic hexokinase, glucokinase, phosphofructokinase, hepatic glycogen, and glucose-6-phosphate.[11] Anderson *et al.*[12] demonstrated that the *in vitro* insulin-potentiating activity found in cinnamon was present in the aqueous fraction. Aqueous extracts of "spent cinnamon," in which many of the organic components found in cinnamon, including cinnamaldehyde, are largely removed when cinnamon oil is extracted from whole cinnamon, has basically the same *in vitro* insulin-potentiating activity as extracts from the cinnamon before the cinnamon oil is removed. In addition, the cinnamon oil has no *in vitro* insulin-enhancing activity in epidydimal fat cells.[12] The structure of a class of water soluble cinnamon polyphenol compounds that display insulin-potentiating, antioxidant and related activities is shown in Fig. 2.

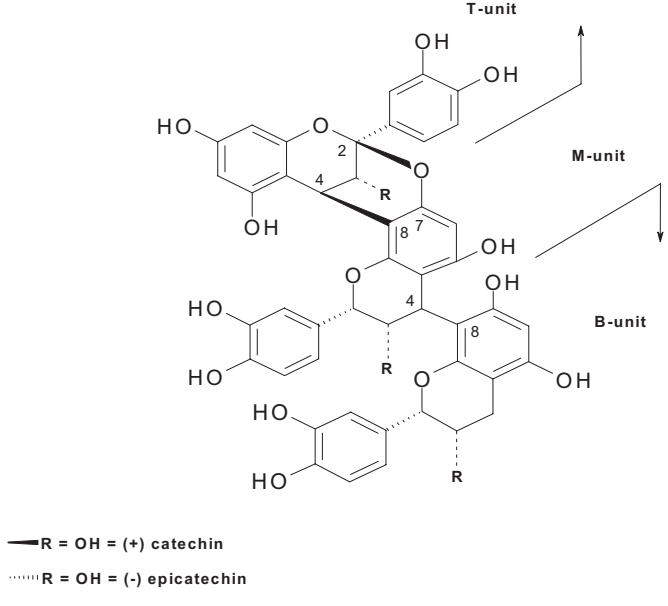


Fig. 2. The structure of a class of water soluble cinnamon polyphenol compounds that display insulin-potentiating and antioxidant activities. One tetramer and four type A trimers have been isolated from cinnamon and all were shown to have *in vitro* insulin-potentiating activity.[12]

MOLECULAR TARGETS

Some of the potential molecular targets of cinnamon are shown in Table 2. Quantitative real-time polymerase chain reaction (PCR) has been used to investigate the effects of aqueous cinnamon extracts on the expression of genes coding for the glucose transporter (GLUT) family, anti-inflammatory tristetraprolin (TTP) family, insulin signal transduction pathway components, adipokines, pro-inflammatory cytokines, and other selected targets in mouse 3T3-L1 adipocytes[13,14] and Raw 264.7 macrophages.[15] Immunoblotting has been used to confirm some PCR results by demonstrating the increased production of some of these proteins.[13–15] The effects of an aqueous cinnamon extract (CPE), high in type A polyphenols, on some of these molecular markers are shown in the next section.

IN VITRO STUDIES

CPE Increases GLUT1 mRNA Levels in Macrophages

Glucose, a major metabolic substrate, is critical for host immunity, and glucose uptake in mammalian cells is facilitated by glucose transporter (GLUT) family proteins.[16] In Raw cells, CPE increases the GLUT1 mRNA levels (the major glucose transporter in Raw cells)[15] more than three-fold.[15] These results suggest an important function in regulating glucose uptake in immunologically important cells.

CPE Increases TTP mRNA and Protein Levels in Adipocytes

Anti-inflammatory TTP binds to some mRNAs and destabilizes transcripts coding for proteins such as TNF-α. The mRNA binding activity of TTP is zinc-dependent and is regulated by post-translational phosphorylation. TTP mRNA and/or TTP protein levels are increased in mammalian cells by a wide range of agents, including insulin. Like insulin, CPE significantly increases TTP mRNA levels in 3T3-L1 adipocytes.[13] TTP mRNA levels in 10 μg/ml CPE-treated cells are approximately two-fold those in the controls, and those in 100 μg/ml CPE-treated cells are approximately six-fold those in the control cells after 0.5–1 hr. CPE induction of TTP mRNA is sustained in adipocytes,

Table 2. Potential molecular targets of cinnamon.

Target Group	mRNA Name	Protein Name
TTP family	TTP (ZFP36)	Tristetraprolin (zinc finger protein 36)
	ZFP36L1	Zinc finger protein 36 like 1
	ZFP36L2	Zinc finger protein 36 like 2
	ZFP36L3	Zinc finger protein 36 like 3
Glucose transporter family	GLUT1 (SLC2A1)	Glucose transporter 1
	GLUT2 (SLC2A2)	Glucose transporter 2
	GLUT3 (SLC2A3)	Glucose transporter 3
	GLUT4 (SLC2A4)	Glucose transporter 4
Insulin signaling pathway	AKT1 (PKB)	Thymoma viral proto-oncogene 1 (protein kinase b)
	GSK3B	Glycogen synthase kinase 3 beta
	GYS1	Glycogen synthase
	IGF1	Insulin-like growth factor 1
	IGF2	Insulin-like growth factor 2
	IGF1R	Insulin-like growth factor receptor 1
	IGF2R	Insulin-like growth factor receptor 2
	INS1	Insulin 1
	INS2	Insulin 2
	INSR	Insulin receptor
	IRS1	Insulin receptor substrate 1
	IRS2	Insulin receptor substrate 2
	PIK3CB	Phosphatidylinositol 3-kinase, catalytic, beta
	PIK3R1	Phosphatidylinositol 3-kinase, regulatory subunit 1
	SHC1	Src homology 2 domain-containing transforming protein 1
	SOS1	Son of sevenless 1
Inflammation and cytokine	COX2 (PTGS2)	Cyclooxgenase-2 (prostaglandin-endoperoxide synthase 2)
	CRP	C-reactive protein
	G-CSF (CSF3)	Granulocyte colony-stimulating factor (colony-stimulating factor 3)
	GM-CSF (CSF2)	Granulocyte-macrophage colony-stimulating factor (colony-stimulating factor 2)
	HuR (ELAVL1)	Hu antigen R (embryonic lethal, abnormal vision-like 1)
	IFNγ	Interferon-gamma

(*Continued*)

Table 2. (*Continued*)

Target Group	mRNA Name	Protein Name
	IL1A	Interleukin 1A
	IL6	Interleukin 6
	IL12B	Interleukin 12B
	PAI1 (SERPINE1)	Plasminogen activator inhibitor I (serine/cysteine peptidase inhibitor 1)
	TNF	Tumor necrosis factor
	VEGFA	Vascular endothelial growth factor A
	VEGFB	Vascular endothelial growth factor B
Adipokines	ADIPOQ	Adiponectin
	LEP	Leptin
	LEPR	Leptin receptor
Other mRNAs	APP	Amyloid beta precursor protein
	RPL32	Ribosomal protein L32
	TAU	Microtubule-associated protein tau

which contrasts with the transient increase by insulin.[14] Treatment of adipocytes with purified type A polyphenols from cinnamon fraction of CPE also increases the amount of TTP protein in the adipocytes.[13] Since TTP gene expression is reduced several-fold in adipose tissue of obese subjects with metabolic syndrome, the induction of TTP by CPE could improve the effects of obesity and related diseases similar to its effects in autoimmune disorders.

CPE Decreases VEGF mRNA Levels in Adipocytes

VEGF mRNA codes for a pro-angiogenic cytokine that is a target for degradation by TTP family proteins both *in vivo* and *in vitro*.[17] Quantitative real-time PCR assays indicate that insulin and CPE reduce VEGF mRNA by approximately 30–50% in adipocytes. VEGF is a mitogenic and angiogenic factor involved in tumor progression, collateral vessel formation in ischemic tissues, inflammation, as well as in the development of diabetic retinopathy.[18] VEGF is also a key mediator of adipogenesis in obesity.[19] These results suggest a potential role of cinnamon in prevention of VEGF-related conditions.

CPE Increases TTP mRNA and Protein Levels in Macrophages

CPE increases TTP mRNA levels in mouse Raw 264.7 cells.[15] TTP mRNA levels in cells treated with 100 μg/ml CPE are approximately 50–100% greater than those of controls. However, insulin does not exhibit any major effects on TTP mRNA levels in Raw cells. Immunoblotting shows that TTP protein is increased in Raw cells treated with CPE but is undetectable in cells treated with insulin for the same length of time. These results indicate that while CPE has insulin-potentiating effects, its effects on some of the inflammatory markers differ from those of insulin.

Cinnamon Inhibits the Formation of Advanced Glycation Endproducts (AGEs)

Several phenolic compounds found in cinnamon, such as catechin, epicatechin, procyanidin B_2, and phenol polymers, all show significant inhibitory effects on the formation of advanced glycation endproducts. Their antiglycation activities are not only brought about by their antioxidant activities but are also related to their trapping abilities of reactive carbonyl species. Studies demonstrate that proanthocyanidins can effectively scavenge reactive carbonyl species and thus inhibit the formation of advanced glycation endproducts. They therefore have great potential to be developed as agents to alleviate diabetic complications.[20]

Cinnamon and Its Components Improve Neural Function

Several hypotheses have been proposed to explain pathogenesis in Alzheimer's disease (AD) including amyloid cascade, excitotoxicity, oxidative stress, mitochondrial dysfunction, metabolic dysfunction and inflammation.[21] One proposed mechanism of Aβ toxicity is through its generation of free radicals, which can initiate lipid peroxidation, damage membrane proteins, and compromise ion homeostasis, all of which can contribute to neurodegeneration. More recently, a disturbance in insulin signaling in the brain has been hypothesized to contribute to the pathology of AD. Reduced CNS expression of genes encoding insulin, insulin

growth factor-I, and insulin growth factor-II, as well as insulin and IGF-I receptors, suggests that AD may represent a neuroendocrine disorder that is similar to, yet distinct from, type 2 diabetes mellitus. This prompted de la Monte's group to propose the term "type 3 diabetes" to reflect the pathogenic mechanism of neurodegeneration linked to AD.[22] Collectively, these results suggest that insulin deficiency in the brain may contribute to the pathology in AD, and therapies that boost insulin, or the responsiveness to it, may be beneficial for AD. Our preliminary studies show that cell death induced by Aβ 25–35 in PC12 neuronal cells is significantly attenuated by CPE as well as insulin. While the combination of CPE and insulin also prevented Aβ-induced cell death, the effects were not synergestic. In addition, Aβ-induced depolarization of the mitochondrial membrane potential was also significantly attenuated by CPE, indicating that the mitochondria mediate some of the protective effects of CPE.

Stroke is a neurological injury in which the blood supply to a part of the brain is interrupted. It involves the sudden loss of neuronal function due to a decline in cerebral perfusion. The part of the brain with decreased blood flow no longer receives adequate oxygen. Ischemic stroke can lead to vascular leakage, inflammation, tissue injury, and necrosis. Associated changes include impairment of metabolism, energy failure, free radical production, excitotoxicity, altered calcium homeostasis, and activation of proteases (see Panickar[23] for review).

Neuronal death and astrocyte swelling are key features of ischemic stroke in the brain. We used oxygen-glucose deprivation (OGD), in neural cultures, to simulate conditions seen in stroke. OGD-induced neuronal death in PC12 cells was significantly attenuated by CPE.[24] Another important consequence of ischemic injury is brain edema.[23] Brain edema represents an abnormal accumulation of fluid in the brain parenchyma resulting in a volumetric enlargement of the brain. A consequence of this volume increase is the development of increased intracranial pressure leading to brain herniation, brainstem compression and, ultimately, death. Edema further impairs cerebral perfusion and oxygenation, and contributes to additional ischemic injuries including neuronal death. Astrocyte swelling is a major component of cytotoxic brain edema in ischemia. CPE significantly attenuated OGD-induced cell swelling in C6 glial cultures.[24] Mitochondrial dysfunction has been hypothesized to

induce cell swelling. OGD-induced depolarization of the mitochondrial membrane potential in glial cultures was significantly attenuated by CPE. Thus, in both Aβ-induced cell death and OGD-induced glial swelling, CPE attenuated mitochondrial dysfunction.

Hepatic encephalopathy (HE) is a major neurological complication in patients with severe liver failure. One of the major consequences of acute HE (fulminant hepatic failure) is brain edema which can lead to increased intracranial pressure, brain herniation, and a mortality approaching 80–90%. Astrocyte swelling is generally believed to be a key component of cytotoxic brain edema in HE as well. While the pathogenetic factors responsible for brain edema in HE are still not clearly known, elevated levels of brain ammonia due to failure of the liver to detoxify it have been strongly implicated as an important element in this condition. C6 glial cultures were treated with ammonium chloride in the presence or absence of CPE and ammonia-induced astrocyte swelling was significantly attenuated by CPE (Fig. 3).

Our results indicate an important neuroprotective role for CPE in Aβ-toxicity, OGD as well as ammonia-neurotoxicity. Based on our results

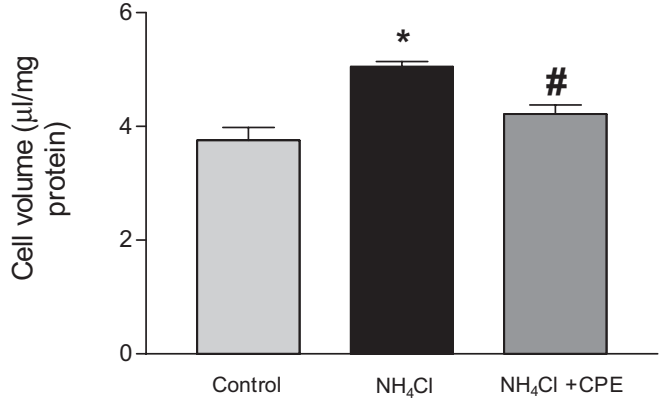


Fig. 3. Cinnamon extract (CPE) prevents ammonia-induced glial swelling in cultures. C6 glial cells were exposed to 10 mM NH_4Cl in the presence or absence or CPE (0.05 mg/ml) for 24 hrs. Cell volume was measured using the 3-O-methyl-[^{3}H]-glucose method. Ammonia significantly increased cell swelling by 33% but such swelling was significantly blocked by CPE.

*$p < 0.05$ versus control; # $p < 0.05$ versus NH_4Cl.

from Aβ exposure and OGD, it appears that some of the neuroprotective effects of CPE might be due to its effects on the mitochondria.

ANIMAL STUDIES

Glucose and Insulin Effects of Cinnamon and Its Components

CPE enhances insulin action via increasing glucose uptake *in vivo*, at least in part, through enhancing the insulin-signaling pathway in skeletal muscle. Cinnamon extract treated rats showed significantly higher glucose infusion rates at 3 mU/kg per min insulin infusions compared with controls (146% of controls).[25] At 30 mU/kg per min insulin infusions, the glucose infusion rate in CPE treated rats was increased 17% over controls. Insulin-stimulated IR-β and the IRS-1 tyrosine phosphorylation levels in skeletal muscle of CPE treated rats were 33% higher, respectively, added to 41% higher IRS-1/PI 3-kinase association. CPE also improved the glucose utilization of normal male Wistar rats fed a high-fructose diet (HFD) for three weeks to induce insulin resistance.[26] At 3 mU/kg/min insulin infusions, the decreased GIR in HFD-fed rats (60% of normal controls) was improved by CPE administration to the same level of controls and the improving effects of CPE on the GIR of HFD-fed rats were blocked by approximately 50% by N-monometyl-L-arginine [an inhibitor of nitric oxide (NO)]. The same tendency was found during the 30 mU/kg/min insulin infusions. The muscular insulin-stimulated IR-β and IRS-1 tyrosine phosphorylation levels and IRS-1 associated with PI 3-kinase in HFD-fed rats were only 70%, 76% and 72% of controls, respectively, and these decreases were significantly improved by CPE treatment. These results suggest that early CPE administration to HFD-fed rats would prevent the development of insulin resistance at least in part by enhancing insulin signaling and possibly via the NO pathway in skeletal muscle. NO has vasodilating effects, and increased blood flow is known to improve insulin sensitivity. Moreover, treatment with CPE significantly increased the decreased activity of hexokinase and glycogen content in the liver and skeletal muscle of fructose-fed rats.[27]

Additionally, CPE improves the impaired insulin action in STZ-induced diabetic rats, and early CPE administration prevents the insulin

resistance in a type 2 diabetes model, Otsuka Long Evans Tokushima Fatty rats (Qin *et al*., unpublished data). Kim *et al*.[10] studied the effects of a cinnamon extract on blood glucose after 2, 4 and 6 wks in db/db mice, another animal model of type 2 diabetes. Fasting blood glucose and postprandial glucose both decreased significantly compared to the control group in a dose-dependent manner. The decline in blood glucose levels reached its maximum after 2 wks and remained almost constant after 4 and 6 wks of cinnamon extract administration. Kannappan *et al*.[27] investigated whether cinnamon bark extract mitigates the adverse effects of fructose loading on glucose metabolism and lipid profile in HFD-fed rats. A cinnamon extract reduced the fasting blood glucose and HbA1c to near normal values. Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was elevated in fructose-fed rats and in cinnamon extract HFD-fed rats, the level was close to normal, which is in agreement with our previous report.[26] Additionally, Talpur *et al*.[28] reported that combined essential oils, all containing cinnamon, lowered circulating glucose levels in both Zucker fatty rats and spontaneously hypertensive rats, suggesting that these natural products are enhancing insulin sensitivity.

Some studies suggest that CPE is able to increase insulin secretion.[10] In STZ-induced diabetic rats, oral administration of cinnamaldehyde significantly increased plasma insulin levels, when compared to untreated diabetic rats.[6] The use of a lower dose of STZ produced an incomplete destruction of pancreatic β-cells even though the rats became permanently diabetic.[29] After treatment with a low dose of STZ, there would be many surviving β-cells, and regeneration is also possible.[30] In the db/db mice model,[10] serum insulin levels were found to be significantly higher in the group with a higher dosage of cinnamon than the control group. The authors suggest that the possible mechanism by which cinnamon extract brings about its hypoglycemic action in diabetic mice may be by potentiating the effect of insulin in serum or by increasing either the pancreatic secretion of insulin from the existing β-cells or its release from the bound form.

Cinnamon Extract Improves Dyslipidemia

The metabolic syndrome is commonly associated with an atherogenic dyslipidemia which accounts for a high risk of atherothrombosis and

cardiovascular events. The fundamental defect of patients with metabolic syndrome is the resistance to insulin action which is involved in the appearance of a combined dyslipidemia (hypertriglyceridemia, low HDL cholesterol, preponderance of small dense LDL particles and postprandial lipidemia).

Cinnamon has potential lipid lowering properties in animal and human studies.[3,27,31,32] In CPE-treated db/db mice, the serum concentration of triglycerides decreased by 45%, the level of HDL-cholesterol increased 1.5-fold, and HDL-total cholesterol ratio was also higher in the CPE-treated than in the control.[10]

In the HFD-fed rat model, CPE inhibited the elevations in cholesterol, triglycerides, free fatty acids and phospholipids associated with feeding with a high fructose diet.[27] Recently, we investigated the effects of a commercial water extract of cinnamon, Cinnulin PF®, on the postprandial lipid metabolism in HFD-fed rats, in an olive oil loading study. Acute Cinnulin PF® (50 mg/kg BW, orally) inhibited serum triglyceride levels and the overproduction of triglycerides and triglyceride rich lipoproteins (TRL)-apoB48. We also found that Cinnulin PF® inhibited the inflammatory factor, TNF-α. TNF-α infusion induced the overproduction of apoB48-containing lipoproteins, which is associated with increased cardiovascular disease risks and these are particularly atherogenic.[33]

Cinnamon Extract Inhibits Systolic Blood Pressure

Many agents (nutrients, nutraceuticals, and drugs) that enhance insulin sensitivity and/or reduce circulating insulin concentrations lower blood pressure. Preuss *et al.*[34] examined the effects of dietary cinnamon on systolic blood pressure (SBP) in spontaneously hypertensive rats (SHR). Diets high in sucrose are associated with insulin resistance and the elevation of SBP. Addition of cinnamon to the diets reduced the SBP of rats eating sucrose-containing diets to virtually the same levels as SHR consuming starch diets.[34] The presence of cinnamon in the diet also decreased the SBP of SHR consuming a non-sucrose-containing diet, suggesting that cinnamon reduces more than just sucrose-induced SBP elevations — perhaps a genetic component or components of the elevated SBP as well.

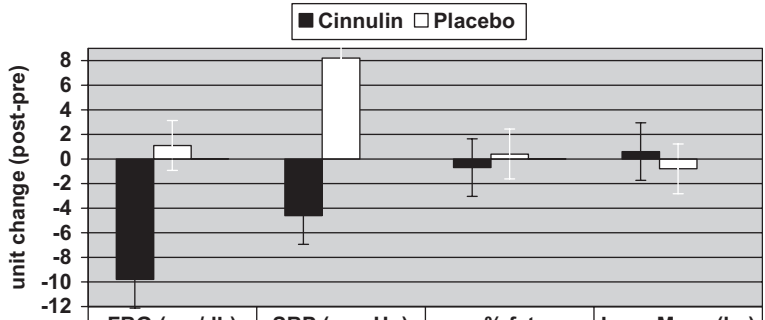
Cinnamon and Its Components as Antioxidants

Cinnamon extracts exhibit antioxidant effects.[12,31] Lin *et al*.[35] reported that an extract from *Cinnamomum cassia* exhibited a greater inhibition on FeCl(2)-ascorbic acid induced lipid peroxidation of rat liver homogenate *in vitro*, and also showed excellent antioxidant activities in enzymatic and nonenzymatic liver tissue oxidative systems. Devi *et al*.[36] investigated the perturbation of oxidant-antioxidant balance in brain synaptosomes of diabetic rats and determined the antioxidant and free radical-scavenging property of *Cinnamomum tamala*, which displayed scavenging activity against superoxide.

CLINICAL TRIALS

Following the observations that cinnamon potentiates insulin action *in vitro*, Khan *et al*. conducted a human study involving 60 people with type 2 DM, 30 males and 30 females who were taking sulfonylurea drugs.[32] Subjects were divided randomly into six groups. Groups 1, 2 and 3 received one, three or six grams of cinnamon as *Cinnamomon cassia* per day for 40 days. From 40 to 60 days, there was a washout period in which subjects did not receive capsules. Groups 4, 5 and 6 received the same number of placebo capsules as the corresponding cinnamon groups. There were three placebo groups since the number of capsules in each of the groups was different. After 40 days, all three levels of cinnamon reduced mean fasting serum glucose (18–29%) (three groups of 10 people), triglycerides (23–30%), total cholesterol (12–26%) and LDL cholesterol (7–27%). There were no significant changes in any of the three placebo groups. Values after the 20-day washout period were returning to baseline but were still significantly lower than the values at the beginning of the study. This study basically confirms the effects of cinnamon since the results of groups 2 and 3 repeated those in group 1, and all groups had their own respective placebo groups.

In a separate study involving 22 subjects with metabolic syndrome, subjects were divided into two groups and given either 500 mg/day of a commercially available aqueous extract of cinnamon (Cinnulin PF®, Integrity Nutraceuticals, Spring Hill, TN) or a placebo for 12 wks.



	FBG (mg/dL)	SBP (mm Hg)	% fat	Lean Mass (kg)
■ Cinnulin	-9.8	-4.6	-0.7	0.6
□ Placebo	1.1	8.2	0.4	-0.8
□ P-value	0.01	0.001	0.02	0.002

Fig. 4. Beneficial effects of aqueous extract of cinnamon on glucose, blood pressure, percentage body fat, and lean mass of subjects with metabolic syndrome.

Source: Ziegenfuss.[37]

An estimated 41 million people in the U.S. are postulated to have metabolic syndrome characterized by elevated blood sugar, hyperlipidemia, hypertension, and obesity. Subjects in the group receiving the capsules containing the aqueous extract of cinnamon displayed decreases in fasting blood glucose, systolic blood pressure, and increases in lean mass, compared with the placebo group. There were also significant decreases in body fat in the cinnamon group (Fig. 4).[37]

Oxidative stress plays an important role in the development of diabetes and cardiovascular diseases.[38] Hyperglycemia causes the auto-oxidation of glucose, glycation of proteins, and the activation of polyol metabolism.[39] These changes accelerate the generation of reactive oxygen species (ROS) and increase oxidative modifications of lipids and proteins.[40] Roussel *et al*.[41] found a significant positive correlation between plasma glucose levels and plasma malondyaldehyde (MDA), a measure of lipid peroxidation in people with metabolic syndrome. The improvement of impaired fasting glucose due to cinnamon was correlated with the antioxidant effects of cinnamon supplementation assessed by plasma MDA, sulfhydryl groups and plasma antioxidant status evaluated using ferric reducing antioxidant power (FRAP).[41] A significant positive correlation

between plasma glucose levels and plasma MDA confirms a previous study showing that plasma glucose levels play a role in determining oxidative status.[42] In subjects with metabolic syndrome, plasma MDA levels were reduced by the aqueous extract of cinnamon, indicating decreased lipid peroxidation, while plasma sulfhydryl groups were increased, indicating a protection of antioxidant sulfhydryl groups against oxidation.[41] In the group receiving cinnamon, plasma SH groups were increased after 12 wks of supplementation, suggesting that cinnamon acts in protecting both lipids and proteins against oxidation. In parallel, the FRAP, which is a measure of the total antioxidant capacity of plasma, was increased, thereby providing a contributory factor to the protective effects of cinnamon supplementation.[41]

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies among women of child-bearing age, affecting 5–10% of the population.[43] Insulin resistance and compensatory hyperinsulinemia are present in 50–70% of women with PCOS and maybe as high as 95% in overweight women. Excess insulin secretion may also be implicated in the increased metabolic and cardiovascular risks reported in this disorder. Since insulin-sensitizing agents such as chromium and troglitazone have been shown to be beneficial in the treatment of PCOS, it was postulated that the insulin-potentiating water-soluble polyphenol compounds found in cinnamon may also be beneficial for women with PCOS.[43] During an 8-wk treatment period, oral cinnamon extract, 1g/day, resulted in a significant reduction in fasting glucose as well as in insulin resistance. Oral glucose tolerance tests (OGTT) also showed a 21% reduction in mean glucose and an increase in Matsuda's insulin sensitivity index. The cinnamon extract improved insulin resistance in the women with PCOS compared to that in the control group (Fig. 5).

Polyphenols from cinnamon also have beneficial effects on subjects with good glucose tolerance (7 healthy males, 26 ± 1 yrs, with BMI of 24.5 ± 0.3 kg/m^2).[44] Cinnamon ingestion led to reduced total plasma glucose responses to oral glucose ingestion as well as improving insulin sensitivity. Effects were significant when the cinnamon was taken with the glucose and if taken 12 hrs before glucose ingestion.[44] Hlebowicz *et al*.[45] also reported beneficial effects of cinnamon on postprandial blood glucose of 14 healthy normal subjects (25.6 ± 4.8 yrs with BMI of

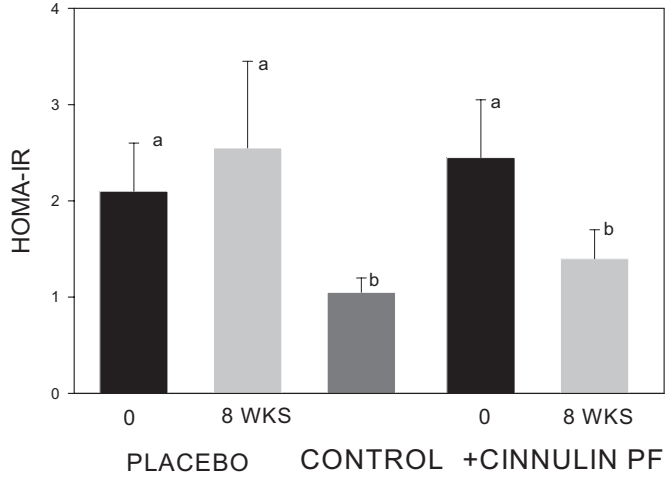


Fig. 5. Aqueous extract of cinnamon (Cinnulin PF) increases insulin sensitivity of women with polycystic ovary syndrome. Fourteen women with polycystic ovary syndrome were divided into two groups and given either placebo or capsules containing 333 mg of aqueous cinnamon extract (Cinnulin PF) three times per day for eight weeks. Homeostasis model insulin resistance HOMA-IR was calculated using the following formula: fasting glucose (mmol/L) × fasting insulin (μU/ml)/22.5. Control is for control women who do not have PCOS. Bars with different superscripts are significantly different at $p < 0.05$.

Source: Wang.[43]

22.6 ± 2.2 kg/m^2) in a controlled cross-over study. There were also effects on gastric emptying that could partially explain the results.

Not all of the clinical trials regarding whole cinnamon or extracts of cinnamon have reported significant effects and a summary of the human studies involving cinnamon are shown in Table 3. Vanschoonbeek *et al.*[46] reported no significant effects of 1.5 g of *Cinnamomum cassia* per day for 6 wks on postmenopausal subjects with type 2 diabetes. All but four of the subjects were taking glucose-lowering agents and most were taking metformin derivatives. Blevins *et al.*[47] also did not observe an effect of *C. cassia* in a study involving 58 subjects with type 2 DM. Seventy-seven percent of the subjects in the cinnamon group and 91% in the placebo group were taking glucose-lowering agents. More than half of the subjects

Table 3. Summary of human studies involving cinnamon and its components.

Authors	Subjects	Amount/Form	Type/Duration	Significant Effects
Khan *et al*.[32]	30M, 30 F; Type 2 DM	1, 3, 6 g/*C. cassia*	PC/40 days; 20 days washout	Glu↓; TG↓; TC↓; LDL↓
Mang *et al*.[50]	79M,F; Type 2 DM	Aqueous extract equiv. 3 g cinnamon	PC4 months	Glu↓; larger effects at higher glucose
Vanschoonbeek *et al*.[46]	25F, Postmenopausal	*C. cassia*; 1.5 g/day	PC/6 wks	No effects
Ziegenfuss *et al*.[37]	11M, 11F; Metabolic syndrome	Aqueous extract (Cinnulin PF)	PC/12 wks	FBG↓; SBP↓; LBM↑; %BF↓
Suppapitiporn *et al*.[53]	60M,F; Type 2 DM	*C. cassia*/1.5 g/day	PC/12 wks	No effects
Hlebowicz *et al*.[45]	8M, 6F; Normal	Rice pudding ± 6 g cinnamon	Meal tolerance test	Post Glu↓; Gastric emptying ↓
Blevins *et al*.[47]	43M,F; Type 2 DM	*C. cassia*/1 g/day	PC/3 months	No effects
Altschuler *et al*.[48]	72 Adolescents; Type 1 DM	Cinnamon/1 g/day	PC/90 days	No effects
Wang *et al*.[43]	15F, PCOS	Aqueous extract (Cinnulin PF)	PC/8 wks	Insulin sensitivity↑
Roussel *et al*.[41]	11M, 11F; Metabolic syndrome	Aqueous extract (Cinnulin PF)	PC/12 wks	FRAP↑; Plasma SH↑; MDA↓

were also taking lipid lowering agents. Altschuler *et al*.[48] also did not report significant effects but this study was with adolescent subjects with type 1 diabetes. The majority of the studies not reporting beneficial effects of cinnamon or the aqueous extract of cinnamon involve subjects taking adequate amounts of glucose-lowering drugs to control blood glucose and maintain normal HbA1c levels and, based on these studies, a meta-analysis reported no effects of additional cinnamon.[49] However, if subjects are taking adequate amounts of glucose-lowering drugs at the beginning of and throughout the study, it is not surprising that a nutraceutical does not have additional effects. Similarly, additional amounts of glucose-lowering drugs would also likely not show significant additional effects if glucose related variables are well controlled.

The beneficial effects of cinnamon are greater in the study of Khan *et al*.[32] than those observed in the studies of Ziegenfuss *et al*.,[37] Mang *et al*.[50] and Wang *et al*.,[43] but the subject populations were very different. Subjects in the Khan study had type 2 DM and were taking sulfonylurea drugs that increase insulin secretion. Since compounds found in cinnamon increase insulin sensitivity, they are likely to have larger effects in subjects taking sulfonylurea drugs. Insulin resistance would also be larger in subjects with type 2 DM than in subjects who are still prediabetic. Mang and others[50] reported similar decreases in blood glucose of subjects with type 2 DM to those of Ziegenfuss *et al*.[37] in a group of subjects with metabolic syndrome. Similar decreases in blood glucose were reported by Wang *et al*.[43] in women with insulin resistance related to polycystic ovary syndrome. There were no significant differences in lipid profiles in these later studies. The duration of the supplementation seems important to consider since, in the studies of Ziegenfuss *et al*.[37] and Roussel *et al*.,[41] there were no effects on blood glucose after a 6-wk intervention with supplementation of an aqueous cinnamon extract (500 mg/day) but only after 12 wks. Similarly, there were no beneficial effects in postmenopausal women with type 2 DM after only 6 wks.[46] Antioxidant effects were also not significant after 6 wks but were after 12 wks in the study of Roussel *et al*.[41]

Vanschoonbeek *et al*.[46] reported no effect of 1.5 g/day of cinnamon powder for 6 wks on indices of glycemic control (fasting blood glucose, insulin, oral glucose tolerance test, HBA_{1c}, insulin sensitivity, and insulin resistance) or blood lipids in 25 postmenopausal women from the

Netherlands. In addition to duration, the study population of Vanschoonbeek *et al.*[46] was different from those of Khan *et al.*,[32] Mang *et al.*,[50] Roussel *et al.*,[41] Ziegenfuss *et al.*[37] and Wang *et al.*[43] in that only postmenopausal females were included as subjects. Whether differences in hormonal milieu affect the potential interaction between cinnamon supplementation and glucose control is unknown at this time. In all of the human studies involving cinnamon or aqueous extracts of cinnamon there have been no reported adverse events, and subjects with the poorest glycemic control appear to benefit the most.

Model of Cinnamon Effects

A model of the potential diverse effects of cinnamon is depicted in Fig. 6.[13] Cinnamon polyphenols affect multiple steps related to glucose and insulin function. CPE activates insulin receptors by increasing their tyrosine phosphorylation activity and by decreasing phosphatase activity that

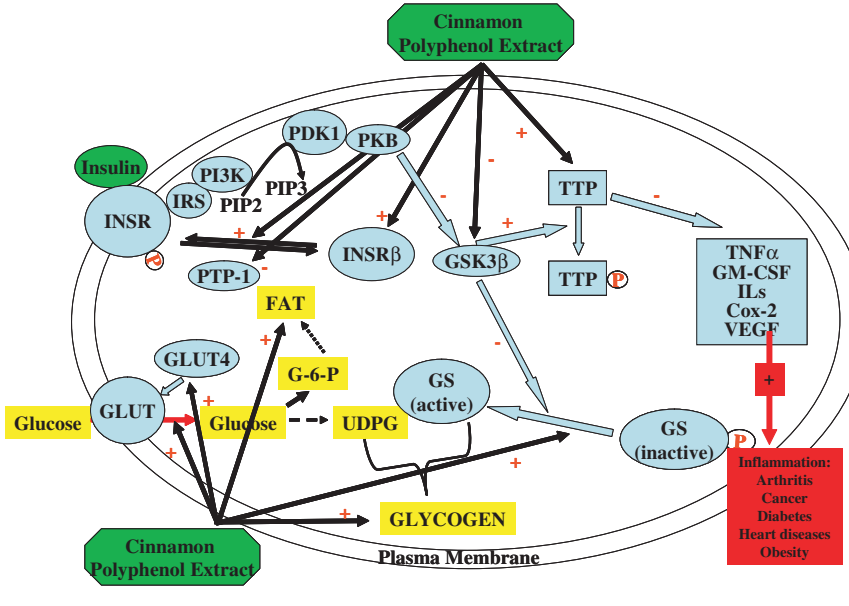


Fig. 6. Model of the effects of CPE ("+" represents positive effects and "–" represents negative effects).

inactivates the insulin receptor.[51] CPE also increases the amount of IRβ and GLUT4 protein.[13] CPE increases glycogen synthase activity and glycogen accumulation[52] with decreased GSK3β activity.[52] CPE also increases the amount of the early response anti-inflammatory protein, tristetraprolin (TTP).[13] All these activities and other potential activities may eventually lead to more efficient glucose transport and utilization. In addition, CP-induced TTP accumulation may provide one of the molecular bases for the beneficial effects of cinnamon in improving the conditions of people with diabetes by down-regulating the synthesis of pro-inflammatory cytokines.

CONCLUSIONS

Compounds found in cinnamon not only improve the function of insulin but also function as antioxidants and may be anti-inflammatory and exert neuroprotective effects as well. CPE regulates multiple genes and the health benefits of cinnamon include effects on glucose metabolism and inflammation as well as neuroprotective effects. Human studies involving subjects with metabolic syndrome, type 2 DM and polycystic ovary syndrome show the beneficial effects of whole cinnamon and aqueous extracts of cinnamon on glucose, insulin, lipids and antioxidant status. There may also be effects of the cinnamon compounds on lean body mass, body composition, and inflammatory response.

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Cloves (Eugenol)

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The active components in cloves are eugenol **1** and isoeugenol **2**. The antioxidant and anti-inflammatory activities of both compounds are well established. We synthesized various dimer forms of eugenol-related compounds, namely eugenol-dimer **3**, isoeugenol-dimers **(**dehydrodi-isoeugenol **4** and alpha-diisoeugenol **5)**, 2-methoxy-4-methylphenol (MMP)-dimer **10** and 2-*t*-butyl-4-methoxyphenol (BHA)-dimer **13**, and their antioxidant, anti-inflammatory and cytotoxic activities, as well as those of curcumin **11**, were investigated. There are numerous potential molecular targets, mostly bioactive proteins, for the anti-inflammatory and cytotoxic activities of phenolic compounds. We focused attention on inhibition by eugenol-related compounds of lipopolysaccharide (LPS)-stimulated cyclooxygenase-2 (COX-2) in RAW 264.7 cells (a mouse leukemic monocyte-macrophage cell line). Compounds **4** and **13** showed potent COX-2 inhibition, similar to that of **11**, followed by **3** and **10**. Among the monophenols, vanillin **8**, but not **1**, **2** or BHA **12**, was a potent inhibitor. The relationships between cytotoxicity towards human submandibular gland carcinoma cells (HSG) or human gingival fibroblasts (HGF) and inhibition rate constant (k_{inh}), a measure of radical activity, were investigated. The cytotoxicity of eugenol-related compounds

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and 2-methoxyphenols was strongly related to k_{inh}, suggesting that their cytotoxicity may be mediated by radical reactions. Also, theoretical parameters were examined by the density function theory (DFT)/B3LYP method. Possible links between cytotoxicity and chemical hardness (η) and between COX inhibition and electrophilicity (ω) were observed. The cytotoxicity of eugenol-related compounds may be a result of cytotoxic radical metabolites whose activities are parameterized by k_{inh} and η. In living systems, oxidized **1** and **2** form dimers and other intermediates by the radical coupling reaction and other reactions. Therefore, the biological activity of monomers **1** and **2** may involve not only the parent monomers but also radical metabolites. Dimer forms **3**, **4**, **10** and **12**, as well as **11**, showing potent COX-2 inhibitory activity, may be useful for the prevention and treatment of various chronic diseases. Clinical trials involving cloves are discussed.

INTRODUCTION

Cloves are an important spice and are widely used. Eugenol **1** is the main component of cloves. Isoeugenol **2** can be produced from **1**, a reaction that occurs naturally in cloves. **1** and **2** are incorporated into numerous household and personal hygiene products, including perfumes, cream lotions, soaps and detergents, and are added as flavoring agents to nonalcoholic drinks, baked foods, and chewing gum. **1** has been used as an antiseptic, antibacterial and analgesic agent in traditional medical practice in Asia. Also, **1** is used as the liquid constituent of zinc oxide-chelate cements (zinc-oxide eugenol cement, ZOE) in dentistry.[1] ZOE is used in pulp capping materials, provisional cements, root canal sealers and impression pastes in dentistry. Although ZOE is readily hydrolyzed in the presence of water to zinc hydroxide and eugenic, ZOE has the reputation of adapting readily to the cavity wall. This cement has a palliative and healing activity due to its bacteriostatic and antiseptic action.[2] Compound **1** possesses antioxidant[3] and antimicrobial[4] activities and also shows inhibition of platelet aggregation and thromboxane synthesis.[5] Beneficial protective effects of **1** against cellular damage to various cell types have been reported,[6–9] as well as anti-inflammatory activity.[10–13]

An FAO/WHO Expert Committee has previously given approval for eugenol intake of up to 2.5 mg/kg/day for humans, concluding that eugenol is not carcinogenic.[14] Also, on the basis of numerous long-term studies, there is no strong evidence for the carcinogenicity of isoeugenol in male mice.[15] However, **1** and **2**, particularly the latter, are known to be important contact allergens.[16] To ameliorate the adverse effects of these compounds, many modified derivatives of eugenol and isoeugenol have been synthesized.[17–19] We have previously synthesized 2-allyl-4-methoxyphenols **6** and their dimers from eugenol **1** or isoeugenol **2**: eugenol-dimer **3** (3,3′-dimethoxy-5,5′-di-2-propenyl-1,1′-biphenyl-2,2′-diol); dehydrodiisoeugenol **4** (2-(3-methoxy-4-hydroxyphenyl)-3-methyl-5-(1-propenyl)-7-methoxy-2,3-dihydrobenzofuran) and alpha-diisoeugenol **5** (*r*-l-ethyl-5-hydroxy-*t*-3-(4-hydroxy-3-methoxyphenyl)-6-methoxy-*c*-2-methylindane). Further, we have synthesized MMP-dimer **10** (3,3′-dimethoxy-5,5′-dimethyl-1,1′-biphenyl-2,2′-diol) from 2-methoxy-4-methylphenol (MMP) **9**.[3,20,21] Investigations in our laboratory have focused on the mechanism of action of a variety of anticancer and antioxidant agents, particularly eugenol, isoeugenol and related compounds, with a view to designing effective protocols for clinical trials. We have investigated the radical production, pro-oxidative and radical-scavenging activities of these compounds, as well as their cytotoxicity, tissue reactions and/or anti-inflammatory activity (NF-κB and COX-2 inhibition).[22–37] Here, we present the results of our experiments, discuss the molecular targets of eugenol and related compounds, and describe clinical trials with these agents.

CHEMICAL CONSTITUENTS

The chemical structures of eugenol-related compounds and the formation and delocalization of eugenol and isoeugenol are shown in Figs. 1 and 2, respectively.

MOLECULAR TARGETS

Extensive research on phytophenols and polyphenols from natural sources during the last half century has identified various molecular targets that can potentially be used not only for the prevention of cancer and chronic

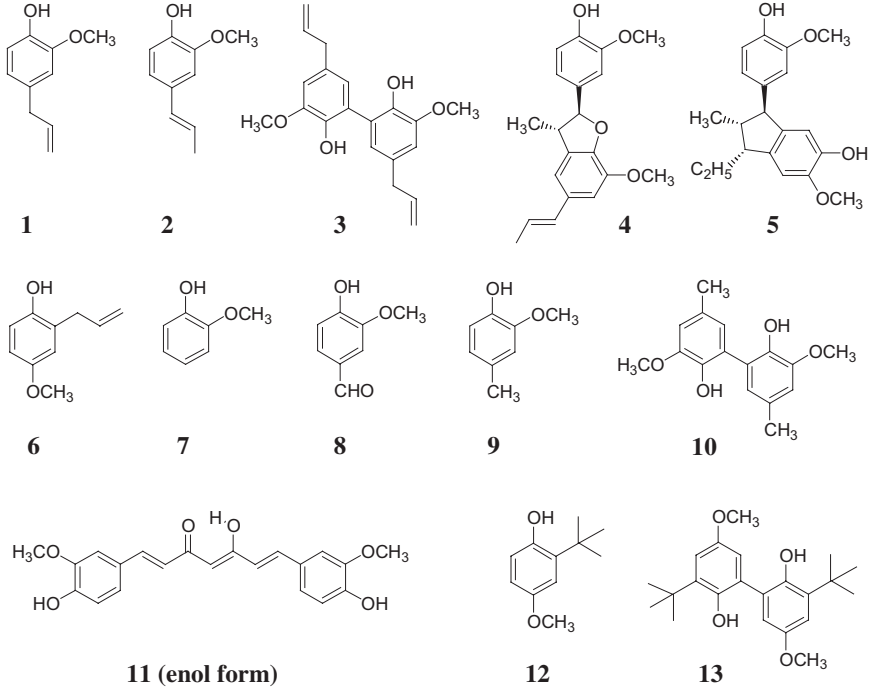

Fig. 1. Chemical structures of eugenol-related compounds.

disease but also for treatment. The anti-inflammatory effects of phytophenols and polyphenols, which frequently behave as nonsteroidal anti-inflammatory drug (NSAID)-like compounds, are associated with numerous potential targets, such as transcription factors (e.g. NF-κB, AP-1, STAT, p53), anti-apoptotic proteins (e.g. Bcl-2, BclXL), apoptotic proteins (caspases, Bax), growth factor signaling pathways (e.g. TNF, ILs, EGF), protein kinases (e.g. IKK, AKT, JNK), cell-cycle proteins (e.g. cyclins, cyclin-dependent kinases), and COX-2.[38] We have focused here on cellular damage produced by phenoxyl radicals and reactive oxygen species (ROS) and on COX-2 as a molecular target of eugenol-related compounds.

Phenoxyl Radical Attack

Hansch[39] suggested that the radical toxicity of phenols proceeds in a two-step reaction. First, radical formation occurs, in which the phenol is

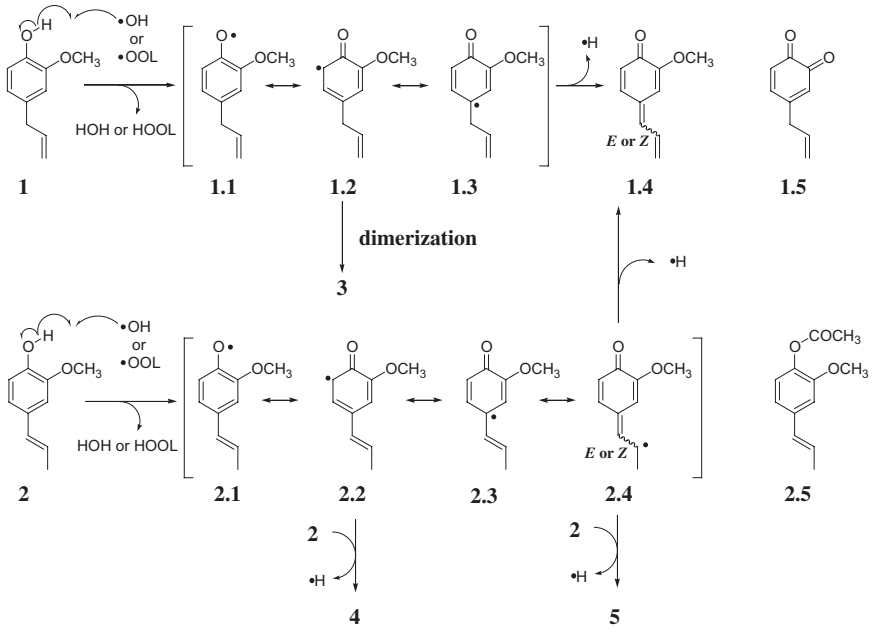

Fig. 2. Antioxidation mechanism of eugenol (**1**) and isoeugenol (**2**) by resonance stabilization of spin density of phenoxy radicals.

converted to a phenoxyl radical in a rate-limiting process. Secondly, the radical migrates and reacts with a sensitive site within the cell. The second step may involve damage to DNA, to proteins, and to lipids, and may also involve non-specific damage due to hydrophobicity. Reaction with DNA as a molecular target of phenolic compounds would account for their estrogenic and carcinogenic actions.

As found previously, ROS and reactive nitrogen species (RNS, ·NO) play important roles in regulation of cell survival. In general, moderate levels of ROS/RNS function as signals to promote cell proliferation and survival, whereas high levels of ROS/·NO can induce cell death. ROS is a term that includes radical oxygen species such as O_2^- and ·OH as well as non-radical derivatives of molecular oxygen (O_2) such as H_2O_2. ROS are mainly generated at mitochondrial electron transport chains during normal cellular metabolism. In addition, various stimuli, including tumor necrosis factor-alpha (TNF-α), FAS ligand, and growth

factors, rapidly provoke ROS accumulation in their target cells. ROS can combine with ·NO or larger molecules to form RNS or lipid peroxy radicals (ROO·). Phenols with antioxidant properties may enhance endogenous defense systems and reduce both initiation and propagation of ROS. The expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione-S-transferase is regulated by ROS stress in a complex way.[40,41]

We investigated intracellular ROS formation induced in living cells by eugenol **1** and isoeugenol **2** after treatment with horseradish peroxidase/hydrogen peroxide (HRP/H_2O_2) or visible-light (VL) irradiation by using the 5-(and 6-)-carboxy-2′,7′-dichlorofluorescein diacetate (CDFH-DA) method.[30] In HRP/H_2O_2-treated human submandibular gland carcinoma cells (HSG) (Fig. 3a), ROS levels were increased at low concentrations of **1** (5–100 μM) compared with the control, but were decreased at a high concentration (500 μM). On the other hand, **2** increased ROS levels over a wide concentration range. Additionally, **1** did not alter intracellular glutathione (GSH) levels in the cells even up to a concentration of 1,000 μM, whereas **2** concentration-dependently decreased GSH levels, with a complete decrease at 1,000 μM.[30] The cytotoxicity of **2** was one order of magnitude greater than that of **1**. The high cytotoxicity of **2** was clearly related to its induction of high levels of ROS production at a lower intracellular GSH level. It is known that exposure to light aggravates the inflammatory reaction (hypersensitivity) triggered by **1** and **2**, particularly the latter.[3] The behavior of **1** and **2** in ROS generation with visible light (VL) irradiation was similar to that with HRP/H_2O_2 (Fig. 3b). At higher concentrations compound **1** scavenged ROS, whereas **2** caused ROS formation across the whole concentration range, although it was relatively less effective at higher concentrations. **2** is the most active in inhibiting ferrous ion-induced, ferric ion-induced and cumene hydroperoxide-induced lipid peroxidation in rat brain homogenates, and also is a significant hydroxyl radical scavenger,[42] but ROS generation by **2** appears to exceed the capacity of intracellular ROS scavenging (Fig. 3). ROS-producing enzymes may be particularly activated by the radical reaction of **2**.

Protective effects of **1** on radical-induced cellular damage have previously been reported. For example, **1** effectively prevents membrane and

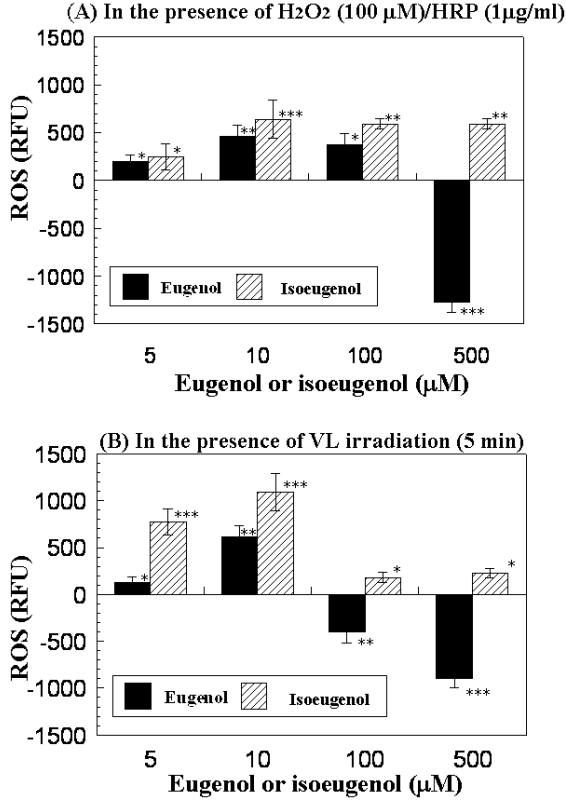

Fig. 3. ROS production in HSG cells induced by eugenol **1** or isoeugenol **2** together with oxidative treatment with (a) H_2O_2 (100 μM)/HRP (1 μg/ml) or (b) VL-irradiation for 5 mins. ROS production in single cells is expressed as relative fluorescence intensity units (RFU).[30] ROS production with only (a) H_2O_2/HRP or only (b) VL-irradiation is expressed as zero. Columns show means ± SD. There is a significant difference between eugenol or isoeugenol and the control (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Controls are expressed as 0. From Atsumi *et al*. (2005). *Toxicol. In Vitro* **19**: 1031 (Copyright Elsevier).

cellular damage in irradiated thymocytes, whereas **2**, ascorbic acid and alpha-tocopherol acetate do not, which is possibly related to the membrane-specific antioxidant activity of **1**.[8] Similarly, **1** inhibits ROS generation, intracellular calcium accumulation, and the subsequent mitochondrial membrane potential collapse, cytochrome *c* release and caspase-3 activation induced by oxidized low density lipoprotein

(oxLDL).[9] Also, **1** shows neuroprotective activity against neurotoxicity induced by N-methyl-D-aspartate (NMDA), oxygen-glucose deprivation, and xanthine/xanthine oxidase in primary murine cortical cultures.[7] Further, **1** shows protective effects against $CC1_4$-induced hepatotoxicity and superoxide radical production.[6] On the other hand, in studies of the antiproliferative and pro-apoptotic activity of eugenol-related biphenyls (**1**, **2**, **3** and brominated biphenyls) on malignant melanoma cells, **1** and **2** did not inhibit the proliferation of melanoma cells. In contrast, **3** showed mild antiproliferative activity and brominated biphenyls had strong activity.[43] This suggests that dimeric forms derived from **1** may have enhanced antiproliferative activity.

As shown in Fig. 3, **1** and **2** may play a dual role as both antioxidants and pro-oxidants: **1** acts as a pro-oxidant at low concentrations but acts as an antioxidant at higher concentrations. In contrast, **2** acts as a potent pro-oxidant over a wide range of concentrations. The different radical-scavenging behavior of **1** and **2** may be related to their phenolic O-H bond dissociation enthalpies (BDE) and ionization potentials (IP). The BDE and IP values of **2** are considerably less than those of **1** (see Table 3), and compounds with lower values are more likely to act as pro-oxidants.

The production of ROS has previously been reported to play a crucial role in cell-cycle progression, including signal transduction cascades and protein ubiquitination and degradation. Low concentrations of ROS result in increased cell-cycle progression, or, in the case of prolonged exposure, growth arrest. At high concentrations of ROS, all of the above processes are activated, in combination with enhanced damage to the building blocks of the cell, leading to apoptosis or even necrosis.[40,41] The protective effects of **1** on radical-induced cellular damage are probably related to its antioxidative activity.

Radical formation and delocalization of **1** and **2** provide crucial information about **1**- or **2**-induced toxicity (Fig. 2). Compound **1** also produces eugenol radical species (**1.1**, **1.2** and **1.3**) and an eugenol quinonemethide (**1.4**). Eugenol radical species can either haptenate proteins or engage in cytotoxic binding to cells by reaction at the unsubstituted *ortho*-position. Also, dimer **3** may be formed from compound **1** by *ortho-ortho* radical coupling reactions. In addition to radical mechanisms, a second mechanism

may involve *in vivo* demethylation by enzymes to form 4-alkyl catechols, which can then haptenate proteins by oxidation to the *ortho*-quinone **1.5**, which acts as an electrophilic Michael acceptor for protein nucleophiles.[17] In contrast, isoeugenol **2** generates several isoeugenol radical species (**2.1**, **2.2**, **2.3** and **2.4**). The benzyl radicals (**2.4**) derived from compound **2** dimerize to form dihydrodiisoeugenol **4** as a result of the radical-radical coupling reaction **2.2** + **2.4**. Also alpha-*O*-dilignol may be formed by the radical-radical reaction **2.1** + **2.4**.[35] Thus, the high cytotoxic activity of **2** may be attributed to benzyl radical formation and its cytotoxic binding to cells.[30] The higher allergenic action of **2** may also be due to a radical mechanism, namely reaction of compound **2.4** with proteins and formation of hapten protein conjugates.[44] **2** possesses a high potency for cytotoxicity, possibly because the radical migrates and reacts with a sensitive site within the cell.

In terms of detoxication mechanisms, in animal tests compound **2** is rapidly metabolized and is excreted predominantly in the urine as phase II conjugates of the parent compound.[45] Butylated hydroxyanisole (BHA) **12** is widely used as an artificial antioxidant. At higher concentrations, BHA is an established carcinogen in animal tests; however, the protective effect of low BHA concentrations on biological systems is presumably a result of its ability to induce phase II detoxifying enzymes such as glutathione S-transferases and quinone reductase.[46] Eugenol-related compounds could probably be detoxified *in vivo* by such enzymes.

COX-2 Inhibition

As found recently, not only ROS but also RNS play important roles in human physiology and pathology. RNS are produced *in vitro* by macrophages incubated in the presence of LPS and interferon alpha. They originate from the semi-essential amino acid L-arginine by the activity of ˙NO synthase. At higher concentrations, RNS have antimicrobial, antitumor, and cytotoxic effects. At low concentrations, they stimulate guanylate cyclase, resulting in effects on blood flow and blood pressure regulation, neural signal transmission, and neuroendocrine activity.[47] COX is the rate-limiting enzyme in the conversion of arachidonic acid to prostaglandins (PG), and in particular COX-2 is induced

in response to many pro-inflammatory stimuli such as cytokines, growth factors, LPS and mitogens *via* activation of *cis*-acting transcription factors such as NF-κB in a variety of cells. Such factors are closely involved in inflammation and cancer.[38] Compounds with COX-2 inhibiting activity possess anti-inflammatory properties. Antioxidant and cyclooxygenase-inhibitory phenolic compounds from *Ocimum sanctum* Linn. have been investigated, showing that eugenol **1** possesses 97% COX-1 inhibitory activity and a slightly higher level of COX-2 inhibitory activity at 1,000 μM.[11] Compound **1** suppresses COX-2 gene expression in LPS-stimulated mouse macrophages and inhibits the proliferation of HT-29 cells and the mRNA expression of COX-2, but not COX-1. This suggested that eugenol might be a plausible lead candidate for further development as a COX-2-inhibiting anti-inflammatory or cancer chemopreventive agent.[12] Also, LPS-dependent expression of COX-2 protein in RAW cells was recently reported to be inhibited markedly by **2**, and less effectively by **1**. Specifically, an increase in the concentration of **1** or **2** did not cause complete inhibition of COX-2 expression; relative to the control, inhibition of COX-2 expression was approximately 50% at 400 μM of **1** and 50 μM of **2**. However, compounds **1** and **2** inhibit LPS-dependent production of NO in RAW 264.7 cells and decrease the LPS-mediated iNOS protein without any toxic effects on cell viability.[12]

We have investigated inhibition of LPS-induced COX-2 expression by eugenol-related compounds in RAW 264.7 cells.[26,27,29,31–35] The results are shown in Table 1. Compounds **4**, **8** and **11** showed potent COX-2 inhibition, followed by **3**, **10** and **11**. In contrast, **1**, **2** and **12** did not inhibit COX-2 even at higher concentrations of 500 μM. This did not accord with the results described above.[12] In contrast, the dimer forms **3**, **4** and **13** clearly inhibited COX-2 expression. When a high concentration is required for COX-2 inhibition, a false-positive result is frequently obtained because of cytotoxicity. There is a general opinion in drug screening that if the 50% COX-2 inhibitory concentration (IC_{50}) of a compound is less than 3 μM, then it can be regarded as a strong enzyme inhibitor. The IC_{50} threshold for candidate compounds should be less than 10 μM.[48] The most potent COX-2 inhibitors **4**, **8** and **11** had IC_{50} values of less than 10 μM.

Table 1. COX-2 inhibition in RAW 264.7 cells and electrophilicity (ω) of eugenol-related compounds.

Name	Compound	ω	LPS-Stimulated COX-2 Inhibition	Reference
Eugenol	**1**	1.200	negative	27
Isoeugenol	**2**	1.655	negative	28
Eugenol-dimer	**3**	1.487	positive, 300 μM	27
Dehydrodiisoeugenol	**4**	1.648	positive, <10 μM	28
α-diisoeugenol	**5**	1.218	negative	31
Guaiacol	**7**	1.157	positive, 500 μM	36
Vanillin	**8**	2.985	positive, 10–100 μM	36
2-methoxy-4-methylphenol (MMP)	**9**	1.114	positive, 500 μM	27
MMP-dimer	**10**	1.381	positive, 200–500 μM	27
Curcumin	**11**	4.159	positive, <10 μM	31
2-t-butyl-4-methoxyphenol (BHA)	**12**	1.402	negative[a]	34
BHA-dimer	**13**	1.830	positive,[a] <10 μM	34

[a] Fimbria of *Polyphyromonas gingivalis*; for compounds see Fig. 1. COX-2, cyclooxigenase-2; LPS, lipopolysaccharide (*Escherichia coil* O111 B4-derived LPS); ω calculated by DFT/B3LYP.

NF-κB is an important transcriptional factor that is activated by phosphorylation-dependent proteolysis of the inhibitor of NF-κB, which regulates inflammatory responses and the expression of various inflammatory cytokine genes.[38,49,50] Cloves have been reported to be able to suppress NF-κB activation by suppressing I-κBα degradation.[10] Compounds **4**, **8** and **3** potently inhibit LPS-stimulated NF-κB. Similarly BHA-dimer inhibits NF-κB activation stimulated by a bacterial component, fimbriae of *Polyphyromonas gingivalis* (an oral anaerobe).[33] Curcumin **11** possesses significant COX-2 inhibiting activity through the suppression of NF-κB.[38,49] Compounds **1**, **2** and **12** do not inhibit NF-κB activation in our studies. Also, **4** inhibits binding of activator protein-1 (AP-1) to the 12-tetradecanoylphorbol-13-acetate-responsive element (TRE) sequence in LPS-stimulated RAW 264.7 cells.[29]

COX-2 inhibition by vanillin **8** and curcumin **11** has recently been evaluated in terms of their molecular orbital energies using chemical

hardness concepts.[35–37] We reinvestigated the relationships between COX-2 inhibiting activity and electrophilicity (ω) for eugenol-related compounds. The ω-term was calculated from data in Table 3. Compounds with potent COX-2 inhibitory activity showed markedly higher ω values, suggesting that the anti-inflammatory activity of these compounds may be related to ω-controlled enzymes. Previously, the toxicity of polyaromatic hydrocarbons, which are electrophilic compounds, has been related to the ω-term,[51] and the ω-term has also been used in understanding the reactivity of HIV-1 nucleocapsid protein p7 (NCp7) with a variety of electrophilic agents.[52] These findings underline the usefulness of appropriate chemical descriptors in understanding the origin of biological activity. One of the well-recognized molecular targets for chemoprevention with NSAIDs is COX-2. Hence, the link between COX-2 inhibition and the ω-term of eugenol-related compounds suggests that COX-2 inhibition may be controlled by the ω-term. The chemical hardness concept may be useful for analyzing and predicting the biological activity of drug candidates.

IN VITRO STUDIES

To clarify mechanisms of eugenol-induced cytotoxicity, we investigated the radical-scavenging activity, theoretical parameters and cytotoxicity of eugenol-related compounds, and also explored quantitative structure-activity relationships (QSARs) between cytotoxicity or radical-scavenging activity and theoretical parameters.

Radical-Scavenging Activity Under Cell-Free Conditions

We have proposed a quantitative model to describe the antioxidant activity of phenolic antioxidants on the polymerization of methyl methacrylate (MMA) initiated by benzoyl peroxide (BPO) under nearly anaerobic conditions as assessed by differential scanning calorimetry (DSC). This induction period method has proved to be reliable for evaluating the activity of these compounds because biological systems have a low oxygen tension.[3,32] Also, as cancer cells have anaerobic metabolism (i.e. they do not utilize oxygen),[53] our biomimetic system under nearly anaerobic conditions may be a good model for evaluating the antioxidant activity of

anticancer drugs. Additionally, a quantitative method to assess 1,1′-diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$) scavenging activity has been widely used for evaluating phenolic antioxidant activity.[54] The radical-scavenging activity of 10 typical eugenol-related compounds (2-methoxyphenols) was investigated by combining two distinct approaches: first, the induction period method for polymerization of methacrylates initiated by PhCOO radicals derived from BPO, and secondly a DPPH radical-scavenging test. The induction time (*IT*) and initial rate of polymerization in the presence (Rp_{inh}) or absence (Rp_{con}) of an antioxidant were determined by the method reported previously.[32] In brief, the experimental resin consisted of MMA and BPO with or without additives. BPO was added at 1.0 mol%, and the additives were used at 0.01 mol% (1 mmol). Approximately 10 ml of the experimental resin (MMA: 9.12–9.25 mg) was loaded into an aluminum sample container and sealed by applying pressure. The container was placed in a DSC (model DSC 3100; Mac Science Co., Tokyo, Japan) maintained at 70°C, and the thermal changes induced by polymerization were recorded for the appropriate periods. The heat due to the polymerization of MMA was 13.0 kcal/mol in these experiments. The conversion of all samples was calculated from DSC thermograms using the integrated heat evoked by polymerization of MMA. Time-exotherm and time-conversion curves of typical methoxyphenols, curcumin, isoeugenol and eugenol are shown in Fig. 4. Time-conversion curves break when a phenolic inhibitor is consumed. These breaks are sharp and provide a reliable measure of the induction period of the inhibitors. The induction time (*IT*) was calculated from the difference between the *IT* of specimens and that of controls. The initial rates of polymerization in the absence (Rp_{con}) and presence (Rp_{inh}) of the phenolic inhibitors were calculated from the slope of the plots during the initial linear phase of the conversion rate of MMA polymerization (a tangent drawn at the early polymerization stage).

Calculation of stoichiometric factor (n) and inhibition rate constant (k_{inh}) was performed as follows.

The relative n value in Eq. (1) can be calculated from the induction time in the presence of an inhibitor, [*IH*]:

$$n = \frac{R_i [IT]}{[IH]}. \tag{1}$$

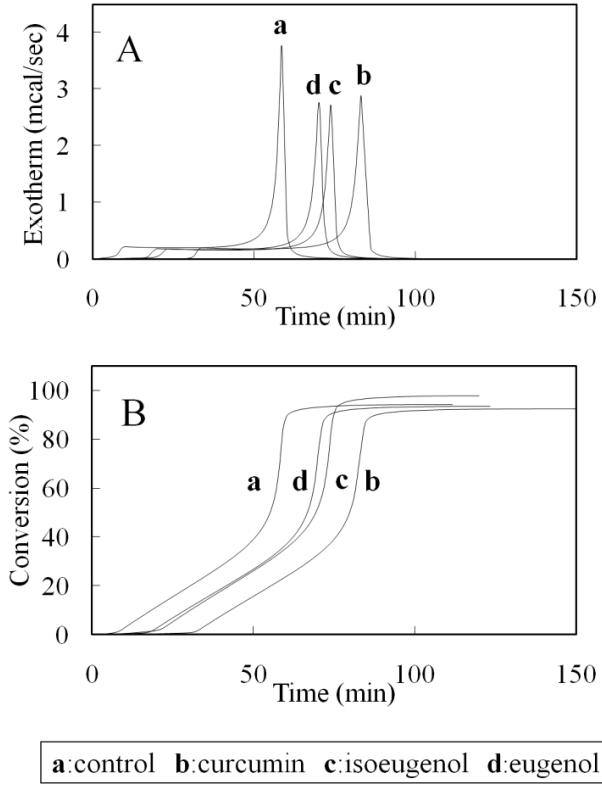

Fig. 4. Typical (a) time-exotherm and (b) time-conversion curves for the polymerization of MMA-BPO in the presence of eugenol **1**, isoeugenol **2** and curcumin **11**. Each compound was added at 1 mM.

The number of moles of peroxy radicals trapped by the antioxidant was calculated with respect to 1 mole of inhibitor moiety unit. The initiation rate R_i value for BPO at 70°C was 2.28×10^{-6} mol $l^{-1}s^{-1}$.

When R_i is constant, i.e. when new chains are started at a constant rate, a steady-state treatment can be applied and the initial rate of polymerization of MMA is given by Eq. (2):

$$Rp_{\mathrm{con}} = \frac{\{k_{\mathrm{p}}[\mathrm{MMA}]R_{\mathrm{i}}^{1/2}\}}{(2k_{\mathrm{t}})^{1/2}}, \tag{2}$$

where MMA represents methyl methacrylate and k_p and k_t are the rate constants for chain propagation and termination, respectively. The Rp_{inh} rates are determined by Eq. (3):

$$Rp_{inh} = \frac{\{k_p[MMA]R_i\}}{\{nk_{inh}[IH]\}} \quad (3)$$

in which Rp_{inh} is the initial rate of inhibited polymerization, [MMA], n, [*IH*] and k_p are defined above, and k_{inh} is the rate constant for scavenging (inhibition) of MMA radicals by an antioxidant. From Eqs. (2) and (3), k_{inh}/k_p can be calculated from Eq. (4):

$$\frac{k_{inh}}{k_p} = \frac{[MMA]}{[IT]\times[Rp_{inh}]}. \quad (4)$$

The results are shown in Table 2. n declined in the order **11** > **10** > **5** > **3** > **6** > **2** > **1** > **9** > **7** > **4**. Dimers with two OH groups in the molecule showed large n values. k_{inh}/k_p declined in the order **4** > **7** > **1** > **9** > **6** > **2** > **3** > **10** > **5** > **11**. In general, as n increased, k_{inh}/k_p decreased.

The anti-DPPH$^{\cdot}$ activities of eugenol-related compounds are also shown in Table 2. Anti-DPPH$^{\cdot}$ activity declined in the order **3** > **11** > **10** > **2** ≃ **5** > **1** ≃ **6** > **9** > **7** > **4**. Dimers also showed high anti-DPPH$^{\cdot}$ activity. The compounds tested showed different rank orders of radical-scavenging activity in the DPPH assay and the induction period method. This may be due to the different characteristics of the radical species, namely DPPH$^{\cdot}$, a stable nitrogen-centered radical, and PhCOO$^{\cdot}$ derived from BPO.

Theoretical Parameters

Computational chemistry is one of today's most rapidly expanding and exciting areas of endeavor in the medical and medicinal sciences. Information available from computational methods may be useful for interpreting the molecular mechanisms of action of eugenol-related compounds. We previously investigated the theoretical parameters of eugenol-related compounds using the semi-empirical PM 3 method.[35] Here, we investigate it at the density functional theory (DFT)/B3LYP levels.

Table 2. Radical-scavenging activity of eugenol-related compounds using the induction period method and DPPH assay, and their cytotoxicity towards human submandibular gland carcinoma cells (HSG) and human gingival fibroblasts (HGF).

				Cytotoxicity		
	Induction Period Method[a]			HSG Cells	HGF Cells	DPPH Assay[b]
Compound	n[c]	Rp_{inh}/Rp_{con}	k_{inh}/k_p	log I/C (M)[d]	log I/C (M)[d]	IC_{50} (mM)[e]
1	1.40	0.81	11.19	3.50	3.54	0.06
2	1.73	0.89	8.78	4.55	4.49	0.05
3	2.30	0.95	7.76	3.82	3.16	0.015
4	0.80	0.94	18.29	5.52	5.51	1.30
5	2.74	0.84	5.87	5.57	5.55	0.05
6	2.0	0.86	10.08	3.65	3.39	0.06
7	1.21	0.87	12.81	3.10	2.25	0.51
9	1.36	0.94	10.57	3.40	2.68	0.09
10	2.38	0.91	6.26	4.33	3.51	0.024
11	3.84	0.78	4.53	5.52	5.48	0.04
2.5	—	—	—	3.59	3.69	—

For compounds see Fig. 1.

[a] Described in the text;

[b] DPPH (2,2′-diphenyl-1-picrylhydrazyl);

[c] The number of moles of peroxy radicals (PhCOO*) trapped by one mole of the inhibitor; Rp_{inh}/Rp_{con}, the ratio of the propagation rate with an inhibitor to that of control; k_{inh}/k_p, the ratio of the inhibition rate constant with an inhibitor to that of control;

[d] 50% cytotoxic concentration of phenols (C);

[e] The amount of inhibitor necessary to decrease the initial DPPH concentration by 50%.

Phenolic O-H bond dissociation enthalpy (BDE) and ionization potential (IP)

Using DFT/B3LYP, calculated BDE and IP values of phenolic compounds became interpretable. *BDE* was calculated as follows. First, the lowest and second-lowest energy conformers of both the phenol derivatives and their phenoxyl radical species were identified as candidates for geometry optimization using the conformer search procedure of the MMFF (Merck molecular mechanics) force fields calculation. Then, the tentative conformers were optimized in geometry by *ab initio* molecular orbital calculations on a Hartree–Fock model with *ab initio* 6-31G* (HF//6-31G*)

level for the phenols and UHF//6-31G* level for the phenoxyl radicals *in vacuo* to afford the respective energetic minimized structures. The electronic energy calculation further proceeded by single point calculation of density functional theory (DFT) using the B3LYP functional on the 6-31G* basis set. Then, $BDE = Hr + Hh - Hp$, where *Hr* is the enthalpy of the phenoxyl radical generated by H-abstraction, *Hh* is the enthalpy of the hydrogen radical, and *Hp* is the enthalpy of the parent phenol.

The energy values of both the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) energy of the fully optimized phenol derivatives were calculated from the HF//6-31G* level basis set molecular orbital calculation. The absolute value of HOMO energy was adopted as an approximate ionization potential value (*IP*) according to Koopmans' theory. All of the molecular modeling and calculation were with Spartan 04 (Wavefunction Inc., Irvine, CA, USA).

The results for *BDE* and *IP* are shown in Table 3. BDE_{1st} declined in the order **7** > **8** > **4** > **1** > **9** ≃ **11** > **2** ≃ **6** > **5** > **10** > **3** > **12** > **13**. IP_{1st} declined in the order **8** > **7** > **1** > **9** > **13** > **12** > **6** > **5** > **11** > **4** > **2** > **3** > **10**. The order of *BDE* did not coincide with that of *IP*, but compounds **8** and **7** showed large values for both *BDE* and *IP*.

In medicinal application of antioxidants, it is important to note that when *BDE* or *IP* become too low, the compounds can act as pro-oxidants rather than as antioxidants.[55] **2**, which has an *IP* value lower than that of **1**, may preferentially act as a pro-oxidant. Compounds **3**, **5**, **10**, **11** and **13**, which have two OH groups, acquire high values of *BDE* and *IP* when undergoing full oxidation. *BDE* and *IP* are essential to understanding the chemical and biochemical behaviors of phenolic compounds. Their electron-donating ability is determined by the one-electron oxidation potential of the parent phenolic compounds.

Chemical hardness (η), electronegativity (χ) and electrophilicity (ω)

These values were calculated using Eqs. (5)–(7), respectively:

$$\eta = \frac{E_{\mathrm{LUMO}} - E_{\mathrm{HOMO}}}{2}, \tag{5}$$

Table 3. HOMO (the highest occupied molecular orbital), chemical hardness η(eV), electronegativity χ(eV), phenolic-O-H bond dissociation enthalpy (*BDE*), ionization potential value of Koopmans' (*IP*) and log *P* for eugenol-related compounds.

Compound	E_{HOMO} (eV)	η	χ	*BDE* (kJ/mol)	*IP* (eV)	Log *P*[b]
1	–5.448	2.841	2.608	346.8	5.45	2.55
2	–5.182	2.377	2.805	339.2	5.18	2.51
3	–5.194	2.479	2.715	336.5, 354[a]	5.19, 5.46[a]	4.75
4	–5.203	2.394	2.809	359.7	5.20	4.06
5	–5.283, –5.243	2.713, 2.565[a]	2.571, 2.699[a]	343.2, 347.2[a]	5.28, 5.30[a]	4.40
6	–5.257	2.525	2.732	339.2	5.26	2.55
7	–5.529	2.923	2.601	364.60	5.53	1.51
8	–6.075	2.339	3.737	361.9	6.08	1.19
9	–5.376	2.854	2.522	344.10	5.38	1.98
10	–5.110, –5.028	2.488, 2.297[a]	2.621, 2.732[a]	338.1	5.11, 5.02	3.59
11	–5.267, –5.366	1.612, 1.559[a]	3.662, 3.807	344, 347[a]	5.27, 5.37[a]	2.51
12	–5.300	2.705	2.597	322.7	5.3	3.14
13	–5.365, –5.148	2.402, 2.115[a]	2.964, 3.003[a]	312.8, 343.5[a]	5.36, 5.15	5.40

For compounds see Fig. 1.

[a]Second oxidation. The methods are described in the text.

[b]From Fujisawa *et al.*[20] and Fujisawa *et al.*[35]

$$\chi = -\frac{E_{\mathrm{LUMO}} + E_{\mathrm{HOMO}}}{2}, \tag{6}$$

$$\omega = \frac{\chi^2}{2\eta}, \tag{7}$$

where E_{LUMO} and E_{HOMO} are the energy levels for the frontier orbitals.

The results for η and χ are also shown in Table 3. The relationship between η and χ for eugenol-related compounds can be described by Eq. (8):

$$\eta = 4.46(\pm 0.21) - 0.68(\pm 0.16)\chi, \tag{8}$$

$n = 13$, $r^2 = 0.623$, $p < 0.01$.

Cytotoxicity

We evaluated the cytotoxicity of eugenol-related compounds using human submandibular gland carcinoma cells (HSG), a cancer cell line, and human gingival fibroblasts (HGF), a normal cell line. The cytotoxicity (50% cytotoxic concentration, C) of these compounds towards HSG and HGF cells was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method. The results are summarized in Table 3.

The cytotoxicity towards HSG cells declined in the order **4** ≃ **5** ≃ **11** > **2** > **10** > **3** > **6** > **1** > **9** > **7**. In contrast, the cytotoxicity towards HGF cells declined in the order **4** ≃ **5** ≃ **11** > **2** > **1** ≃ **10** > **6** > **3** > **7**. Compounds, **1**, **6**, **7**, **9** and **10** showed more pronounced cytotoxicity towards HSG cells than to HGF cells; in other words, tumor cells were more sensitive to these compounds. Compounds **4**, **5** and **11** were the most cytotoxic towards both cell lines, with 100-fold greater cytotoxicity than that of **1** and 10-fold greater than that of **2**. The log *P* values of **4**, **5** and **11** were 4.06, 4.40 and 2.51, respectively. Also, for primary cells (HGF) as well as tumor cells (HSG), the three 2-methoxyphenols **1**, **2** and **11**, each with a similar log *P* of 2.5, showed large differences in cytotoxicity, implying that the cytotoxicity of these compounds is not related to log *P*.

QSAR studies of phenolic compounds causing apoptosis showed that cytotoxicity involved minimal apoptosis for most phenols.[57] We previously reported the comparative cytotoxicity of **11** and **5** for HSG and human promyelocytic leukemia cells (HL-60); the CC_{50} (μM) for **11** and **5** was 1.7 and 1.0, respectively, for HL-60 cells, whereas that of the corresponding compounds for HSG cells was 3.1 and 2.7.[31] The cytotoxicity of both compounds was similar in both cell types, but that of **11** involved significantly greater extents of apoptosis than that of **5** (Fig. 5). Why induction of apoptosis by **11** is different from that by **5** needs to be explained. The chemical properties of curcumin, a redox-cycling phenol, have attracted considerable attention. Phenoxyl radical generation by **5** and **11** would be determined primarily by the *BDE*, which is determined in part by resonance stabilization of the steric hindrance of abstraction of the phenolic hydrogen by free radicals. However, BDEs calculated by DFT/B3LYP for these two compounds were similar (Table 3). In contrast,

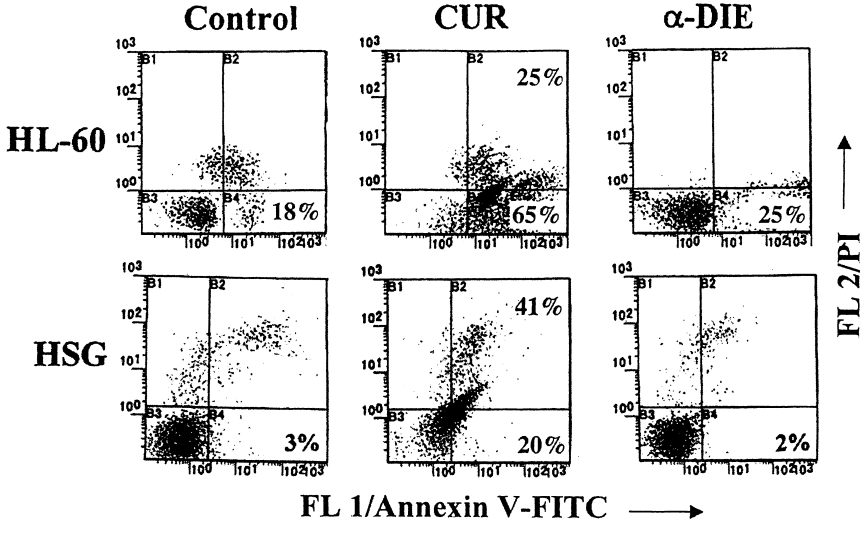

Fig. 5. Proportions of early apoptotic or late apoptotic/necrotic HL-60 and HSG cells after treatment with curcumin **11** or alpha-diisoeugenol **5** at 10 μM. Cells were stained with both annexin V-FITC and PI (propidium iodide). The proportions of early apoptotic and late apoptotic/necrotic cells were calculated from quadrants B4 and B2 (this quadrant contains both late apoptotic and necrotic cells), respectively. The upper value in the quadrant represents cell number (%). From Atsumi *et al*. (2005). *Anticancer Res*. **25** by kind permission of the Editor.

their η-term, the HOMO-LUMO gap parameter, was greatly different between the two compounds, with **11** showing a markedly lower η-value. We previously reported that the cytotoxic potency of 2-methoxyphenols is clearly controlled by their η-value, as judged by PM3 calculations.[35] When the HOMO-LUMO gap was smaller, the cytotoxicity was greater. Together, these findings suggest a possible link between induction of apoptosis and the η-term for **11**. Curcumin-induced cell death is mediated by ROS, and the antiapoptotic protein Bcl-2 plays a crucial role in the early stages of curcumin-triggered apoptotic cell death.[58] Antiapoptotic, apoptotic, cell-cycle and other proteins has previously been reported to be involved in curcumin-induced apoptosis.[38] The activity of bioactive chemicals has previously been reported to depend on η- and χ-controlled enzymes,[59] and we infer from our results that the induction of apoptosis may be related to η-controlled proteins. Also, **11** exerts pro-oxidant properties

after metabolic activation[56] and causes high levels of intracellular ROS generation in HSG cells.[26] Such behaviors of **11** may be parameterized by the η-term. Next, we investigated QSARs as tools to distinguish the mechanisms of cytotoxicity of eugenol-related compounds.

QSAR Studies

QSARs have been used to predict a large number of biological endpoints and shed light on mechanisms of action, whether toxicological or pharmacological.[60] The phenolic hydroxyl group of eugenol-related compounds has a wide range of cellular activities that have not been clearly examined. Phenolic compounds not only act as antioxidants or radical-scavengers but also as pro-oxidants. Thus, phenols have been implicated in problems associated with estrogen toxicity and carcinogenesis. The QSAR paradigm has been useful in clarifying the mechanisms of chemical-biological interaction in various biological systems. We investigated QSARs on the basis of data for cytotoxicity, radical-scavenging activity and theoretical parameters shown in the previous section, and discuss them in the light of these results.

Dependence on inhibition rate constant, k_{inh}

Many researchers have used QSARs to investigate the mechanisms underlying the toxicity of phenolic compounds. For example, QSARs for phenolic compounds in L1210 leukemia cells have been interpreted in terms of *BDE* and overall hydrophobicity (octanol-water partitioning coefficient, log *P*) of the phenols, suggesting that phenol-induced toxicity correlates with radical reactions.[61] Also, in addition to *BDE* and log *P*, acceptable QSARs for the cytotoxicity of phenolic compounds in terms of ionization constants (pK_a) or the Brown variation of the Hammet electronic constant (σ^+) were reported.[39,62] Although a meaningful QSAR for the cytotoxicity of simple phenols in terms of *BDE* and σ^+ was exhibited, *ortho*-substituted or multi-substituted complicated phenols behaved quite differently from the simple phenols; QSAR of these phenols with *BDE* or σ^+ yielded poor results, whereas correlation with the Taft steric parameter ($E_{S\text{-}2}$) or Otsu's radical parameter (E_R) yielded good results.[63] These results

provided valuable guidance for our QSAR studies of complicated phenols. We need E_R and other radical parameters if we are to derive meaningful QSARs for complicated 2-methoxyphenols, because we cannot afford to ignore free radical effects in living systems.

We previously investigated the relationships between cytotoxicity and radical activity for 2-methoxyphenols[3,21,35]; however, their cytotoxic mechanisms at the molecular level have not been clearly elucidated. To clarify the relationships between cytotoxicity and radical activity of phenolic compounds, it is necessary to evaluate precisely their radical-scavenging activity. The cytotoxicity and inhibition rate constant (k_{inh}) data in Table 2 led to the formulation of QSARs 1 and 2:

$$\log 1/C_{HSG} = 6.70\ (\pm 0.43) - 0.29\ (\pm 0.05)\ k_{inh}/k_p, \qquad \text{QSAR (1)}$$

$n = 9$, $r^2 = 0.806$, $p < 0.01$, outlier: **4**,

$$\log 1/C_{HGF} = 6.67\ (\pm 0.75) - 0.33\ (\pm 0.10)\ k_{inh}/k_p, \qquad \text{QSAR (2)}$$

$n = 9$, $r^2 = 0.630$, $p < 0.05$, outlier: **4**.

The coefficient of QSAR (1) was greater than that of QSAR (2). Strong dependence of cytotoxicity, particularly towards HSG cells, on k_{inh}/k_p was observed. There were no relationships between cytotoxicity and n, Rp_{inh}/Rp_{con} or anti-DPPH activity (IC_{50}). Our results for the dependence of cytotoxicity on the parameter k_{inh}/k_p suggest that the cytotoxicity of 2-methoxy-substituted phenols might depend on the radical-trapping rate, k_{inh}.

The cytotoxicity of *ortho*-substituted complicated phenols may be correlated with a radical-associated parameter. Dehydrodiisoeugenol was an outlier in QSARs (1) and (2). This may be related to the C-H bond abstraction, but not the O-H bond abstraction, of this compound.[35] The strong dependence of cytotoxicity on k_{inh}/k_p suggests that the cytotoxicity of the 2-methoxyphenols might be due to radical reactions.

Phenol-induced toxicity has been shown to be a nonspecific toxicity related to hydrophobicity and formation of phenoxyl radicals.[39] However, the cytotoxicity data in Table 2 show no relationships between cytotoxicity

and log *P*. The cytotoxic effects of *ortho*-substituted phenols may not be related to their hydrophobicity.

In general, radical-mediated phenolic toxicity is a multi-step process and is affected by the nature of the rate-determining step. Previously, it was shown that one or a combination of mechanisms, i.e. phenoxyl radical or intermediates (quinone methides, quinone), contribute to phenol toxicity.[62] The formation of intermediates probably affects the cytotoxic binding step.

The QSAR of phenolic compounds in terms of *BDE* was previously investigated.[61] *BDE* is a parameter that strongly implicates a particular mechanism of toxicity. However, the cytotoxicity of eugenol-related compounds was not related to *BDE* or to *IP*, as shown by the poor QSAR in terms of *n* value. The cytotoxicity of methoxyphenols may not only be dependent on the formation of phenoxyl radicals, as estimated by *BDE* or *IP*, but also on the radical-trapping rate, k_{inh}. k_{inh} values depend on two factors, *n* and Rp_{inh}, as shown by Eq. (4).

Dependence on chemical hardness, η

The cytotoxicity of simple monophenols towards leukemia cells was correlated with their HOMO-LUMO gap parameter, suggesting a possible link between cytotoxicity and radical metabolites as parameterized by the HOMO-LUMO gap.[61] Further, QSARs for the toxicity of various aromatic compounds such as phenol and benzene derivatives have previously been developed using a two-parameter approach (LUMO and log *P*).[60] This model provided a mechanistic basis for toxicity in terms of partitioning and bioreactivity.

The cytotoxicity data in Table 3 led to the formulation of QSARs (3) and (4), with two isoeugenol dimers, dehydroisoeugenol **4** and alpha-diisoeugenol **5**, as outliers:

$$\log 1/C_{HSG} = 8.45\ (\pm 0.35) - 1.81\ (\pm 0.33)\eta, \qquad \text{QSAR (3)}$$

$n = 8$, $r^2 = 0.831$, $p < 0.01$, outlier: **4**, **5**,

$$\log 1/C_{HGF} = 9.19\ (\pm 0.40) - 2.23\ (\pm 0.36)\eta, \qquad \text{QSAR (4)}$$

$n = 8$, $r^2 = 0.867$, $p < 0.001$, outlier: **4**, **5**.

The cytotoxicity of the 2-methoxyphenols may overall be related to η-controlled enzymes. The acceptable QSAR of estrogen-like bisphenol A analogs for breast cancer MCF-7 cells was reported previously using the quantum chemical descriptors η and χ.[59] In the present study, the η-term was a much better parameter than the χ-term for evaluating the cytotoxicity of eugenol-related compounds. However, further QSAR studies will be necessary to explain their biological activities on the basis of theoretical parameters.

The dependence of the cytotoxicity of eugenol-related compounds on k_{inh} or the η-term indicates that the cytotoxicity of these compounds might be mediated by radical reactions.

ANIMAL STUDIES AND CLINICAL TRIALS

Cloves are the unexpanded flower bud of the tree. The oil from cloves is rich in essential oils, particularly eugenol **1** and isoeugenol **2**. **1** is both a primary irritant and a sensitizer, and is also found in cinnamon oil. The comparative histopathological effects of **1** and its dimer **3** have been evaluated on oral mucous membranes in mice.[23] **1** caused severe irritation, including hyperkeratosis, parakeratosis, cellular edema and patchy chronic inflammation, indicating a high mitotic activity. Comparatively, **3** caused mild hyperkeratosis and parakeratosis, but the shape and arrangement of basal layer cells were normal. Compound **3** was considerably less toxic than **1** at the tissue level. To assess the usefulness of **3** in dentistry, the physical properties of new zinc oxide eugenol cement (ZOE) together with **3** were investigated.[22] The addition of **3** to the cement did not decrease the physical properties of ZOE. This new cement containing both **1** and **3** may be clinically useful because **3** possesses an NSAID-like property,[25] but clinical trials have not yet been carried out.

For years, compound **1** has been used as the main component of ZOE cements in dental practice to relieve pain arising from a variety of sources, including pulpitis and dental hypersensitivity. The antinociceptive potential of eugenol **1** has been demonstrated in various pain models (formalin and Eddy's hot plate) in mice.[64] Although **1** shows allergenic properties, these findings support the traditional application of eugenol as a dental analgesic. ZOE cements with calcium hydroxide are used as pulp capping

cements in dental practice. **1** produces eugenol radicals under alkaline conditions, as judged by ESR spectroscopy.[3] Eugenol radicals (Fig. 2) formed under alkaline conditions arising from the reaction of calcium hydroxide with water may preferentially attack bacteria on the cavity wall and may enhance the antibacterial activity of eugenol itself.[24] Brauer[65] found many alternatives to eugenol, including 2-ethoxy benzoic acid (EBA), methoxyphenols, *n*-hexyl-vanillate, and beta-diketones. The most significant of these compounds is 2-ethoxy benzoic acid (EBA), which, in combination with **1**, is an effective cement (i.e. EBA cement). We previously used ESR spectroscopy to show that EBA is able to scavenge eugenol radicals.[24] EBA significantly but not completely scavenged free radicals, ROS and eugenol radicals, and therefore the combination of EBA and **1** may be beneficial by improving the biocompatibility of EBA cements. EBA cements may prevent stomatitis and allergic eczematous eruptions in patients treated with ZOE cements. However, further studies at the clinical level should be performed to clarify its mechanism.

Eugenol radicals can be scavenged by cysteine, N-acetyl cysteine (NAC), sodium ascorbate, beta-carotene and retinol, but cannot be scavenged by lycopene, gallic acid, alpha-tocopherol and trolox.[3] Indians and Southeast Asians use cloves, containing **1** and related compounds, to flavor the betel nut. The causal relationship between the use of betel nut and oral cancer[66] may be due to the pro-oxidant activity of eugenols. Since ascorbic acid and beta-carotene are contained in various fruits and vegetables, daily intake of these materials might help to reduce the toxic effects of eugenol and other dental materials. We previously found that a sample of **1** that had been stored for one year in a refrigerator at 4°C had a darkened yellowish color and produced a higher radical intensity than did freshly prepared eugenol. Also, **1** and **2** are highly light-sensitive even under visible light. These observations suggest the occurrence of eugenol oxidation during storage and consequently the formation of eugenol degradation products, including polymers. It is reasonable to recommend that dental materials should use fresh eugenol stored under light shielding conditions, which will produce a lower number of radicals.

The cytotoxicity of **2** towards HSG and HGF cells was 10-fold greater than that of **1**. In contrast, the cytotoxicity of isoeugenyl acetate **2.5** was similar to that of eugenol (Table 2). Isoeugenyl acetate is present in perfumes

and aftershaves, in some products in significant amounts, and may be used in place of **2**. Isoeugenyl acetate is probably metabolized in the skin to **2** and gives positive patch test reactions in one-third of isoeugenol-sensitized individuals. Although this compound has been proposed as an alternative to **2**, there is a high degree of concomitant reactivity with isoeugenol.[67]

A reduction of sensitization potency achieved by dimerization may lead to development of safer cosmetic ingredients. Isoeugenol dimers such as **4** are not currently used for fragrance chemicals. However, the dimerization of isoeugenol may yield a promising candidate as a cosmetic ingredient with low sensitization risk.[68]

Effects of **1** on the central nervous system were previously reported, suggesting its possible application to the treatment of Alzheimer's disease, depression, and Parkinson's disease.[69] **1** has an antidepressant-like activity comparable to that of imipramine, as judged by the forced swim test and tail suspension test in mice. These compounds have been tested for their ability to inhibit proliferation of melanoma cells, and **1**, but not **2**, was found to be a potent inhibitor of melanoma cell proliferation *in vitro* and *in vivo* (in mice).[70] Well-controlled clinical studies of eugenol in patients with Alzheimer's disease, depression, Parkinson's disease or melanoma have not yet been performed.

Vanillin **8**, BHA-dimer **13** and dehydrodiisoeugenol **4**, as well as curcumin **11**, have been shown to possess potent anti-inflammatory activity in our studies. These compounds may be applicable for chemoprevention of oral diseases such as periodontitis, lichen planus and leukoplakia, a precancer. Further experimental and clinical studies are required in this area.

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6

Coriander

Sanjeev Shukla and Sanjay Gupta

Coriander is one of the most ancient culinary herbs known to mankind. It is a member of the *Apiaceae* family and grows wild in southeastern Europe, India and China. The entire coriander plant, including the leaves, stems, seeds and roots, is used in producing a popular spice with a pleasing lemony flavor. The dried seeds of coriander contain many phenolic compounds, mainly flavonoids, coumarins and phenol carboxylic acids, which are thought to contribute to its medicinal properties. Coriander preparations are traditionally ingested or applied externally for a wide range of human ailments, including digestive and gastric complaints, coughs, chest pains, bladder complaints, leprosy rash, fever, dysentery, headaches, oral and pharyngeal disorders, and post-partum complications. It has been shown to have hypoglycemic, hypolipidemic, antihypertensive, antimicrobial, antihelminthic and antimutagenic effects, and also has been shown to relieve symptoms of rheumatism and painful joints. In this chapter, we describe the traditional and therapeutic uses of coriander, evaluating its curative, preventative and health-promoting properties, and discussing the clinical trials that have been conducted to evaluate its efficacy as a medicinal agent.

INTRODUCTION

Coriander sativum is an annual herb that belongs to the family *Apiaceae*. The name "coriander" in a culinary context may refer to either the seed of the plant (used as a spice) or to its leaves (used as an herb). In North America, the name "cilantro" is given to the leaves, and "dhania" in the Indian subcontinent. Other common names for coriander are Mexican parsley, Arab parsley, Chinese parsley and Yuen sai, used in Middle East, Mediterranean, and Latin American and African continents.

HISTORY OF CORIANDER

Coriander is probably one of the first herbs to be used by mankind, perhaps dating back to 5000 BC.[1] It is also mentioned in early Sanskrit writing from about 1500 BC. Fifteen desiccated mericarps were found in a pre-pottery Neolithic blend in the Nahal Hernel cave in Israel, which may be the oldest archeological finds of coriander.[2] Mericarps of coriander have been recovered from an ancient Egyptian tomb, providing evidence of its cultivation by the ancient Egyptians.[3] The Bible mentions coriander in Exodus 16:31, and the Romans spread it throughout Europe. Coriander seems to have been cultivated in Greece as far back as the second millennium BC. It is believed that coriander is one of the earliest herbs planted in North America — in 1670 in Massachusetts — and it soon appeared in Latin America, where the leaves rather than the seeds have become popular.

PLANT DESCRIPTION

Coriander is native to southwestern Asia and west to North Africa. It is a soft, hairless plant which grows to 50 cm (20 inches) tall. The leaves are variable in shape, broadly lobed at the base of the plant, and slender and feathery higher on the flowering stem. The flowers are arranged in small umbels, white or very pale pink, asymmetrical and with petals that point away from the center of the umbel being longer (5–6 mm) than those pointing to the middle of the umbel (only 1–3 mm long); the fruit is a globular schizocarp 3–5 mm diameter. The dry fruit are known as

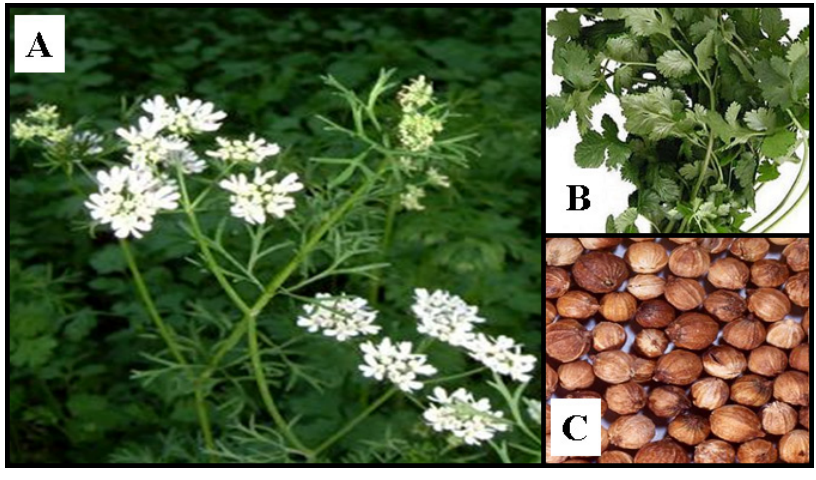


Fig. 1. Coriander blossoms comprising (a) the whole plant, (b) the leaves, and (c) the fruits used for culinary and medicinal purposes.

coriander seeds. The seeds have a lemony citrus flavor, often described as warm, nutty and spicy (Fig. 1).

BIOACTIVE CONSTITUENTS OF CORIANDER

The most important constituents of coriander are essential oils and fatty oils.[4,5] In addition, several water-extracted compounds have been isolated and identified from coriander.[6] The chemical composition of the coriander fruit is shown in Table 1. The content of fatty oil varies from 9.9% to 27.7%; the major fatty oil constituents are petroselinic acid (68.8%), linoleic acid (16.6%), oleic acid (7.55%) and palmitic acid (3.8%). Minor fatty oil constituents include stearic acid, vaccenic acid and myristic acid (<2%). The essential oil content of the ripe and dried fruit of coriander varies between 0.03 and 2.6%.[4] The principal essential oil components of the ripe fruit of coriander are linalool, a naturally-occurring terpene alcohol that comprises 68% of this category, and lesser components of α-pinene, γ-terpinene, camphor, geranyl acetate, and geraniol. Minor essential oil constituents are camphene, β-pinene, sabinene, myrcene, limonene, p-cymene, and terpinolene. The percentages of these essential oils are shown in Table 2.

Table 1. Components present in coriander fruits.

Component	Percentage
Crude fiber	28.43
Fat	19.15
Crude protein	11.49
Water	11.37
Starch	10.53
Pentosans	10.29
Mineral constituents	4.98
Sugar	1.92
Essential oils	0.84

Table 2. Essential oil content in the ripe fruits of coriander.

Major Components	Percentage	Minor Components (>2%)
Linalool	67.7	β-pinene
α-pinene	10.5	Camphene
γ-terpinene	9	Myrcene
Geranyl acetate	4	Limonene
Camphor	3	p-cymol
Geraniol	1.9	Dipentene
		α-terpinene
		n-decyl aldehyde
		Borneol
		Acetic acid esters

Analysis by gas chromatography mass spectrometry has led to the identification of 26 major essential oils in coriander.[7] The chemical constituents and essential oil composition of coriander is greatly influenced by the age of the plant, genetic and environmental factors, the analytical methods used, and the extraction processes employed.[6] The water soluble portion of the methanolic extract of coriander has been found to contain 33 constituents, including monoterpenoids, monoterpenoid glucosides, sulfates, and glycosidases.[5,7] HPLC analysis has shown the presence of 21 phenolic compounds, mainly flavonoids, coumarins and phenol carboxylic acids.[8]

BIOLOGICAL PROPERTIES OF CORIANDER

Constituents of coriander have been demonstrated to possess diverse biological properties.[9] Phenol carboxylic acid acts as a vasorelaxant and antioxidant, and there are reports that caffeic acid and ferulic acid inhibit lipid peroxidation induced by superoxide anions. Coumarins have been shown to possess anti-cancer and antimicrobial properties. Coriander grass contains several flavonoids, primarily apigenin, luteolin, rutin, hesperidin, epicatechin and epigallocatechin, which have been used in various therapeutic settings including treatments for leukemia and treatment of patients with HIV infection. The structure, contents, and biological properties of these bioactive agents are shown in Table 3.

CULINARY USES OF CORIANDER

The most common use of coriander seeds in India is in curry powders, often in a rough ground form that provides a crunchy texture. The seeds can also be used in stews and soups. Coriander blended into smoked meat is commonly used in traditional English black pudding recipes and Italian mortadella sausage. Coriander is an ingredient of Indian "garam masala," pickling spices and pudding spices and is used in cakes, breads and other baked foods. Sugared comfits made from the seeds are a traditional sweetmeat and breath freshener. Coriander spice is used extensively in Arab cookery and is commonly found in lamb, kid and meat stuffings. *Taklia* is a well-known Arab spice mixture of coriander and crushed fried garlic. Coriander with cumin is a common combination typically found in *falafel* and in the Egyptian appetizer *dukka*, which consists of spices plus sesame seeds, hazelnuts, salt and pepper. Coriander compliments ham and pork dishes very well, especially when orange is included. It is also a very nice addition to fish dishes, and a combination of coriander with other spices makes a delicious coating for spiced fish or chicken, rubbed into the scored flesh and grilled. Coriander combined with chili results in *harissa*, a hot North African red pepper sauce, which may be added to cream or cottage cheese. Coriander leaves are always used fresh, in Spanish, Middle Eastern, Indian, Oriental and South American cookery. They are sprinkled like parsley on cooked dishes or minced or pureed into sauces,

Table 3. Structure, content and biological properties of polyphenolic compounds present in coriander grass.

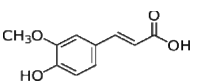
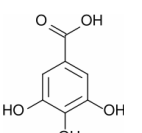
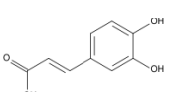
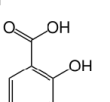
Identified Compounds	Content (%)	Molecular Formula	Structure	Biological Properties
Phenolcarboxylic acids				
Ferulic acid	4.28	$C_{10}H_{10}O_4$		Antioxidant, anti-tumor
Gallic acid	2.04	$C_7H_6O_5$		Antioxidant, anti-inflammatory
Caffeic acid	1.65	$C_9H_8O_4$		Antioxidant, anti-tumor, antimicrobial
Salicylic acid	2.19	$C_9H_8O_4$		Antibiotic

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Table 3. (*Continued*)

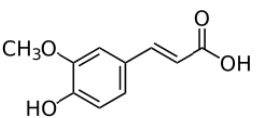
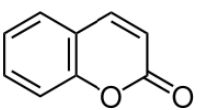
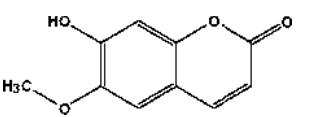
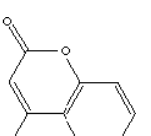
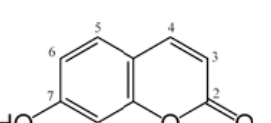
Identified Compounds	Content (%)	Molecular Formula	Structure	Biological Properties
Coumarins				
Esculin	1.94	$C_{15}H_{16}O_9$		Antimicrobial
Esculetin	1.96	$C_9H_6O_2$		Photo-mutagenic
Scopoletin	1.79	$C_{10}H_8O_4$		Anti-inflammatory, antihypertensive
4-hydroxycoumarin	2.03	$C_9H_6O_3$		Anti-tumor, photo-mutagenic
Umbelliferone	1.4	$C_9H_6O_3$		Photo-mutagenic

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Table 3. (*Continued*)

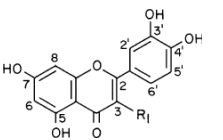
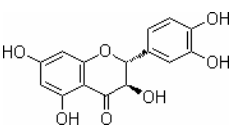
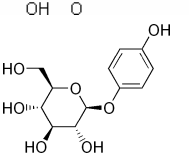
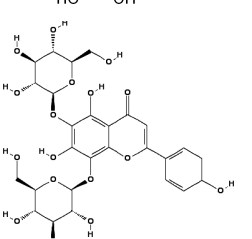
Identified Compounds	Content (%)	Molecular Formula	Structure	Biological Properties
Flavonoids				
Luteolin	6.93	$C_{15}H_{10}O_6$		Antioxidant, anti-mutagenic, anti-inflammatory, immunomodulator
Dihydroquercetin	3.9	$C_{15}H_{12}O_7$		Antioxidant, anti-mutagenic, anti-inflammatory
Arbutin	3.61	$C_{12}H_{16}O_7$		Anti-inflammatory, Antibacterial
Vicenin	3.54	$C_{27}H_{30}O_{17}$		Antioxidant

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Table 3. (*Continued*)

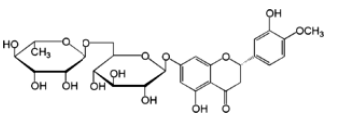
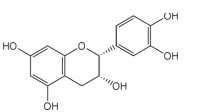
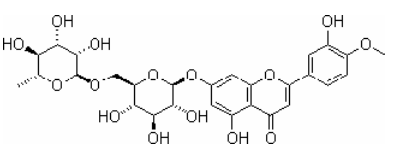
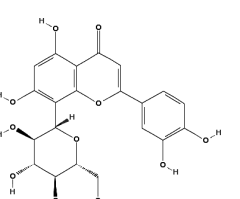
Identified Compounds	Content (%)	Molecular Formula	Structure	Biological Properties
Hesperidin	2.83	$C_{28}H_{34}O_{15}$		Antioxidant, anti-inflammatory, anti-hypertensive
Epicatechin	2.62	$C_{15}H_{11}O_6$		Antioxidant, anti-mutagenic, anti-inflammatory
Diosmin	1.84	$C_{28}H_{32}O_{15}$		Antioxidant, anti-mutagenic, anti-inflammatory
Orientine	1.67	$C_{21}H_{20}O_{11}$		Antioxidant, anti-proliferative

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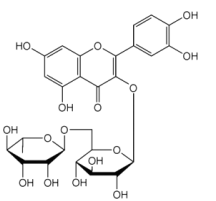
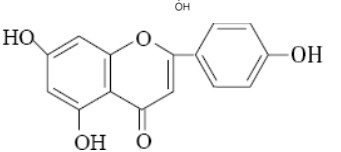
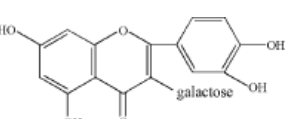
Table 3. (*Continued*)

Identified Compounds	Content (%)	Molecular Formula	Structure	Biological Properties
Rutin	1.38	$C_{27}H_{30}O_{16}$		Antioxidant, anti-tumor
Apigenin	1.02	$C_{15}H_{10}O_5$		Antioxidant, anti-inflammatory, anti-mutagenic
Hyperoside	0.56	$C_{21}H_{20}O_{12}$		Antioxidant, anti-microbial

soups and curries, especially roasted. Seeds and leaves can both be used in salads. Thai people use the coriander plant roots to flavor meat and curries. Coriander is also used in perfumes, liqueurs and gin. The culinary uses of coriander are detailed in a monograph on coriander by Diederichsen.[10]

TRADITIONAL HEALTHCARE USES OF CORIANDER

Coriander leaves are thought to have antispasmodic properties and are used for dyspeptic complaints, loss of appetite, and upper abdominal discomforts.[11] Coriander leaf preparations have also been ingested or applied externally for the treatment of coughs, chest pains, bladder complaints, leprosy rash, fever, dysentery, headaches, oral and pharyngeal disorders, halitosis, post-partum complications, and arthritic complaints. Coriander mixed with poppy seed has been used to treat vertigo. Coriander has also been reputed to have aphrodisiac effects.[11,12]

Galenical preparations of coriander seed for similar applications have been incorporated into traditional Chinese, Indian and Greco-European medicines.[12–15] In Ayurvedic medicine, coriander seeds are usually combined with caraway and cardamom seeds, among others, while in European cultures it is usually combined with caraway, fennel and anise to treat digestive ailments.[12,13] Chinese herbalists use coriander seeds to treat indigestion, anorexia, and stomach ache, and recommend the use of coriander to treat influenza in which there is no sweating. Chinese folk medicine uses coriander leaves and seeds to alleviate bad breath and to help remove unpleasant odors from the genital area. Ground coriander seeds may be used to treat skin and mouth ulcers. Before the invention of toothpaste, coriander seeds were chewed as a breath sweetener. In Germany, coriander is used as a medicinal tea and a component of carminative and laxative remedies, in alcoholic distillate and drops dosage forms, often combined with anise, caraway, or fennel.[15] In the United States, coriander is used as a carminative or digestive component of compounds in confection, infusion, syrup, and tincture dosage forms. It is also used in laxative compound preparations to counteract or modify harsh stomach upsets.[16] The German Commission E has approved the ingestion of coriander seeds for treatment of dyspeptic complaints,

loss of appetite, and various upper abdominal discomforts, such as a feeling of distension, flatulence, and mild cramp-like gastrointestinal upsets. In France, the Annex II has approved the use of coriander seed for similar indications.[11,17]

Coriander seeds contain essential oils which are thought to have many medicinal properties, such as antibacterial properties. The essential oil of coriander stimulates the secretion of gastric juices and is a carminative and spasmolytic.[11–13] It is often combined with purgatives to alleviate spasms that may accompany their use. In Asia, the essential oil is used in pastes as a treatment for mouth ulceration and as a poultice for other ulcers.[14,15] German pharmacopeial grade coriander seed must contain not less than 0.6% (v/m) volatile oil. The Austrian Pharmacopoeia requirement is not less that 0.5% oil. It is believed that the stomachic, spasmolytic and carminative effects of coriander are due to its essential oil content.

BIOLOGICAL EFFECTS OF CORIANDER BASED ON SCIENTIFIC EVIDENCE

Preclinical Studies

Antioxidants properties: The antioxidant potential of coriander fruit aqueous extract has been investigated in comparison with known antioxidant ascorbic acid in an *in vitro* study. The findings suggested that 370 μg of aqueous extract coriander was equivalent to 260 μg ascorbic acid in scavenging 50% of the superoxide radicals in the *in vitro* system. The amount needed to inhibit 50% of lipid peroxide was 4,500 μg of coriander, which was a smaller and safer amount as compared to 5,000 μg ascorbic acid. Furthermore, 1,250 μg coriander was required to inhibit 50% of hydroxyl radicals compared to 4,500 μg ascorbic acid. The daily use of coriander fruits in various forms is very common in India and may be a beneficial food additive with strong antioxidant potential.[18] Another study has observed the superoxide scavenging effects of spices and further revealed that the linalool content of coriander inhibits nitroblue tetrazolium (NBT) reduction maximum by 28%, which has a role in the xanthine-xanthine oxidase system. In contrast, piperine (black pepper) and turmeric extracts

(aqueous and acid) fail to scavenge superoxide anions. It has been reported that linalool (coriander), piperine (black pepper) and cuminaldehyde (cumin) at concentrations of 600 μM has marginal inhibitory effects on lipid peroxidation in rat liver microsomes.[19]

Antiproliferative properties: Nakano *et al*.[20] screened the methanolic extracts of *Umbelliferae* plants for polyacetylenic compounds using ELISA for panaxytriol, and its antiproliferative activity was determined by MTT assay using the tumor cell lines MK-1, HeLa and B16F10. The presence of antiproliferative polyacetylenes activity was observed in the fruit of *Coriandrum sativum* as well as in other fruits of the *Umbelliferae* family.

Photobiologic properties: Ashwood-Smith *et al*.[21] isolated furoisocoumarin (coriandrin), a naturally occurring coumarin from coriander, and evaluated its photo-biological properties. Photosensitized lethal and mutagenic effects of coriandrin on bacteria indicated that it was more active than psoralen. *In vitro* studies in mammalian cells suggested that coriandrin photosensitized more actively than psoralen, even though preliminary evidence from interrupted radiation experiments and DNA analysis suggested that coriandrin does not form DNA interstrand crosslinks. Coriandrin appears to be metabolized more rapidly by hepatic mixed function oxidases than furocoumarins.

Antimicrobial properties: Gill *et al.*[22] demonstrated that the essential oils extracted from the coriander plant have significant effects on the growth of a Gram-positive bacterium *Listeria monocytogenes* on vacuum-packed ham. Coriander oil treatment, present as a 6% gel, inhibited growth of *L. monocytogenes* on the ham samples. Samples receiving this treatment had populations of *L. monocytogenes* 1.3 log CFU/ml lower than controls after week 1 of storage; however, no difference between treatments was observed from 2 wks onward. In another study, Cortés-Eslava *et al*.[23] studied the effects of aqueous crude coriander juice against several aromatic amines, specifically 4-nitro-o-phenylenediamine, m-phenylenediamine and 2-aminofluorene, all of which are metabolically activated into mutagenic compounds by both animal and plant systems. The mutagenic

activities of these aromatic amines were investigated using the Ames reversion mutagenicity assay (his- to his+) with the *S. typhimurium* TA98 strain as an indicator organism. Aqueous crude coriander juice significantly decreased the mutagenicity of metabolized aromatic amines in the following order: 2-aminofluorene (92.43%) > m-phenylenediamine (87.14%) > 4-nitro-o-phenylenediamine (83.21%). The concentration of coriander juice (50–1000 μL/coincubation flask) was neither toxic nor mutagenic. In recent studies, Reuter *et al.*[24] demonstrated the anti-inflammatory effects of coriander oil on skin when used to counteract the effects of ultraviolet light. The observations suggested that skin tolerates the application of coriander oil very well. Lotion containing coriander oil displayed a mild anti-inflammatory effect in erythema tests and the investigators concluded that it could be useful in the concomitant treatment of inflammatory skin diseases.

Digestive properties: Ramkrishna Rao *et al.*[25] demonstrated the beneficial use of spices on the digestive system. In this study using a rat model, 14 spices at different concentrations (curcumin, capsaicin, piperine, garlic, onion, ginger, mint, coriander, cumin, ajowan, fennel, fenugreek, mustard, and asafetida) were used to assess the activities of digestive enzymes produced by the rat pancreas and small intestine. All spices induced enhanced activity of pancreatic lipase and amylase. From these findings it was inferred that these spices have stimulatory effects on the production and activity of these digestive enzymes.

Hypoglycemic properties: Gray and Flatt[26] reported that derivatives of the coriander plant have hypoglycemic effects. When incorporated into the diet (62.5 g/kg) and drinking water (2.5 g/L, prepared by 15 mins decoction) of streptozotocin-diabetic mice, coriander reduced their hyperglycemia. In isolated murine abdominal muscle, coriander (1 mg/mL) aqueous extract increased 2-deoxyglucose transport by 1.6-fold, glucose oxidation by 1.4-fold, and incorporation of glucose into glycogen by 1.7-fold, an effect comparable to that of 10^{-8} M insulin. In addition, a 0.25–10 mg/mL aqueous extract of coriander induced a stepwise 1.3–5.7-fold stimulation of insulin secretion from a clonal B-cell line.

Metal chelation properties: Aga *et al*.[27] demonstrated the metal chelating effect of coriander on lead deposition in male ICR mice administered lead acetate trihydrate (1000 ppm) in their drinking water for 32 consecutive days. Coriander was administered to the mice by gastric intubation after seven days from the start of lead exposure for a total of 25 days. Animals were then sacrificed for comparison of lead distribution. The highest concentrations of lead were found in the femur. Administration of coriander significantly decreased lead deposition in the femur and reduced the severity of lead-induced injury in the kidneys. Meso-2,3-dimercaptosuccinic acid (DMSA), a control, significantly reduced the lead deposition. Urinary excretion of delta-aminolevulinic acid (ALA), which is known to increase with lead intake, was significantly decreased after coriander administration. Further *in vitro* studies with methanolic extract of coriander also demonstrated reductions in lead-induced inhibition of delta-aminolevulinic acid dehydratase (ALAD) activity. This study suggests that coriander, by acting as a chelating agent, can reduce lead deposition.

Hypolipidemic properties: Weber *et al*.[28] studied the effects on rats of consumption of coriander oil as compared with consumption of oleic acid, and found that rats ingesting coriander oil had significantly greater liver weights, but no significant differences in body weight, the weights of heart, liver, kidneys, spleen or testes, lipid content of heart, or total cholesterol, HDL cholesterol and triacylglycerol concentrations of blood plasma were observed when compared with groups fed various levels of oleic acid. cis-Vaccenic acid, a naturally occurring isomer of oleic acid, is a minor constituent oil of plant origin, whereas seeds of Parsley (*Petroselinum rubrum*), fennel (*Foeniculum vulgare*) and coriander (*Coriandrum sativum*) contain high levels of petroselinic acid as part of triacylglycerols. Dietary petroselinic acid, which is stored extensively in adipose tissues, has been found to decrease the level of arachidonic acid in the liver and heart, and is potentially beneficial in specific nutritional states to counteract the detrimental vasoconstrictive effects of overproduction of arachidonic acid and increased biosynthesis of eicosanoids such as thromboxane A2.[29] These studies have confirmed that ingestion of coriander oil containing high proportions of a positional

isomer of oleic acid, i.e. petroselinic (cis-6-octadecenoic) acid, significantly reduced the proportions of arachidonic (all cis-5,8,11,14-eicosatetraenoic) acid in cellular lipids.

Anxiolytic properties: Emamghoreishi *et al.*[30] reported that coriander has traditionally been recommended for relief of anxiety and insomnia in Iranian folk medicine. The pharmacological efficacy of coriander aqueous extract as an anxiolytic agent were evaluated in mice. Aqueous extracts administered at 50, 100 and 500 mg/kg significantly reduced spontaneous activity and neuromuscular coordination, compared to a control group. The results of the study suggested that an aqueous extract of coriander seed has an anxiolytic effect and may have potential sedative and muscle relaxant effects.

Antiparasitic properties: Harve and Kamath[31] observed the larvicidal activity against *A. aegypti* larvas of acetone and petroleum ether extracts from four plants: *Murraya koenigii, Ferula asafoetida, Trigonella foenum graceum* and *Coriandrum sativum*. They reported some synergistic larvicidal activity when the extracts were combined with synthetic agents such as *temephos* and *fenthion*, but poor activity was observed when the extracts were tested individually.

In another study by Eguale *et al.*,[32] the anti-helmintic activities of coriander seed crude aqueous and hydro-alcoholic extracts were investigated on the egg and the adult nematode parasite *Haemonchus contortus*. Both coriander extracts inhibited hatching of eggs completely in sheep infected with *Haemonchus contortus*, at a concentration of less than 0.5 mg/mL. The coriander aqueous extract ED_{50} was 0.12 mg/ml while the hydro-alcoholic extract was 0.18 mg/mL. No statistically significant differences were noted between aqueous and hydro-alcoholic extracts ($p > 0.05$). The hydro-alcoholic extract showed better *in vitro* activity against adult parasites than the aqueous extract. Kim *et al.*[33] tested essential oils from 28 plant species for their nematicidal activities against the *Bursaphelenchus xylophilus* (a pinewood nematode). Essential oils of coriander (*Corundum sativum*), Oriental sweetgum (*Liquidambar orientalis*), and valerian (*Valerian wallichii*) showed good nematicidal activity against *B. xylophilus*. Gas chromatography mass spectrometry analysis of

these plants led to the identification of 26 compounds from coriander, 11 from Oriental sweetgum, and four from valerian oils. Compounds which showed strong nematicidal activity were benzaldehyde, trans-cinnamyl alcohol, cis-asarone, octanal, nonanal, decanal, trans-2-decenal, undecanal, dodecanal, decanol, and trans-2-decen-1-ol.

Clinical Studies

Very few clinical studies have been conducted on coriander. The approved modern therapeutic applications for coriander seed are supportable based on its long history of use in well-established systems of traditional medicine, pharmacological studies in animals, nutrient composition and dietary value studies, and phytochemical investigations. Bub *et al.*[34] conducted a double-blind, placebo-controlled study on 86 nursing home residents with chronic constipation, who received an herbal dietary supplement *Smooth Move* containing *Glycyrrhiza glabra* and *Coriander sativum* fruit along with other species for a period of 28 days. Compared to placebo, a statistically significant increase in the number of bowel movements was observed in 42 subjects receiving *Smooth Move* with an average of 4.14 more bowel movements compared to the 44 placebo subjects. These results demonstrate the use of coriander fruit in chronic constipation.

In another clinical study by Otoom *et al.*[35] 310 diabetic Jordanian patients were interviewed, and it was learned that 96 of them had used herbal products on a daily basis, nearly half of them using these remedies on the recommendation of a friend. The most commonly used herbal products were *Trigonella foenum graecum* (22.9%), *Lupinus albus* (14.6%), *Allium sativum* (11.5%), *Allium cepa* (5.2%), *Nigella sativa* (7.3%), *Zea mays* L. (6.3%), *Urtica dioica* L. (8.3%), *Eucalyptus globules* LA (9.4%), *Olea europea* L. (3.1%), *Cumminum cyminum* (9.4%), *Coriandrum sativum* (10.4%), *Salvia officinalis* L. (3.1%), and *Tilia cordata* (1%). About one third of the patients reported side effects, including headache, nausea, dizziness, itching, palpitations, and sweating. The great majority of patients using these herbal products (86.5%) were satisfied with their diabetes control.

Vejdani *et al.*[36] conducted a study of 32 patients with irritable bowel syndrome, providing some with Carmint, an extract of *Melissa officinalis*,

Mentha spicata, and *Coriandrum sativum*, which reputedly has antispasmodic, carminative, and sedative effects, and providing a control group with a placebo. Patients were also administered loperamide or psyllium, based on their predominant bowel function, for 8 wks. It was found that the severity and frequency of abdominal pain/discomfort was significantly less in the Carmint group than in the placebo group at the end of the treatment ($P = 0.016$ and $P = 0.001$, respectively), as were the severity and frequency of bloating ($P = 0.02$ and $P = 0.002$, respectively). This pilot study suggested that Carmint plus loperamide or Carmint plus psyllium (depending on the irritable bowel syndrome subtype) might be effective in patients with irritable bowel syndrome.

ADVERSE EFFECTS AND SAFETY ISSUES

Several studies of the adverse effects of coriander and other spices have been reported. Ebo *et al.*[37] observed anaphylaxis effects in one patient. The patient developed urticaria, angio-edema, rhinoconjuctivitis and bronchospasm during handling of coriander and fenugreek. Specific IgE, skin tests and basophil activation tests showed positive results in the patients, whereas control patients were negative. The clinical manifestations in temporal relationship to ingestion of coriander and handling of coriander and/or fenugreek, the positive specific IgE results, skin tests and basophil activation assays all support the diagnosis of allergy to both spices. Another study was conducted by Stäger *et al.*[38] on 70 patients with positive skin tests to birch and/or mugwort pollens and celery. Skin scratch tests to test for allergy to aniseed, fennel, coriander, and cumin showed that 24 patients were allergic. Forty-one patients with positive RAST (radioallergosorbent test) were observed by specific serum IgE to these spices from the *Apiaceae* family. van Toorenenbergen *et al*.[39] demonstrated the IgE response to coriander, dill and anise spices through a major IgE-binding component from coriander which had an isoelectric point of pH5. After incubation of SDS-PAGE-separated spice extracts with serum from a patient with an occupational allergy to spices, a closely related pattern of IgE binding to coriander, dill and anise extract was observed. These results suggest that the botanically related spices, coriander, anise and dill contain common IgE-binding structures. A case study

by García-Gómez *et al*.[40] reported that after three years of occupational exposure to powdered coriander, a woman developed respiratory symptoms of immediate hypersensitivity type. Skin tests, nasal and bronchial challenge tests and the RAST were positive to coriander. Column chromatography, enzymatic digestion of the fractions and skin testing suggested that the allergen was a protein. Another occupational study on 45 female spice factory workers suggested that immunologic reactions to spices are frequent and may be related to acute symptoms and lung function changes but not to chronic changes.[41]

CONCLUSIONS

The use of coriander as a medicinal agent over many generations has suggested that it may have a number of effects that are beneficial to humans. Studies in various animal models, cell cultures and clinical trials have provided support for its usefulness as an antioxidant, anti-inflammatory, antimutagenic, anti-hypertensive, hypolipidemic, diuretic, antimicrobial, antiparasitic, antihelminthic and photobiologic protector. Essential oils extracted from coriander are reputed to improve memory and to provide analgesic, antispasmodic, carminative and anti-stomachache properties. Flavonoids in the coriander plant may be valuable in combating a number of pathologic conditions. There is considerable interest in diet-derived natural compounds that can effectively treat various human ailments, and in this regard coriander may be an ideal plant. However, establishing whether or not the therapeutic effects of coriander are beneficial to patients will require research and the generation of scientific evidence. Without such evidence, it will remain unclear whether these untested and unproven medical treatments are truly beneficial. Proper use of coriander preparations may safely provide some therapeutic benefits; however, indiscriminate or improper use of coriander and its derivatives may be ill-advised.

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Fenugreek (Diosgenin)

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Fenugreek is a spice derived from the dry seeds of *Trigonella foenum-graecum* Linn., a member of the legume family Fabaceae. There is ample evidence from traditional practices of medicine such as apothecary, Ayurveda, Chinese and Native American about the potential of fenugreek to prevent and control chronic diseases such as diabetes, high cholesterol, inflammation and gastrointestinal ailments. However, scientific research over the past three decades has expanded the scope of fenugreek and its major bioactive constituents as promising candidates for pre-clinical and clinical studies for its development as evidence-based phytomedicine. Beneficial effects of fenugreek can be attributed to its bioactive molecules, which include saponins, alkaloids, flavonoids, mucilaginous fiber, lysine-rich proteins, and volatile oils. Diosgenin, a steroid saponin found in fenugreek seeds, is the most bioactive component. Fenugreek and diosgenin have mostly been studied for their properties to prevent and/or control metabolic diseases such as type-1 and -2 diabetes, dyslipidemia and obesity, and in some cases have led to clinical investigations with promising results. Recently, there has been a surge to evaluate the cancer modulating properties of fenugreek and diosgenin using various biomarkers and experimental models. The biological mode of action of fenugreek and diosgenin has been found to have several molecular targets, making them poly-target phytochemicals. Either fenugreek or

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diosgenin modulates multiple cell signaling events involving critical molecular candidates taking part in carbohydrate and lipid metabolism, growth, differentiation, apoptosis, inflammation and oncogenesis. Fenugreek and its bioactive components hold promise as functional foods and nutraceuticals and in the prevention of various chronic illnesses.

INTRODUCTION

Trigonella foenum-graecum, commonly called fenugreek (Latin: greek hay) and belonging to the family Fabaceae, is a semi-arid plant native to many Asian and East European countries.[1] The common use of fenugreek in cooking has been dated to 1500 BC as recorded in the Egyptian papyrus. The rhombic yellow to amber colored fenugreek seed (commonly called *methi* in Hindi), with a bitter taste and a strong characteristic pungency, is frequently used in the preparation of pickles, curry powders and pastes, and is often encountered in the cuisine of the Indian subcontinent. The young leaves and sprouts of fenugreek are eaten as greens, and the fresh or dried leaves are used to flavor other dishes as condiments. There is ample evidence from apothecary, Ayurveda, Chinese and Native American medical practices to support fenugreek seeds as a herbal medicine in the prevention and control of chronic diseases such as metabolic disorders, diabetes, high cholesterol, inflammation, and gastrointestinal ailments.[1,2] Based on popular folklore, fenugreek may also be used for breast enhancement and as a lactation stimulant, as well as to combat indigestion and baldness. In western countries, there has been a surge in the use of fenugreek. Firstly, fenugreek seeds (Fig. 1a) are being used as a complementary and alternative medicine for lowering blood glucose, cholesterol, and triglycerides and are available as several commercial tablets, pills, decoctions, and tonics. Secondly, fenugreek seeds are used as a flavoring agent to imitate the taste of maple. Over the past two decades, substantial scientific evidence has accumulated to validate that fenugreek and its major bioactive constituent, diosgenin (Fig. 1b), could control chronic diseases such as obesity, diabetes and cancer. This chapter describes experimental studies in animals and humans on the effect of fenugreek seeds and diosgenin in (a) metabolic diseases and (b) cancer, with more emphasis being placed on the latter with a detailed description

(a)

(b)

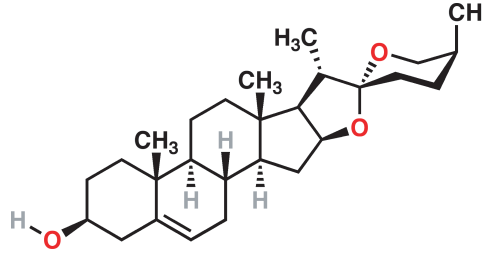

Fig. 1. (a) Dry seeds of fenugreek, and (b) structure of diosgenin, a spirostanol saponin [(25R)-spirost-5-en-3b-ol].

of the molecular modes of action. The effect of fenugreek in the prevention and control of metabolic diseases has been reviewed earlier.[3]

BIOACTIVE COMPONENTS

The chemical composition of fenugreek seeds includes but is not limited to mucilaginous fiber, lysine-rich proteins, saponins, alkaloids, flavonoids, and volatile oils. Two major constituents of fenugreek that have demonstrated potential in alleviating chronic diseases are (a) diosgenin [(25R)-spirost-5-en-3β-ol] and (b) 4-hydroxyisoleucine (4-OH-Ile).[4,5] Both of these compounds have an exclusively plant origin and are not synthesized in mammalian tissues. Diosgenin is used (~60%) for the commercial synthesis of cortisone, pregnenolone, progesterone, and other steroid products. Thus, there is a general claim that diosgenin-containing foods have an estrogenic effect in humans, but there is no scientific evidence

to validate this.[6,7] Furthermore, diosgenin is not metabolically converted into bioactive steroids in the mammalian body and, therefore, is considered safe. In a study that assessed the safety of diosgenin-containing yam, it was reported that the expected upper limit for the concentration of diosgenin was 3.5% (wt/wt). At this dose, diosgenin did not show systemic toxicity, genotoxicity, or estrogenic activity.[6] The seeds of fenugreek yielded as high as 0.28–0.92% diosgenin in certain cultivars.[4] The safety and toxicological effects of fenugreek seeds are discussed in detail by Basch *et al*.[3] Other bioactive fenugreek chemicals include: volatile oils such as 3-hydroxy-4,5-dimethyl-2-furanone, dihydrobenzofuran, dihydroactinidiolide, muurolene, elemene and selinene; alkaloids such as trigonelline, gentianine and carpaine; flavonoids including vitexin and its glycosides and esters, isovitexin, orientin, vicenins 1 and 2, quercetin and luteolin; and mucilage mostly in the form of galactomannan.

METABOLIC DISEASES

A summary of selected studies that demonstrate the beneficial effects of fenugreek against metabolic diseases is shown in Table 1. Fenugreek has been shown to lower serum cholesterol, triglyceride, and low-density lipoprotein in animal models of hypercholesterolemia.[8–10] Findings of several human intervention trials have demonstrated that dietary fenugreek lowered serum cholesterol and triglyceride levels.[11–14] Oral supplements of fenugreek have been shown to ameliorate most metabolic symptoms associated with type-1 and type-2 diabetes in both humans and relevant animal models.[3] Diosgenin, the active steroid saponin in fenugreek, has been shown to elicit hypocholesterolemic and hypotriacylglycerolemic activity.[15,16] In a recent study, Son *et al.* demonstrated that diosgenin controlled hypercholesterolemia in rats fed a high-cholesterol supplemented diet by improving the lipid profile as well as by modulating oxidative stress.[17]

The effect of fenugreek seeds in lowering blood glucose level has been demonstrated by various groups in various animal model systems.[18–24] Human intervention studies also confirmed the hypoglycemic effect of fenugreek seed powder in type-1 diabetes patients[25,26] and in type-2 diabetes patients.[27–29] In addition, dietary supplement of fenugreek seeds to normal Wistar rats resulted in significant increase in blood

Table 1. Effect of fenugreek and diosgenin on metabolic diseases.

Disease State	Fenugreek/ Diosgenin	Experimental Model/Human	Reference
Diabetes	Fenugreek	Alloxan diabetic rats	18–20, 24
	Fenugreek	Alloxan diabetic dogs	21
	Fenugreek	Streptozotocin diabetic mice	22
	Fenugreek	Type-2 diabetic rats	23
	Fenugreek	Type-1 diabetes patients	25, 26
	Fenugreek	Type-2 diabetes patients	27–29
Hypercholesterolemia	Fenugreek	Alloxan diabetic dogs	9
	Fenugreek	Hypercholesterolemic rats	10
	Fenugreek	Hypercholesterolemic dogs	8
	Fenugreek	Type-1 diabetes patients	11
	Fenugreek	Type-2 diabetes patients	14
	Fenugreek	Coronary heart disease patients	12
	Fenugreek	Hypercholesterolemic patients	13
	Diosgenin	High cholesterol-fed rats	15, 17
	Diosgenin	High fat-fed rats	16
Hyperinsulinemia	Fenugreek	Normal rats	30, 31
Hepatic steatosis	Fenugreek	Zucker obese (fa/fa) rats	32

insulin, demonstrating fenugreek as a potential stimulant of insulin-producing pancreatic islet β-cells.[30] The insulinotropic function of fenugreek was attributed to the special amino acid 4-hydroxyisoleucine.[31]

In a recent study using Zucker obese (fa/fa) rats as a model of fatty liver disease, it was demonstrated that fenugreek significantly alleviated hepatic steatosis in obese rats.[32] The hepatotropic beneficial effect of fenugreek was accompanied by reduced plasma tumor necrosis factor (TNF)-α and altered expression of hepatic TNF-α and TNF receptor-II exclusively in obese rats.[32]

CANCER

In the past few years, several pre-clinical studies have demonstrated the anticancer effects of fenugreek and diosgenin as summarized in Table 2.

Table 2. Anti-cancer effects of fenugreek and diosgenin.

Endpoints/ Cellular Effects Targets	Regulation: Block/ Up/Down	Cancer Model/Cells	Reference
***In vivo* studies**			
Tumorigenesis			
Colon ACF	Block/Down	AOM-induced rat colon cancer model	33
Colon tumors	Block	AOM-induced rat colon cancer model	36
Colon tumors	Down	DMH-induced rat colon cancer model	34, 35
Breast tumors	Down	DMBA-induced rat breast cancer model	37
***In vitro* studies**			
Growth inhibition	Block	KBM-5 chronic myelogenous leukemia cells	43
	Block	MCF-7 breast carcinoma cells	48
	Block	MDA breast carcinoma cells	48
	Block	1547 osteosarcoma cells	49, 50
	Block	HT-29 colon carcinoma cells	33
	Block	HCT-15 colon carcinoma cells	51
	Block	HCT-116 colon carcinoma cells	52, 53
	Block	K562 leukemia cells	54
	Block	HEL erythroleukemia cells	55
Modulation of cell cycle			
Sub-G_1 phase	Block	KBM-5 chronic myelogenous leukemia cells	43
G_1 phase	Block	1547 osteosarcoma cells	50, 58
G_2/M phase	Block	HEL erythroleukemia cells	59
Proapoptotic effects			
p53 activation	Up	MCF-7 breast carcinoma cells	48
	Up	1547 osteosarcoma cells	50
	Up	M4Beu melanoma cells	60
	Up	HEp-2 laryngocarcinoma	60
NF-κB activation	Up	KBM-5 chronic myelogenous leukemia cells	43
	Up	1547 osteosarcoma cells	50

(*Continued*)

Table 2. (*Continued*)

Endpoints/ Cellular Effects Targets	Regulation: Block/ Up/Down	Cancer Model/Cells	Reference
p21 expression	Up	HEL erythroleukemia cells	59
	Up	HCT-116 colon carcinoma cells	53
Caspase-3	Up	HT-29 colon carcinoma cells	33
activation`	Up	MDA breast carcinoma cells	48
	Up	K562 leukemia cells	54
	Up	HEL erythroleukemia cells	55
Bcl-2	Down	HT-29 colon carcinoma cells	33
modulation	Down	MDA breast carcinoma cells	47
PARP cleavage	Up	HCT-116 colon carcinoma cells	53
	Up	HEL erythroleukemia cells	55, 60
	Up	K562 leukemia cells	61

IN VIVO STUDIES

There have been limited research investigations to demonstrate the cancer chemopreventive efficacy of fenugreek and diosgenin *in vivo*. In the azoxymethane (AOM)-induced rodent colon cancer model, 1% fenugreek supplemented in the diet (wt/wt) inhibited the formation of colonic aberrant crypt foci (ACF), putative precancerous lesions of the colon, when administered either during initiation/postinitiation or promotion stages.[33] Diosgenin at 0.05% and 0.1% wt/wt in the diet also inhibited ACF formation in both initiation/postinitiation and promotion stages. The demonstrated ability of fenugreek and diosgenin to inhibit both the total population of ACF and large ACF (those with crypt multiplicity of four or more) suggests that it could effectively retard and cease the appearance and growth of precancerous lesions in the colon.[33] Furthermore, it was also demonstrated that the lower dose of 0.05% diosgenin was as effective as the higher dose of 0.1% in blocking ACF formation during the early stages of colon carcinogenesis.[33] Devasena and Menon reported that fenugreek seeds (at a dose of 2 g/kg body weight) administered in the diet for 30 wks significantly reduced the percent of incidence and

multiplicity of colon tumors in 1,2-dimethylhydrazine (DMH)-injected Wistar rats.[34] Supplementation of fenugreek also decreased the activities of β-glucoronidase in the colon, intestine and liver, and both β-glucoronidase and mucinase in the intestine/colon contents, suggesting a protective role during carcinogen/co-carcinogen release and hydrolysis of mucin.[34] In a more recent report, fenugreek was shown to protect against DMH-induced colon carcinogenesis by modulating hepatic oxidative stress.[35] In a double-blind study designed to assess the tumor-modulating potential of diosgenin using the AOM-induced rodent colon cancer model, Malisetty *et al*. reported that 0.1% of diosgenin suppressed the incidence of both invasive and non-invasive colon tumors by up to 60%.[36] In addition, diosgenin decreased colon tumor multiplicity (adenocarcinomas/rat) compared to controls. In part, these *in vivo* effects were shown to be related to a lower proliferating cell nuclear antigen (PCNA) index in colonic tumors, suggesting that diosgenin attenuates tumor, cell proliferation.[36] Therefore, studies aimed at understanding the chemopreventive ability of diosgenin against colon cancer through major signal transduction pathways are warranted. It still remains unclear in the pre-clinical setting whether diosgenin would decrease colonic tumors of familial origin or prevent metastasis. Nevertheless, the study by Malisetty *et al*. is the only one that has attempted to understand the pre-clinical efficacy of diosgenin to inhibit colon tumorigenesis and has paved the way for studying the tumor-inhibiting ability of diosgenin in other organ sites using well-established models.[36] In terms of addressing the protective role of fenugreek in breast cancer, Amin *et al*. demonstrated that the water extract of fenugreek seed (at a dose of 200 mg/kg body weight) administered orally reduced the percent of incidence, multiplicity, and latency of 7,12-dimethylbenz(α)anthracene (DMBA)-induced breast tumors.[37] It remains unclear as to what role fenugreek and diosgenin may have in cancers of other tissue sites *in vivo*. Several studies have shown the ability of diosgenin to antagonistically influence the inflammatory process in relevant animal models. For instance, diosgenin dose-dependently attenuated subacute intestinal inflammation and normalized bile secretion in indomethacin-induced intestinal inflammation in rats.[38] The role of chronic inflammation on carcinogenesis is vital[39]; thus, the study by Yamada *et al.* demonstrating the ability of diosgenin to effectively treat

inflammation could be extrapolated to its prospective chemopreventive action against cancers.[38]

IN VITRO STUDIES

In the following sub-sections, the research outlining the effects of diosgenin on different cancer cell types *in vitro* through modulation of growth and proliferation, apoptotic machinery, COX/LOX pathway, cholesterol biosynthetic pathway, etc. and the implications for cancer chemoprevention will be discussed. Many molecular candidates critical to tumorigenesis are affected by diosgenin. How they correspond to cellular effects as chemopreventive in function is depicted in Fig. 2.

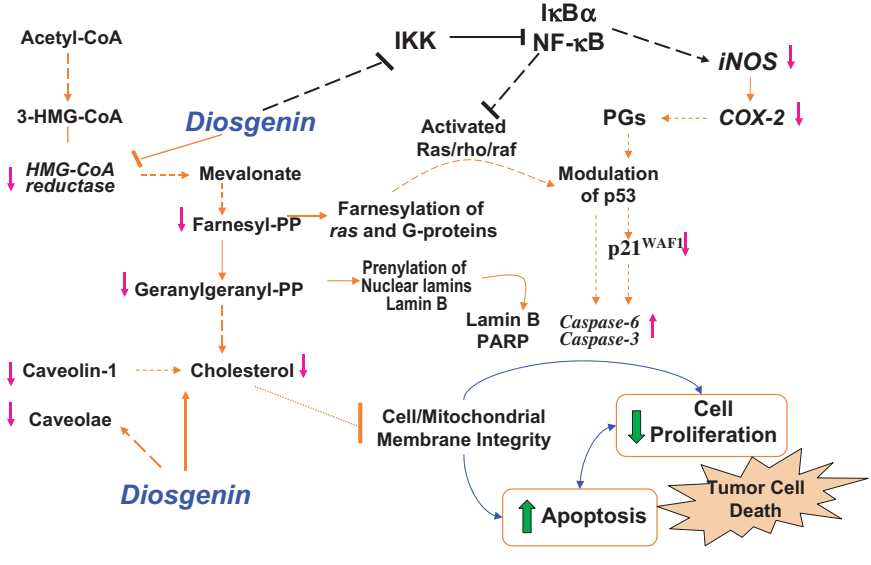

Fig. 2. Possible mechanism(s) of diosgenin leading to reduced tumor cell proliferation and increased cell apoptosis. (a) Suppression of inflammatory signaling pathways through NF-κB and COX-2, (b) inhibition of activation of oncogenic molecules through the modulation of HMG-CoA reductase-prenylation, and (c) suppression of tumor cell membrane integrity and cell-receptor signaling through reduced cholesterol and caveolin-1 synthesis.

Cell Growth

The first report of the ability of diosgenin to inhibit tumor cell growth comes from a study by Chiang *et al*.: dioscin, protodioscin, and methyl protodioscin, all analogs of diosgenin, showed cytotoxicity on cultured C6 rat glioma cells by PRE assay.[40] In addition, Hu *et al*. demonstrated the cytotoxic action of diosgenin analogs against human myelogenous leukemic K562 cells.[41,42] At a dose level of 25 μM, diosgenin caused 50% cytoxicity in chronic myelogenous leukemia KBM-5 cells.[43] To systematically evaluate its potential anticancer activity, Hu and Yao tested the *in vitro* cytotoxicity of protodioscin against 60 human cancer cell lines in the anticancer drug screening program of the USA National Cancer Institute (NCI).[44] As a result, protodioscin was found to be cytotoxic against most cell lines from leukemia and solid tumors in the NCI's human cancer panel; 50% growth inhibition was achieved with doses ≤ 2.0 μM against one leukemia line (MOLT-4), one non-small cell lung cancer (NSCLC) line (A549/ATCC), two colon cancer lines (HCT-116 and SW-620), one CNS cancer line (SNB-75), one melanoma line (LOX IMVI), and one renal cancer line (786–0).[44] Furthermore, methyl protodioscin was found to induce cytotoxic effects in human breast cancer MCF-7 cells.[45] Similar cytotoxic effects of diosgenin were demonstrated in breast carcinoma cells[46–48] and in other human cancer cell lines of various organ types: osteosarcoma,[49,50] colon carcinoma,[33,51–53] leukemia[54] and erythroleukemia.[55] Lee *et al.* recently reported that diosgenin, at doses up to 50 μM, inhibited melanogenesis in B16/BL6 murine melanoma cells; however, even at the highest dose of 50 μM studied, no cytotoxicity was observed in these cells.[56]

Cell Cycle and Apoptosis

A major anticancer mechanism of several phytochemicals involves the perturbation of the cell cycle machinery resulting in cell cycle arrest leading to (or independently inducing) apoptosis.[57] The anti-proliferative effect of diosgenin has been demonstrated *in vitro* principally through inhibition of cell cycle signaling and induction of apoptosis in a number of human cancer cell lines. For instance, diosgenin inhibited the growth of

1,547 osteosarcoma cells through G_1 phase cell cycle arrest and induction of apoptotic demise; the mechanism involved the activation of p53 and binding of NF-κB to DNA independent of PPAR-γ.[50,58] Furthermore, chronic myelogenous leukemia KBM-5 cells were arrested at the sub-G_1 phase and inhibited TNF-dependent NF-κB activation and TNF-induced degradation and phosphorylation of I-κBα (the inhibitory subunit of NF-κB); the cell cycle arrest was also correlated with a downregulation of TNF-induced cyclin D1.[43] Diosgenin also arrested the growth of HER2-overexpressing AU565 human breast adenocarcinoma cells at the sub-G_1 phase.[47] The effect of diosgenin in suppressing osteoclastogenesis in Raw 264.7 cells was reported to follow a pro-apoptotic mechanism through receptor activated NF-κB ligand (RANK-L) induction.[43] Interestingly, in human erythroleukemia TIB-180 (HEL) cells, diosgenin caused apoptosis induction through G_2/M cell cycle arrest and p53-independent p21 upregulation.[59] Conversely, the pro-apoptotic mechanism of diosgenin in laryngocarcinoma HEp-2 and melanoma M4Beu cells was through p53-dependent cell demise.[60] In estrogen receptor-positive MCF-7 human breast cancer cells, diosgenin induced p53 tumor suppressor protein, while the pro-apoptotic mechanism of diosgenin in estrogen receptor-negative MDA human breast carcinoma involved the activation of caspase-3.[48] Caspase-3 activation-dependent apoptosis was also seen in HT-29 human colon adenocarcinoma,[33] K562 leukemia,[54] and HEL erythroleukemia cells.[55] Diosgenin caused poly (ADP-ribose) polymerase (PARP) cleavage-mediated pro-apoptotic effects in HEL cells,[55,61] and K562 erythroleukemia cells.[62] Selective apoptosis in human breast cancer cells overexpressing HER2 oncoprotein was demonstrated mechanistically through PARP cleavage involving the downregulation of phosphor-Akt and phosphor-mTOR, and upregulation of phosphor-JNK independent of p38 and ERK phosphorylations.[47] Bertrand *et al*. reported that diosgenin induced K562 cells to apoptotic death over a wide period of time ranging from 4–48 hrs, thus demonstrating a variable kinetics after diosgenin treatment.[63] In the same study, the authors reported a decrease in phospho-ERK expression in diosgenin-treated cells.[63] In addition, the antiapoptotic Bcl-2 was inhibited by diosgenin in HT-29 human adenocarcinoma cells[33] and MDA human breast carcinoma cells.[48] In a recent study, HCT-116 cells, a highly metastatic human colon carcinoma cell

line, possessing mutations of K-ras and β-catenin oncogenes and positive for TGF-β1 and -β2 expressions, responded to the cytotoxic actions of diosgenin, in part, through suppression of p21 ras and β-catenin resulting in cell growth arrest and cleavage of PARP leading to apoptotic cell demise.[53] From the studies reviewed, it appears that diosgenin could follow several mechanisms to inhibit cancer cell growth and/or promote cell death depending on the cell type involved (Fig. 2). Other mechanisms of cell death, such as switch-on of anoikis machinery, are yet to be understood in the role of diosgenin as an anticancer agent. In addition, there is little evidence about the effect of diosgenin on DNA damage and repair mechanisms.

Cyclooxygenase and Lipoxygenase

The cyclooxygenase (COX) and lipoxygenase (LOX) systems are the two principal machineries in the biosynthesis of eicosanoids.[64] COX-2, one of the two COXs playing a role in the conversion of arachidonic acid (AA) into prostaglandins (PG) and thromboxane (TX) A_2, is purported to be the inducible form, and its involvement in inflammation and tumorigenesis is well known. The literature is replete with evidence demonstrating the cancer chemopreventive activity of specific COX-2 inhibitors,[65] and several phytochemicals, such as curcumin, are known inhibitors of COX-2.[57] Moalic *et al*. demonstrated that diosgenin inhibited COX-2 activity and expression in human osteosarcoma 1547 cells.[49] Studies have also abolished both basal and TNF-induced COX-2 gene products in KBM-5 cells in a time-dependent fashion.[43] By contrast, diosgenin increased the synthesis of AA in erythroleukemia HEL cells leading to COX-2 overexpression, which was accompanied by apoptosis induction.[59] In a recent study, it was reported that 10 μM diosgenin increased the expression of COX-2 and TX synthase in HEL cells. Diosgenin also stimulated PG-E_2 and TX-B_2 production.[66] Similarly, diosgenin induced apoptosis in non-cancerous human rheumatoid arthritis synoviocytes (RAS) through the overexpression of COX-2 protein and a concomitant increase in the level of PG-E_2.[67] In apoptotic mechanisms involving COX-2 overexpression in human RAS cells, a combination with MEK inhibitor (U0126) treatment has been demonstrated to augment the effects of diosgenin.[68]

Interestingly, diosgenin induced COX-2-independent apoptosis through activation of the p38 MAP kinase signaling pathway and inhibition of NF-κB binding in COX-2 deficient K562 cells.[62]

The LOX pathway, comprising a group of isozymes, plays a role in the conversion of AA to hydroperoxyeicosatetraenoic acids and leukotrienes. Like COX-2, 5-LOX is an inducible enzyme and is well-implicated in carcinogenesis. Furthermore, its inhibition is purported to be vital to the action of several cancer chemopreventive agents.[69] In a study by Nappez *et al.* it was reported that diosgenin treatment in HEL cells did not affect 5-LOX mRNA or 5-LOX activating protein (FLAP) mRNA at the transcriptional level.[70] However, when HEL cells undergoing differentiation were incubated with diosgenin in the presence of indomethacin (a COX inhibitor), the growth inhibitory effect of diosgenin was reversed and an exponential growth kinetic of undifferentiated cells was observed.[70] Taken together, these studies provide valuable clues as to the role of 5-LOX in diosgenin's modulation of growth and differentiation in HEL cells; however, how these results could be extrapolated to other cancer cell types remains unanswered and warrants further investigation. Other plant saponins, such as saikosaponins, structurally different from diosgenin by the absence of steroid but the presence of triterpene moiety, have been demonstrated to inhibit AA metabolism by targeting both COX and LOX pathways; however, the effect appears to be mainly mediated through the LOX pathway.[71] Both COX-2 and 5-LOX are implicated in carcinogenesis and showcase as vital targets for cancer chemoprevention and therapy. It is likely that the chemopreventive activity of diosgenin against several cancer types may follow the inhibition of either or both of these pathways in concert or independently.

Fatty Acid and Cholesterol Biosynthetic Pathway

Regulation of key enzymes involved in the *de novo* biosynthesis of fatty acids is known to be critical in cancer pathogenesis. Fatty acid synthase (FAS), a key lipogenic enzyme, is upregulated in most human cancers principally by conferring growth and survival advantages rather than functioning as an anabolic energy-storage pathway, hence appearing oncogenic in function.[72] Intrigued by this unique function of FAS, chemicals

capable of selectively aiming at inhibition of FAS were synthesized as new therapeutics for treating and preventing cancer.[73] Many herbs used in complimentary medical practices for the management of obesity do so by inhibiting FAS.[74] Diosgenin, a known hypolipidemic agent,[15–17] was thus studied for its potential to inhibit FAS in human breast cancer *in vitro*.[47] It was demonstrated that diosgenin inhibited the expression of FAS in HER2 overexpressing breast cancer cells,[47] suggesting that FAS could be a potential molecular target of diosgenin in other cancer cells that possess abundance of FAS expression.

It has been demonstrated that the upregulation of candidates involved in the cholesterol biosynthetic pathway lead to the stimulation of DNA synthesis during tumorigenesis.[75,76] The activity and expression of 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis, is increased several-fold in tumors compared with normal tissues.[77–79] Several *in vitro* and *in vivo* studies have shown that statins, inhibitors of HMG-CoA reductase, inhibit tumor cell growth,[80] thus identifying HMG-CoA reductase as a crucial molecular target in the therapeutic efficacy of drugs. Rao *et al.* demonstrated that the phytochemicals farnesol and lanosterol modulate tumor cell growth by inhibiting HMG-CoA reductase.[81] Diosgenin was recently tested for its anticancer ability via modulation of HMG-CoA reductase.[53] In HCT-116 human colon carcinoma cells, diosgenin at sub-maximal inhibitory doses suppressed both mRNA and protein expression of HMG-CoA reductase, suggesting that it targets the cholesterol biosynthetic pathway as a possible mode of action related to its growth inhibitory effect.[53] However, these studies did not examine the effect of diosgenin on the kinetics of HMG-CoA reductase. It remains unclear as to whether the anticancer mechanisms of diosgenin are similar to those of other plant sterols and isoflavones that have been demonstrated to affect HMG-CoA synthase and reductase protein and gene expression,[82] leading to modulation of cell survival through the sterol regulatory element binding protein-dependent mechanism.[83] Another theory would be that diosgenin-like HMG-CoA reductase inhibitors may reduce the formation of mevalonic acid, leading to a possible decrease in the levels of dolichol and isoprenoids.[84] While dolichol is involved in the process of N-linked glycosylation of membrane-targeted proteins, isoprenoids are necessary for

the prenylation, subsequent membrane anchoring, and activity of downstream growth factor signaling components such as Ras.[85] Thus, like statins, diosgenin may exert its inhibitory effects on tumor cell growth, in part, via modulation of post-translational glycosylation and isoprenylation. Another important role of cholesterol biosynthesis is the maintenance of cell membrane assembly through cholesterol-rich membrane caveolae or lipid rafts (Fig. 2). Caveolae, a source of vital membrane receptors, are known to be affected by HMG-CoA reductase inhibitors.[86] How diosgenin alters membrane lipid composition or the function of caveolae via inhibition of HMG-CoA reductase in preneoplastic or neoplastic lesions is an important field to be explored.

CONCLUSION AND FUTURE RESEARCH

Fenugreek is an ancient food and medicine that is currently receiving scientific attention. Studies conducted so far support fenugreek as an excellent candidate for the control and prevention of metabolic diseases including type-1 and -2 diabetes, dyslipidemia and fatty liver disease. While there are numerous studies including clinical intervention trials to demonstrate this, the underlying molecular mechanisms of action are still unclear and warrant thorough investigation. Using experimental models, diosgenin, the major steroid saponin constituent of fenugreek, has been identified for its cancer chemopreventive efficacy as well as its lipid lowering effects. A number of phytochemicals that prevent initiation of normal cells to precancerous states, and thereby halt the progression to neoplastic growth and expansion, are known to target complex intracellular signaling pathways. Many of them are known to act on one primary target (such as an enzyme) leading to modulation of secondary targets. A newer approach is to search for compounds (both synthetic as well as naturally occurring) with multiple targets acting in concert at the cellular and molecular levels for effective cancer chemoprevention. In this regard, diosgenin has been demonstrated to target multiple pathways by inhibiting or stimulating specific signaling candidates conducive to cancer prevention or therapy in the preclinical setting. Overall, the multiple signaling pathways affected by diosgenin in tumor cells could plausibly lead to repression of transformation, induction of apoptosis, suppression

of proliferation and growth, cessation of the inflammatory process, and disruption of cell membrane integrity. There has been substantial effort in addressing the role of diosgenin in modulating the growth and proliferation of human cancer cell types and its potential mechanism(s) of action *in vitro*, but there is only limited information on its *in vivo* effects. Further studies utilizing physiologically relevant (*in vivo*) and/or human attainable doses of fenugreek/diosgenin that will selectively inhibit tumor cell growth or induce apoptosis through the mechanisms observed in the reported *in vitro* experiments are required. There are only a handful of studies that have used the whole seeds of fenugreek to understand its efficacy in protecting against cancers, and hence extensive research is warranted to determine the efficacy of both fenugreek and its main bioactive component diosgenin utilizing relevant *in vivo* models of different organ sites. Interestingly, there is no evidence for the role of fenugreek and diosgenin against tumor angiogenesis and metastasis. In addition, there is little information regarding its bioavailability, pharmacokinetics and pharmacodynamics in relation to its anticancer effects. Whether fenugreek and diosgenin potentiate the effects of existing cancer therapeutic drugs as an adjuvant or elicits synergistic or additive effects in combination treatments is not known. Trouillas *et al.* tested the biological activities of diosgenin and other structurally-related steroid saponins and alkaloids *in vitro*, and the anti-cancer activity of diosgenin was proposed to be related to the presence of a hetero-sugar moiety and the 5,6-double bond in its structure together with structural conformation at C-5 and C-25 carbon atoms.[87] To determine the structure-function relationship of diosgenin in other cancer cell types and to understand whether and how the synthetic changes brought about could augment its biological activity in favor of its role as a cancer chemopreventive compound may be yet another explorative field in this area. Our scientific knowledge in this area is limited, and extensive preclinical and clinical research will help to establish not only whether fenugreek and diosgenin are safe and efficacious as chemopreventive agents against several human cancers, but also to develop and evaluate standards of evidence for health claims for foods containing diosgenin and fenugreek as they become increasingly popular and enter the marketplace labeled as nutraceuticals and functional foods.

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8

Diallyl Sulfide from Garlic

Girija Kuttan* and Punathil Thejass

Garlic, *Allium sativum* L. has been used as common food and for the treatment of many diseases since ancient times. The consumption of garlic has long been associated with a reduced risk in the occurrence of cancer at various sites, including the prostate, lung, breast and colon. This protective effect is attributed to organosulfur compounds (OSCs) present in garlic that are generated upon its processing. Diallyl sulfide (DAS) is one of the potent oil soluble OSCs present in garlic and preclinical studies have provided convincing evidence to indicate that DAS is highly effective in affording protection against cancer in laboratory animals induced by a variety of chemical carcinogens. Inhibition of carcinogen activation through inhibition of Phase I enzymes and/or acceleration of carcinogen detoxification via induction of phase II enzymes (glutathione transferases, quinone reductase, etc.) are believed to be responsible for protective effects of DAS against chemically induced cancers. However, it is becoming clear that there are multiple mechanisms activated in response to DAS, including induction of apoptotic pathways, suppression of cell cycle progression, stimulation of immune system, inhibition of angiogenesis and anti-inflammatory activity. Moreover, these mechanisms seem to have some degree of interaction to synergistically afford chemoprevention and made DAS an effective inhibitor of cancer process.

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INTRODUCTION

Allium is the largest and most important representative genus of the Alliaceae family and comprises 450 species, widely distributed in the northern hemisphere. The wild progenitor of garlic is believed to have originated in the high planes of west Central Asia or the Kirgiz Desert of western Russia. The term *garlic* originates from the Anglo- Saxon "gar-leac" or spear plant. The genus *Allium* is derived from the Celtic word *all*, meaning pungent or burning. The species name *sativum* signifies planted, cultivated or sown.[1] Besides the well-known garlic and onion, several other species are widely grown for culinary use, such as leek (*Allium porrum* L.), scallion (*Allium fistulosum* L.), shallot (*Allium ascalonicum* Hort.), wild garlic (*Allium ursinum* L.), elephant garlic (*Allium ampeloprasum* L. var. *ampeloprasum*), chive (*Allium schoenoprasum* L.), and Chinese chive (*Allium tuberosum* L.).

Garlic, *Allium sativum* L., has been used as common food and for the treatment of many diseases since ancient times. The first citation of this plant is found in the Codex Ebers (1550 BC), an Egyptian medical papyrus reporting several therapeutic formulas based on garlic and onions as useful remedy for a variety of diseases such as heart problems, headache, bites, worms and tumors. Cloves of garlic have been found in the tomb of Tutankhamun and in the sacred underground temple of the bulls of Saqqara. Apart from Egyptians, Babylonians, Phoenicians, Vikings, Chinese, Greeks, Romans and Indians used garlic frequently.[2] They took garlic as a remedy for intestinal disorders, flatulence, worms, respiratory infections, skin diseases, wounds, symptoms of aging, and many other ailments.

Scientific research on these plants started in the second half of the 19th century with the work of Louis Pasteur (1858), who noted antibacterial properties of garlic.[3] To date more than 3,000 publications from all over the world have gradually confirmed the traditionally recognized health benefits of garlic.

The scientific community has now become interested in the pharmacologic properties of *Allium* vegetables and their chemical constituents, particularly with regard to their effects on the cardiovascular system and in the prevention of cancer. Garlic has antihypertensive and antiarrhythmic properties and exerts an antithrombotic effect through fibrinolytic activity and the reduction of platelet aggregation. Ingestion of garlic has also been

reported to lower the concentration of triglycerides, cholesterol, and low-density lipoproteins and to increase the concentration of high-density lipoproteins in blood. These findings suggest that garlic has a preventive effect against atherosclerosis and its complications, including stroke, myocardial infraction, and thrombotic disorders. In addition, garlic stimulates immune function through activation of macrophages and induction of T-cell proliferation, alters blood glucose level, offers protection against microbial, viral and fungal infections, and possesses potential anti-aging effects.

Cancer prevention by natural products has received considerable attention in recent years. Chemoprevention, which is referred to as the use of natural products to intervene in multistage carcinogenesis, has emerged as a promising approach to reducing the risk of cancer and its progression. One of the first plants whose constituents were reported to possess antitumor properties was garlic. Experimental and epidemiological studies have provided evidence in support of the association between garlic intake and reduced cancer risk,[4] including reduction of esophageal, mammary, skin, pulmonary, forestomach, colon, and lung tumors. You *et al*.[5] provided evidence for the anticancer effect of *Allium* vegetables by population-based observational study in China. After analyzing 564 patients with stomach cancer and 1,131 controls, they documented an inverse correlation between dietary intake of *Allium* vegetables and cancer risk. A more recent population-based, case-control study involving 238 cases with histologically confirmed prostate cancer and 471 control subjects, conducted in Shanghai, China, found that men with high intake of total *Allium* vegetables (> 10 g/day garlic, onions, scallions, chives, and leeks) had a statistically significant lower risk of prostate cancer than those with low intake (< 2.2 g/day).[6]

Anticancer as well as other medicinal properties of *Allium* vegetables including garlic are attributed primarily to organosulfur compounds (OSCs), which are released upon processing of these vegetables. Kelloff *et al*.[7] categorized the chemopreventive agents into three classes: (a) antimutagenic (acts at the activation and DNA adduction of the mutagen), (b) antimitogenic reagents (acting at the stimulation of the proliferation signal pathway by mitogens), and (c) antioxidants. It is very difficult to assign a particular organosulfur compound acting as chemopreventive agent to a particular class because all these agents are believed to act through a combination of two or more mechanisms.

CHEMISTRY OF GARLIC

The chemistry of garlic is quite complex and likely developed as a self-protective mechanism against microorganisms and other insults. The main components of fresh garlic are water, carbohydrates, protein, fiber, fat and aminoacids. In addition, garlic contains calcium (50–90 μg/100 g), copper (0.02–0.03 μg/100 g), iron (2.8–3.9 μg/100 g), potassium (100–120 μg/100 g), magnesium (43–77 μg/100 g), germanium (14 μg/100 g), chromium (0.3–0.5 mg/100 g), manganese (0.2–0.6 mg/100 g), boron (0.3–0.6 mg/100 g), barium (0.2–1 mg/100 g), aluminum (0.5–1 mg/100 g), sodium (10–22 mg/100 g), phosphorous (390–460 mg/100 g), zinc (1.8–3.1 mg/100 g), selenium (15–35 μg/100 g), thiamine (0.25 mg/100 g), riboflavin (0.08 mg/100 g), vitamin C (5 mg/100 g), nicotinic acid (0.5 mg/100 g), retinal (15 μg/100 g) and energy (39–140 cal/100 gwet wt.).[8] Garlic is rich in OSCs and at least 33 different types have been identified in garlic. The amount and composition of OSCs vary with different strains of garlic. In addition, environmental factors such as climate and use of fertilizers also modify the OSC content in garlic. Two pathways are involved in the conversion of natural garlic to sulfur compounds. The first pathway is natural aging bioconversion, which leads to the formation of mainly water soluble sulfur compounds such as S-allylcysteine (SAC) and S-allylmercaptocysteine (SMAC). The second pathway is cell decomposition to allicin, which again breaks down rapidly under uncontrollable chemical reactions to produce odorous oil-soluble sulfur compounds, namely diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS) and ajoene.[2]

The primary sulfur-containing compounds in intact garlic are γ-glutamyl-S-alk(en)yl-L-cysteines, which can be hydrolyzed and oxidized to form alliin (S-alk(en)yl-L-cysteine sulfoxide). In addition (1)-S-methyl-L-cysteine sulfoxide (methiin), (1)-S-(trans-1-propenyl)-L-cysteine sulfoxide, S-(2-carboxypropyl)glutathione, γ-glutamyl-S-allyl-L-cysteine, γ-glutamyl-S-(trans-1-propenyl)-L-cysteine and γ-glutamyl-S-allyl-mercapto-L-cysteine are also present in garlic cloves. Alliin, which accumulates naturally during storage of garlic bulbs, is the odorless precursor of the OSCs and accounts for about 80% of the cysteine sulfoxides in garlic. When garlic is cut, chopped or crushed, the clove's membrane disrupts and alliin is transformed enzymatically into allicin by allinase. Allicin, an

alkyl alkane thiosulfinate, is responsible for the characteristic odor and taste of garlic. It is highly unstable and instantly decomposes to form oil-soluble OSCs including diallyl, methyl allyl, and diethyl mono-, di-, tri-, tetra-, penta-, and hexasulfides, the vinyldithiins, and (E)- and (Z)-ajoene. The formation of thiosulfinates is very rapid and has been found to complete in 60 secs. Simultaneously, γ-glutamyl cysteines are also converted to the water-soluble OSCs, SAC and SAMC (Fig. 1).[2,9] The total allicin yield has been determined as 2.5 mg/g of fresh crushed garlic or about 5–20 mg per clove. Further transformation of organosulfur compounds

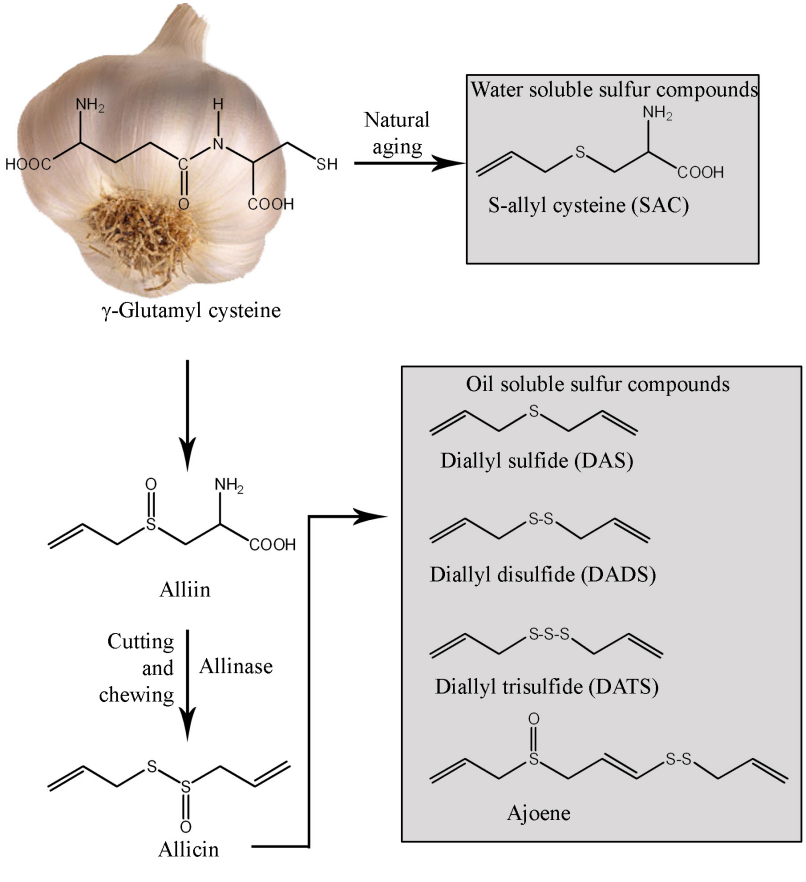

Fig. 1. Chemical reactions in processed *Allium* vegetables and generation of organosulfur compounds.

(OSCs) can occur after interaction with free sulfhydryl groups, including those present in cysteine, glutathione or proteins. Incubation of cysteine with the DAS group produces allylmercaptane.[10] Allyl methyl sulphate (AMS) and DAS have been found in human breath after ingestion of garlic.[11] DAS is a lipophilic thioether and can further undergo extensive oxidation at three positions, i.e., the sulfur atom, the allylic carbon and the terminal double bonds. Cytochrome P450 (CYP) enzyme-mediated oxidation at the sulfur atom of DAS sequentially produces diallyl sulfoxide (DASO) and diallyl sulfone (DASO2).[12] Garlic also contains several low molecular weight polar compounds that are stable to cooking and storage and also exhibit several biological activities. These are of phenolic and steroidal origin, often glycosylated and include sapogenins, saponins, and flavonoids.

ANTICARCINOGENIC ACTIVITY OF DAS

Considerable evidence points to the ability of garlic to suppress a myriad of chemically induced tumors. Garlic extract has been found to inhibit two-stage chemical carcinogenesis induced by 7,12-dimethyl-benzanthracene (DMBA) and croton oil on mice skin with significant reduction in papilloma formation.[13] Hussain *et al*.[14] have shown that oral administration of garlic significantly reduced methylcholanthrene-induced uterine cervical carcinoma in Swiss albino mice. Preclinical studies have provided convincing evidence to indicate that DAS is highly effective in affording protection against cancer induced in experimental animals by a variety of chemical carcinogens. Diallyl sulfide was found to inhibit 2-dimethylhydrazine (DMH)-induced colon cancer in female C57BL/6J mice by reducing the colon nuclear damage.[15] DAS also significantly inhibited nuclear aberration formation (a measure of nuclear damage) over a radiation dose range of 0.5 to 10 Gy and thus reduced gamma-ray exposed acute colonic mucoasal injury in female C57BL/6J mice.[16] Singh and Shukla[17] documented significant protection from neoplasia by topical application of DAS indimethylbenz(a)anthracene (DMBA)/benzo(a)pyrene (B(a)P)-exposed animals. The antitumor activity of DAS was of a much higher magnitude in B(a)P-induced carcinogenesis in comparison to animals exposed to DMBA in terms of tumor incidence, cumulative number of tumors and average number of tumors per mouse.[17] Topical application of

DAS and garlic oil during the initiation phase of skin cancer reduced the number of tumors per mouse. The results showed that the treatment with DAS can effectively delay the onset of tumorigenesis and reduce the cumulative number of tumors and the average number of tumors per mouse. In groups in which DAS was applied prior to initiation or promotion, a significant population of the animals remained tumor-free till the termination of experiment.[18] In another study by Dwivedi *et al*.[19] it was shown that the topical application of DAS or DADS significantly inhibited skin papilloma formation from the ninth week of promotion and significantly increased the rate of survival in the murine model. These findings suggest that DAS can effectively inhibit chemically-induced mouse skin carcinogenesis.

Hadjiolov *et al*.[20] demonstrated the inhibitory effect of DAS on aristolochic acid (AA)-induced tumors in rats and also conducted experiments to establish the effects of DAS administration on AA-derived DNA single-stranded regions and DNA adduct formation in the forestomach of such animals. Forestomach, urinary bladder and thymus tumors were induced in male BD-6 rats after oral treatment with AA. Administration of DAS intragastrically prior to AA treatment significantly reduced the number of rats with forestomach tumors. The co-administration of DAS significantly reduced the incidence of AA-induced forestomach tumors to 10%, which was 60% when AA was administered alone. The high dose of DAS markedly inhibited the formation of squamous cell carcinomas in the forestomach. In addition, DAS co-administration decreased the accumulation of single-stranded regions in rat forestomach DNA.[20] DAS has been shown to inhibit 7,12-dimethylbenz[a]anthracene (DMBA) induced buccal pouch and forestomach cancer in hamsters. DAS resulted in a significant reduction in buccal pouch tumor frequency, buccal pouch tumor burden, buccal pouch gamma GT lesion frequency, and forestomach tumor frequency. In a separate experiment, DAS also reduced the level of autoradiographically quantified unscheduled DNA repair synthesis (UDS) in pieces of hamster buccal pouch concurrently exposed *in vitro* to the potent buccal pouch carcinogen N-methyl-N-benzylnitrosamine (MBN).[21] Hong *et al*.[22] demonstrated that DAS inhibited the metabolic activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) — a potent tobacco carcinogen believed to be important in the etiology of human oral cancer in tobacco chewers and lung cancer in cigarette smokers — in rat

lung and nasal mucosa microsomes. DAS pretreatment significantly decreased the incidence of NNK-induced lung tumors (37.9% versus 100%) and the tumor multiplicity (0.6 versus 7.2 tumors/mouse). In pulmonary metabolism of NNK, DAS pretreatment reduced the rates of formation of keto aldehyde, keto alcohol, NNAL-N-oxide, and NNK-N-oxide by 70–90%. In addition, the formation of NNK oxidative metabolites from NNK in the liver microsomes from DAS-pretreated mice was remarkably reduced. DAS also inhibited the metabolism of NNK in mouse lung microsomes *in vitro*.[22] Sengupta *et al*.[23] showed that DAS inhibited azoxymethane-induced colon carcinogenesis in Sprague–Dawley rats by significantly reducing the incidences of aberrant crypt foci by 43.65%.

Wargovich and Imada[24] have shown that DAS significantly inhibited nitrosamine N-nitrosomethyl-benzylamine (NMBA)-induced esophageal carcinogenesis. DAS significantly reduced the mutagenicity of NMBA in the rat esophagus. NMBA is specific in inducing tumors in the rat esophagus and has been used in many studies investigating the mechanism and prevention of this cancer. The type of mutation induced by two 2-mg/kg subcutaneous injections of NMBA in the lacI gene of "Big Blue" rats is G:C→A:T transitions.[25] Administration of DAS to rats resulted in the inhibition of microsome-mediated mutagenicity of aflatoxin (AFB1). The cytosolic fraction from DAS-treated rats produced a significant inhibition of AFB1-8,9-epoxide-induced mutagenicity and significantly increased the cytosolic formation of AFB1-glutathione conjugates.[26] Singh *et al*.[27] demonstrated the inhibitory effect of DAS on the development of diethylnitrosamine (DEN)-initiated and 2-acetyl-aminofluorene (2-AAF)-promoted preneoplastic altered hepatic foci (AHF) in Wistar rats. In this study DAS-supplemented rats were found to restore the near-normal levels of enzymes GST-P and GGT when exposed to DEN and 2-AAF. DAS administration following DEN and 2-AAF exposure led to the restoration of enzymic activity of ATPase, G6 Pase and AlkPase, as evidenced by number and area of the foci, suggesting the protective role of DAS in rat hepatocarcinogenesis by suppressing DEN- and 2-AAF-induced AHF development. Collectively, preclinical investigations demonstrate consistently that cancer chemoprevention by DAS is clearly evident and appears to be independent of the organ site or the carcinogen employed.

MOLECULAR MECHANISMS (TARGETS) FOR ANTICANCER ACTIVITY OF DAS

Elucidation of the mechanism(s) by which DAS offers protection against chemically-induced cancer has been the topic of intense research in the past two decades. DAS may intervene at one or more steps in the carcinogenic process such as initiation, promotion or progression. The protective action of DAS may not be attributed to a single mechanism but explained on the basis of the following probable mechanisms.

Inhibition of Phase 1 Enzymes

Many of the carcinogens are not chemically reactive as such but undergo metabolic activation to form electrophilic reactants. Once they enter the human body, they are subjected to metabolism by enzymatic processes that occur mainly through oxidation but also, to a lesser extent, through reduction and hydrolysis, which causes the chemical molecules to become more hydrophilic. This physiological event is called phase 1 metabolism and is primarily catalyzed by cytochrome P450 enzymes (CYPs). Phase 1 enzymes typically carry out oxidation and reduction reactions that make carcinogens more water soluble, but at the same time they are capable of activating compounds to electrophilic species. These reactive electrophilic species can interact with a nucleophilic group in DNA to induce point mutation or other genetic lesions, thus leading to the activation of proto-oncogenes and inactivation of their tumor repressor genes. As a consequence, procarcinogens are usually converted into highly reactive intermediates that can bind to critical macromolecules such as DNA, RNA and protein. So far, 57 CYPs have been identified in humans based on their similarity of DNA sequence and some protein functions.[28] Organosulfur compounds (OSCs) derived from garlic, including DAS, have been shown to inhibit experimental cancers in various animal models, primarily through inhibition of phase I enzymes. DAS and its metabolites diallyl sulfoxide and diallyl sulfone competitively inhibited the activity of cytochrome P 450 2E1 in a time-dependent and NADPH-dependent manner using pseudo-first-order kinetics.[29] DAS administration

protected against hepatotoxicity caused by exposure to P 450 2E1 substrates, including N-nitrosodimethylamine (NDMA).[30] Moreover, DAS with selective P 450 HE1 inhibitory activity lessened the mutagenicity of the vinyl carbamate (VC) and NDMA-induced mutagenesis in *Salmonella typhimurium* TA100 in a concentration-dependent manner. The suppression of VC- and NDMA-induced mutagenesis by DAS was correlated with their inhibition of P 450 IIE1-mediated p-nitrophenol hydroxylation and NDMA N-demethylation.[31] The rat nasal cavity is one of the known target organs for carcinogenesis by NDMA, N-nitrosodiethylamine (NDEA) and tobacco carcinogen 4-(methylnitrosamino)1-(3-pyridyl)-1-butanone (NNK), and Hong *et al*.[32] documented significant decrease in the oxidative metabolism of NDEA and NNK in nasal mucosa by administration of DAS to male rats. Since P 450 2E is vital in catalyzing the activation of N-nitrosodimethyl amine (NOMA), DMH, benzene, alkanes, halogenated hydrocarbons, and many other low molecular weight environmental chemicals, inhibition of this enzyme is expected to block toxicity and carcinogenesis of these compounds.

Induction of Phase II Enzymes

Cells also have a chemical protection mechanism against carcinogenesis and mutagenesis and other toxicity by induction of the enzymes involved in the metabolism, particularly phase II enzymes such as NAD(P)H quinone reductase and glutathione S-transferases (GSTs). One of the most important mechanisms of chemoprevention by organosulfur compounds is the induction of phase II enzymes. These phase II enzymes often add large polar groups to the primary metabolite thus limiting further transformation and enhancing elimination, thereby leading to detoxification. Cancer chemopreventive action is the result of balance between activating and detoxifying reactions of phase I and phase II enzymes, respectively. This balance under normal circumstances is genetically controlled but gets modulated by a variety of factors such as age, hormones, and exposure to drugs. Quinone reductase (QR) catalysis is a two-electron transfer to a wide variety of the redox cycling species including quinones, transferring them into dehydrodiols and thereby preventing the mutation of the DNA and reducing cancer risk. There are monofunctional as well as bifunctional inducers.

Monofunctional inducers of phase II enzymes decrease the incidence of carcinogenesis through scavenging of electrophilic compounds whereas bifunctional inducers elevate both phase II and phase I enzymes. Since a bifunctional inducer can activate procarcinogens to ultimate reactive form, a monofunctional inducer is always desirable. Evidence has demonstrated the role of DAS in the prevention of chemically-induced cancers by inhibiting carcinogen activation and enhancing detoxification of activated carcinogenic intermediates through the induction of phase 2 enzymes, including glutathione transferases (GSTs). Hu *et al*.[33] showed that administration of DAS, DADS and DATS to A/J mice induced the expression of Alpha (mGSTA3-3, mGSTA1-2, mGSTA4-4), Mu (mGSTM1-1) and Pi class GST, (mGSTP1-1) in the liver, lung and forestomach, and it was found that OSC-mediated prevention of BP-induced forestomach tumorigenesis, but not lung neoplasia, in A/J mice is most closely correlated with the induction of mGSTP1-1. In addition, DAS and DADS were found to be potent inducers of quinone reductase activity and protein level in the forestomach and/or lung of A/J mice.[34] Green *et al*.[35] showed that DAS inhibited diethylstilbestrol (DES) induced DNA damage by altering the expression of DES metabolizing genes including GST. DES has induced mammary tumors in female ACI rats and is associated with an increased risk of developing breast cancer in humans. DES decreased the expression of GST by 23%, whereas DAS and DAS/DES treatments increased the expression of GST by 12- and 16.7-fold, respectively. DAS reduced the mutagenicity of (+)-anti-7beta, 8alpha-dihydroxy-9alpha, 10alpha-oxy-7, 8, 9, 10-tetrahydrobenzo[a] pyrene (BPDE) and styrene oxide (SO) by enhancing the induction of phase II enzymes including total glutathione S-transferase (GST) activity, GST mu activity, and quinone reductase (QR) activity.[36] Sheen *et al*.[37] demonstrated that DAS inhibited aflatoxin B(1) (AFB(1))-induced DNA damage in primary rat hepatocytes by increasing GST and glutathione peroxidase (GPx) activities as compared with the AFB(1) only treated controls. Results of immunoblot analysis of cytosolic GST isoenzyme indicate that the levels of GST isoform Ya, Yb2 and Yc were markedly increased after treatment with DAS compared with the AFB(1) control. Thus, it is reasonable to conclude that the induction of phase II enzymes, especially GST, represents another potential mechanism to explain OSC-mediated prevention of chemically-induced cancers.

Inhibition of DNA Adduct Formation

Formation of DNA adduct is believed to be an initial step in carcinogenesis by chemicals. Garlic powder has been shown to decrease the occurrence of DMBA-DNA adducts in rat mammary gland and the amounts of total and individual adducts correlated positively with mammary tumor incidence.[38] DNA adducts induced by incubation of human bladder tumor cells with 2-aminofluorene (2-AF) were inhibited by DAS and DADS.[39] DAS and DADS also inhibited 2-AF-induced DNA adduct formation in human promyelocytic leukemia cells (HL-60) in a dose dependent manner.[40] N-nitroso compounds, a class of potential human carcinogens that can be synthesized in humans from precursors present in the diet, are metabolized to alkylating agents that can bind to DNA. DAS reduced N-nitrosopiperidine (NPIP)-induced oxidative DNA damage in the human hepatoma cell line HepG2 through the modulation of phase I and II enzyme activities but did not protect against N-nitrosodibutylamine (NDBA)-induced oxidized purines. The formation of formamidopyridine-DNA glycosylase (Fpg) sensitive sites induced by NPIP was prevented by DAS and the maximum reduction of the formation of Fpg sensitive sites induced by NPIP was observed with diallyl disulfide (DADS).[41] Further, DAS has been shown to be protective towards the oxidative DNA damaging effect of two N-nitrosamines, N-nitrosopyrrolidine (NPYR) and N-nitrosodimethylamine (NDMA) in the single-cell gel electrophoresis (SCGE)/HepG2 assay. NPYR and NDMA incubated with formamidopyrimidine-DNA glycosylase (Fpg) caused a significant increase in oxidative DNA damage, and NPYR exerted greater genotoxic effects than NDMA. DAS reduced the genotoxic effects of the N-nitrosamines in a dose-dependent manner. DAS reduced NDMA-induced oxidative damage by 53% and the effect of NPYR was attenuated by about 61%.[42]

Nigam and Shukla[43] demonstrated that topical application of DAS inhibited DMBA-induced DNA strand breaks in mouse skin. The pretreatment of DAS (10 mg/kg body weight) showed 68.35% protection and post-treatment showed 59.49% protection, at an intermittent period of 48 hrs, against DMBA-induced DNA strand breakage. Wilson *et al.*[44] documented the inhibitory effect of DAS on 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP)-induced DNA strand breaks in

normal human breast epithelial cell lines, MCF-10A. PhIP is the most abundant heterocyclic amine (HCA) found in the human diet. Heterocyclic amines (HCAs) are formed when meat products such as beef, chicken, pork and fish are cooked at high temperatures. PhIP causes mammary carcinomas in female rats and mice and is associated with an increased risk of developing colon, breast and prostate cancer in humans. PhIP is metabolized by cytochrome P 450s producing N-OH-PhIP. N-OH-PhIP can be esterified by phase II enzymes forming an arylnitrenium ion that binds to DNA causing adducts. Furthermore, N-OH-PhIP may be reduced by cytochrome b^5 reductase producing superoxide anions and hydroxyl radicals, causing DNA strand breaks. PhIP produced reactive oxygen species causing DNA strand breaks and DAS inhibited PhIP-induced DNA strand breaks by inhibiting the production of reactive oxygen species.[44]

DAS also inhibited diethylstilbestrol (DES)-induced DNA adducts in microsomes, mitochondria and nuclei isolated from breast tissue of female ACI rats. The percent inhibition ranged from 86% in microsomes to 93% in nuclei. DES produced DNA adducts in mtDNA and nDNA. DAS completely inhibited the DES-induced mtDNA adducts and caused a dose dependent decrease in nDNA adduct formation.[45] DAS and crushed garlic have been demonstrated to inhibit O6-methylguanine formation in liver DNA of dimethylnitrosamine-treated rats. DAS produced 89% inhibition of O6MG formation in liver DNA and in lung and kidney the mean inhibitions were found to be 98% and 74%, respectively. Feeding 2.5% garlic for seven days inhibited DMN-induced O6MG formation in liver DNA by 46%.[46] These findings suggest that DAS can effectively check the mutations induced by environmental toxicants.

Induction of Cell-Cycle Arrest

Uncontrolled cellular division caused by the transformation of genetic material is a primary cancer characteristic. Non-neoplastic cell division is governed by a tightly controlled process that is regulated at several checkpoints by internal and external signals. The progression of the cell cycle through the four phases, G1, S, G2 and M, is regulated by cyclin dependent kinase (CDK) molecules and cyclins, which drive the cell from one

phase to the next and in turn are regulated by inhibitors. This process offers many potential targets for chemopreventive agents including DAS. Cell-cycle arrest occurs in response to cellular stress through activation of signal transduction pathways commonly referred to as checkpoints. Checkpoints are activated in the G1/S phase to prevent replication of damaged DNA or in the G2/M phase to prevent segregation of damaged chromosomes during mitosis. Checkpoints ensure completion of phase-specific events and help maintain genetic integrity. In normal cells, transition from the G1 to S phase during the cell cycle requires the activity of two classes of CDKs, CDK4/6 and CDK2. CDKs exist predominantly in multiple quaternary complexes, each consisting of a cyclin, a CDK, a proliferating cell nuclear antigen (PCNA) and cyclin-dependent kinase inhibitors (CDKIs). CDK4/6 associates with D-type cyclins, whereas CDK2 associates with either cyclin E initially or with cyclin A later in the cell cycle. CDK activity can be inhibited by two different families of cyclin-dependent kinase inhibitors, the INK4 and the Cip/Kip family. INK4 members specifically bind to the catalytic subunits of CDK4 and CDK6 and prevent their association with D-type cyclins. The INK4 family includes four proteins: p16INK4a, p15INK4b, p18INK4c and p19INK4d. In contrast to INK4, Cip/Kip proteins have a broader function in the cell cycle by affecting activities of cyclin D-, E- and A-dependent kinases. These include p21Waf1/Cip1, p27Kip1 and p57Kip2 and were identified as potent inhibitors of cyclin E- and A-dependent CDK2 but as possible positive regulators of cyclin D-dependent kinases.[47]

There is evidence that one of the mechanisms by which garlic extract and isolated organosulfur compounds including DAS exert their anticarcinogenic effects is by arresting the cell cycle at different stages of its progression. Sriram *et al.*[48] demonstrated that treatment of human colon cancer cells Colo 320 DM with DAS resulted in the cell-cycle arrest at the G2/M phase. DAS has been shown to enhance the ability of breast tissue to repair diethylstilbestrol (DES)-induced DNA damage by regulating the expression of Gadd45a, PCNA and DNA polymerase, which were down-regulated by DES.[49] Further, DAS was found to upregulate the levels of cyclin-dependent kinase inhibitor p21/Waf1, which in turn enhances the expression of the tumor suppressor wild-type (wt) p53 in DMBA-induced skin tumors in Swiss albino mice. p21/Waf1 is a downstream effector

molecule of p53.[50] Collectively, these studies indicate that cell-cycle arrest is a common cellular response to DAS.

Induction of Apoptosis

Apoptosis, or programmed cell death, is a physiological process characterized by cell shrinkage, blebbing of plasma membrane, nuclear condensation and DNA fragmentation. By eliminating damaged cells, regulation of cell number can be achieved by apoptosis, which turns out to be important in embryonic development, metamorphosis, tumor growth and hormone-dependent atrophy. Apoptosis is usually mediated through the activation of caspases. Mechanistically, two different types of apoptosis have been described: one that is caspase-8-dependent and receptor-mediated (type I or death receptor or extrinsic pathway), and the other that is caspase-9-dependent and usually mediated through the mitochondria (type II or mitochondrial or intrinsic pathway). Activation of the intrinsic apoptotic pathway is regulated by the Bcl-2 family of anti-apoptotic (e.g. Bcl-2 and Bcl-xL) and proapoptotic (e.g. Bax and Bak) proteins. In addition to numerous proteins including TNF/Fas ligands, p53, the Bcl-2 family and the caspases, the integrity of mitochondria is also important in the regulation of apoptosis. Disruption of mitochondria causes the release of cytochrome *c*, which in concert with ATP and apoptosis protease-activating factor 1, activates caspase-9, triggering a cascade of events culminating in the destruction of cells. Notably, the suppression of apoptosis by tumor promoters underlies the importance of apoptosis in the progression of tumors.[51]

Evidence has shown that organosulfur compounds including DAS exhibit their anticancer activity by inducing apoptosis. DAS has been shown to trigger apoptosis by modulating the levels of Bcl-2 proteins. Treatment with DAS increased the ratio of Bax/Bcl-2 in SH-SY5Y neuroblastoma cells, as well as in H460 and H1299 lung cancer cells compared with untreated controls.[52,53] DAS-induced apoptosis was associated with mitochondrial release of cytochrome *c*, increase in cytosolic Smac/Diablo, and down regulation of inhibitor-of-apoptosis proteins and nuclear factor-κB (NF-κB). DAS also activated caspase-9 and caspase-3, indicating its effect on the intrinsic pathway of apoptosis. Influence on calcium homeostasis is also believed to contribute to apoptosis by DAS. It is generally

accepted that excessive intracellular calcium is frequently associated with the activation of Ca2+-dependent endonucleases and apoptosis. Treatment with DAS resulted in an increase in intracellular Ca^{2+} level, which also contributed to DAS-induced apoptosis in SH-SY5Y neuroblastoma cells.[52] Treatment of human glioblastoma cells T98G and U87MG with DAS triggered the production of reactive oxygen species (ROS) that induced apoptosis with the phosphorylation of p38 MAPK and activation of the redox-sensitive JNK1 pathway together with overexpression of Bax, downregulation of Bcl-2 and some BIRC proteins, mitochondrial release of cytochrome *c* and Smac into the cytosol, and activation of calpain, caspase-9, and caspase-3. DAS treatment also enhanced intracellular Ca^{2+} level.[54]

DAS has also been shown to induce apoptosis in human colon cancer cells, Colo 320 DM, through increased production of ROS and expression of caspase-3 and suppression of extracellular regulatory kinase-2 (ERK-2) activity.[55] The aberration of tumor suppressor gene p53 and the ras oncogene have been linked to the induction of multiple signaling pathways and to the resistance offered by cancer cells to apoptosis. Kalra *et al*.[56] demonstrated the involvement of multiple signaling pathways in DAS-mediated apoptosis in DMBA-iduced mouse skin tumors in Swiss albino mice. They showed that DAS upregulated the expression of tumor suppressor protein p53 (wt p53), its downstream target molecule p21/Waf1, and proapoptotic protein Bax, and downregulated DMBA-induced antiapoptotic protein expression, survivin and Bcl-2. DAS also significantly reduced the expression of ras oncoprotein and DMBA-induced protein expressions of PI3K/Akt and p38MAPK, but it did not alter the expression of JNK1 and ERK1/2.[56]

Inhibition of Tumor Angiogenesis

Angiogenesis, the development of new blood vessels from the endothelium of a pre-existing vasculature, is a critical process required by most solid tumors to support their localized growth and metastatic dissemination within the host. Without angiogenesis, tumor expansion cannot proceed beyond 1–2 mm because tumor proliferation is severely limited by nutrient supply to, and waste removal from, the tumor into the surrounding medium. Thus, antiangiogenic therapy is a promising diversion

in the treatment of cancer. It is now widely accepted that angiogenesis is balanced between pro- and antiangiogenic molecules present in the body. The "angiogenic switch" is "off" when the effect of proangiogenic molecules is balanced by that of antiangiogenic molecules and "on" when the net balance is tipped in favor of angiogenesis. The proangiogenic factors which stimulate the growth of capillary vessels include vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), angiogenin, estrogens, basic and acidic fibroblast growth factor (bFGF and aFGF), transforming growth factor-α (TGF-α), TGF-β, tumor necrosis factor, platelet-derived endothelial growth factor, hepatocyte growth factor, angiogenin, interleukin 8, placenta growth factor, and matrix metalloproteinases (MMPs). Vascular endothelial growth factor (VEGF), a survival factor for endothelial cells by inhibiting apoptosis, is a key angiogenic factor frequently used by tumors and tissues to switch on their angiogenic phenotypes. This potent and unique angiogenic protein stimulates capillary formation, endothelial cell migration and proliferation as well as increases vascular permeability. DAS has been shown to inhibit B16F-10 melanoma induced tumor specific capillary formation in C57BL/6 mice by blocking the VEGF signaling pathway. DAS significantly reduced the highly elevated circulating levels of VEGF immediately after tumor challenge by downregulating VEGF mRNA expression. DAS also decreased the circulatory levels of proinflammatory cytokines such as IL-1β, IL-6, TNF-α and GM-CSF, which act as autocrine growth factors for tumor angiogenesis. Studies with human umbilical vein endothelial cells (HUVECs) demonstrated that DAS blocks angiogenesis by inhibiting VEGF-induced endothelial cell proliferation, migration and invasion. Further, DAS significantly inhibited VEGF-induced tube formation of HUVECs on matrigel. Incubation of HUVECs on matrigel, a replica of the extracellular matrix (ECM), with VEGF resulted in the formation of elongated and tube-like structures. The formation of tubes on matrigel involves endothelial cell attachment, migration and production of enzymes capable of remodeling ECM. DAS effectively reduced the width and length of endothelial tubes. Recent studies have clearly demonstrated that MMPs, a family of Zn-dependent endopeptidases that are able to degrade the extracellular matrix (ECM), mediate the release

and accumulation of VEGF from the cell matrix and trigger the angiogenic switch by rendering VEGF bioavailable to its receptors. DAS has been found to regulate the levels of MMPs by significantly promoting the levels of tissue inhibitor of metalloproteinase-1 (TIMP 1), an endogenous inhibitor of MMPs.[57] DAS was also found to inhibit the angiogenesis in Ehrlich ascites (EA) tumor bearing Swiss albino mice in a dose-dependent manner.[58]

Modulation of Immune System

Tumor development, outgrowth and metastasis are under the surveillance of the immune system. The fate of host-tumor interactions depends on the balance between the intrinsic metastatic potential of the tumor and the strength of the host immune response. Tumor cells evade host defenses by the production of soluble factors that downregulate the function of lymphocytes, macrophages and NK cells. In fact, cancer can be considered to be a result of the failure of human immune surveillance. Immunomodulators are substances that modify the activity of the immune system. They have biphasic effects: some tend to stimulate the immune system, which are low; others inhibit host parameters, which are normal or already activated. DAS is a potent immunomodulator and it has been demonstrated to stimulate the immune system of BALB/c mice by enhancing total WBC count, bone marrow cellularity and the number of α-esterase positive cells. Further treatment with DAS along with the antigen sheep red blood cells (SRBC) produced an enhancement in the circulating antibody titer and the number of plaque forming cells (PFC) in the spleen of animals.[59] Jeong and Lee[60] reported the protective effect of DAS on the N-nitrosodimethylamine (NDMA)-induced immunosuppression of humoral and cellular responses in BALB/c mice. Oral administration of DAS prior to NDMA treatment for 14 consecutive days blocked the NDMA-induced suppression of the antibody response to a T-cell-dependent antigen, sheep erythrocytes, and the lymphoproliferative response to the T-cell and the B-cell mitogens in a dose-dependent manner.

One of the major drawbacks of current cancer therapeutic practices, such as chemotherapy and radiotherapy, is immunosuppression. DAS has

been shown to prevent radiation-induced immunosuppression in Swiss albino mice. Further administration of DAS reduced the serum content of alkaline phosphatase (ALP) and lipid peroxides, which were elevated after irradiation. DAS also downregulated the elevated level of glutamate pyruvate transaminase (GPT) in liver after irradiation. In addition, DAS significantly enhanced the glutathione (GSH) content in liver and intestinal mucosa, which was drastically reduced after irradiation.[59] DAS was also found to alleviate cyclophosphamide (CTX)-induced urotoxicity in Swiss albino mice. Morphological analysis of the urinary bladders of the CTX-treated group showed severe inflammation and dark coloration whereas the DAS-treated group showed almost normal bladder morphology. Moreover, DAS significantly enhanced GSH content (which was drastically reduced by CTX administration) in both bladder and liver.[62]

Modulation of Inflammation

Although chronic inflammation and carcinogenesis are thought to be mechanistically linked, very little data are available on the anti-inflammatory effects of DAS. Chronic inflammation and infections lead to the up-regulation of a series of enzymes and signaling proteins in affected tissues and cells. These proinflammatory enzymes include the inducible forms of nitric oxide synthase (iNOS) and cyclooxygenase (COX-2). NO is synthesized by oxidative deamination of L-arginine by a family of three distinct nitric oxide synthases (NOSs). Unlike the constitutively expressed and Ca^{2+}-dependent neuronal (NOS1) and endothelial (NOS3) isoforms, the expression of Ca^{2+}-independent iNOS (NOS2) is inducible by bacterial endotoxins and cytokines to provide a sustained release of NO. Although iNOS provides a benefit to the organism in terms of immune surveillance, aberrant or overproduction of NO has been implicated in the pathogenesis of cancer via reactive NO-species-mediated reactions like nitrosative deamination of DNA bases, lipid peroxidation and DNA strand breaks. NO can also act as an endothelial growth factor to mediate tumor vascularization and tumor blood flow. COX-2 is responsible for the conversion of arachidonic acid to prostaglandins (PGs). Elevated levels in the expression of the inducible COX-2 have been detected in various tumor types and may account for excessive PG production. In addition to their

role as proinflammatory mediators, PGs have been demonstrated to suppress immune functions, inhibit apoptosis, enhance proliferation and increase the invasiveness of cancer cells. Consequently, inhibition of expression and enzymatic activity of COX-2 and downregulation of PG levels is regarded as a rational and feasible strategy in cancer chemoprevention with the first positive results in human trials. TNF-α and other inflammatory cytokines have also been shown to stimulate tumor promotion and progression of initiated cells as well as of preneoplastic lesions. Thus, TNF-? can be considered as an endogenous tumor promoter and a central mediator in cancer development.

Sengupta *et al*.[63] demonstrated that DAS reduced colon carcinogenesis by reducing the incidence of aberrant crypt foci by 43.65% through the reduced expression of cyclooxygenase-2 and inducible nitric oxide synthase. DAS was demonstrated to attenuate bleomycin-induced pulmonary fibrosis in Wistar rats by reducing bleomycin-induced activation of inducible nitric oxide synthase (iNOS) and nuclear factor kappa-B (NF-κB) and downregulating the elevated levels of the early inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), in the lung tissues.[64] Chang and Chen[65] reported that DAS decreased stimulated NO and PGE2 production in lipopolysaccharide (LPS)-activated Raw 264.7 cells by inhibiting inducible NO synthase and cyclooxygenase-2 expressions. DAS also downregulated the levels of proinflammatory cytokines including TNF-α, interleukin (IL)-1β, IL-6 and IL-10 in lipopolysaccharide (LPS)-activated Raw 264.7 cells.[66] DAS was found to inhibit monosodium urate (MSU) crystals and IL-1β-induced joint tissue inflammatory responses by downregulating the expression of COX-2 and NF-κB. MSU crystals upregulated COX-2 expression and PGE2 in HIG-82 cells and this was inhibited by co-incubation with DAS. DAS also inhibited MSU crystal and IL-1β-induced elevation of COX-2 expression in primary synovial cells and chondrocytes.[67]

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Research over last two decades has revealed that garlic derived OSCs including DAS does not only inhibit chemically-induced cancers but can

also suppress the growth of cancer cells in culture and *in vivo*. Studies in experimental animals indicate that the benefits of DAS are not limited to one tissue or carcinogen, and it can interfere with several cell-signaling pathways, including inhibition of carcinogen-activating enzymes, induction of carcinogen-detoxifying enzymes, induction of apoptosis, arrest of cell-cycle progression, inhibition of inflammation and angiogenesis, as well as several other mechanisms that are not yet fully described. These mechanisms show the remarkable ability of DAS to act on the process of carcinogenesis by affecting the three phases: tumor initiation, promotion and progression, and suppressing the final steps of carcinogenesis, i.e. angiogenesis and metastasis. At an early stage, DAS can modulate the enzymes that are required for the activation or detoxification of many carcinogens, and it has been shown to induce the activity of phase II enzymes (GSTs) and/or inhibit phase I enzymes (cytochrome P450s). Furthermore, recent evidence illustrates that DAS acts to arrest cancerous cell-cycle progression and hence may also retard the development of later stages of carcinogenesis. Cell-cycle arrest by DAS occurs through an irreversible G2/Mphase arrest via a mechanism involving a reduction of key G2/M-regulating proteins, whereas overexpression of Bax, downregulation of Bcl-2 and activation of caspase-8 and caspase-9 are implicated in DAS-mediated apoptosis. Finally, DAS may exert anticarcinogenic effects by the regulation of genes involved in the inflammation pathway and endothelial cell functions, including the proliferation, invasion and migration of endothelial cells, tubular formation and MMP production. The molecular targets for the anticancer activity of DAS are summarized in Fig. 2.

There are several issues to be addressed before the development of this OSC as a drug in actual treatment of cancer. The first and perhaps most important issue relevant to clinical application of DAS is whether the micromolar concentrations shown to be effective against cultured cancer cells are achievable in humans. In addition, a detailed toxicology of DAS is equally important for its clinical development. To this end, more clinical trials are needed to validate the use of this agent both alone and in combination for cancer chemotherapy. One of the most difficult problems of chemopreventive drug testing is lengthy trials due to the long development period of the majority of cancers. Hence, a comprehensive stepwise

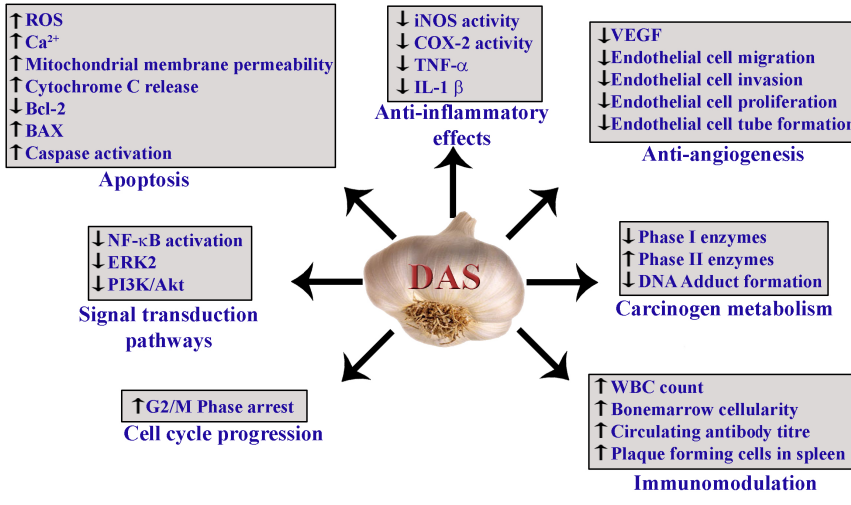

Fig. 2. Molecular targets for the anticancer activity of DAS.

approach must be taken to elucidate how dietary modifications and chemoprevention can be harnessed effectively for cancer prevention and control.

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9

Ginger (6-gingerol)

Nidhi Nigam, Jasmine George and Yogeshwer Shukla*

Ginger, the rhizome of *Zingiber officinalis*, one of the most widely used species of the ginger family, is a common condiment for various foods and beverages which is equally reputed for its medicinal properties. Ginger has been traditionally used from time immemorial for varied human ailments in different parts of the globe, to aid digestion and treat nausea and vomiting in pregnancy. Some pungent constituents present in ginger and other zingiberaceous plants have potent antioxidant, anti-inflammatory, antiemetic, antiulcer, cardiotonic, antihypertensive, hypoglycemic, antihyperlipidemic and immunostimulant properties, and some of them exhibit cancer preventive activity in experimental and clinical trials. These properties of ginger are attributed to the presence of certain pungent vallinoids, *viz.* [6]-gingerol and [6]-paradol, as well as some other constituents like shogaols and zingerone. Experimental studies have also revealed that ginger and its most active constituent, [6]-gingerol, regulate the molecules in the cell signal transduction pathways including nuclear factor-kappa B (NF-κB), activator protein-1 (AP-1), growth factors, chemokines, mitogen-activated protein kinase (MAPK), p53, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) pathways. By modulating cell signaling pathways, these components, among other mechanisms, activate cell regulatory signals and also induce apoptosis in precancerous or cancer cells, resulting in the inhibition of cancer development and/or

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progression. This chapter provides comprehensive information on the biological and therapeutic role of ginger ([6]-gingerol) and reviews current studies regarding the effects of ginger on cancer and other disease-related molecular pathways. Although all the evidence from research on ginger is very promising, future studies are necessary to fully understand its contributions to human health, and advise its regular consumption in our diets, in which it is currently limited and sporadic.

INTRODUCTION: OTHER NAMES AND TRADITIONAL USES

The past several decades have presented mounting evidence that points to dietary habits as an important determinant of disease risk. Although the linkages with diet are intriguing, the literature is also laden with inconsistencies. The reasons for these inconsistencies are likely multi-factorial but probably reflect variations in the ability of bioactive constituents to reach or affect critical molecular targets.[1] Fluctuations in the foods consumed not only influence the intake of particular bioactive components but may alter metabolism and potentially influence the sites of action of both essential and nonessential nutrients.[2] Natural dietary agents including fruits, vegetables and spices have drawn a great deal of attention from both the scientific community and the general public due to their various health promoting effects, notably including suppression of cancers. Dietary agents consist of a wide variety of biologically active compounds that are ubiquitous in plants and many of them have been used as traditional medicines for thousands of years.[1]

Ginger is native to India and China. It takes its name from the Sanskrit word *stringa-vera*, which means "with a body like a horn," as in antlers. The English botanist William Roscoe (1753–1831) gave the plant the name *Zingiber officinale* (family: Zingiberaceae), in an 1807 publication. Ginger has been important in Chinese medicine for many centuries and is mentioned in the writings of Confucius. It is also named in the Koran, the sacred book of the Muslims, indicating it was known in Arab countries as far back as 650 AD. It was one of the earliest spices known in Western Europe, used since the 9th century. It became so popular in Europe that it was included at every table setting, like salt and pepper. The other names of the spice are:

East Indian pepper; Jamaica ginger; Jamaica pepper; *gingembre* (French); *Ingwer* (German); *zenzero* (Italian); *jengibre* (Spanish); cheung, chiang, jeung (Burmese); *adruk* (green), *ard(r)ak(h)* (green), *sont(h)* (dried) (Indian); *aliah* (Indonesian); *mioga*, *myoga*, *shoga* (Japanese); *k(h)ing* (green) (Thai).[3]

Ginger is a perennial creeping plant, with a thick tuberous rhizome, producing an erect stem 30–100 cm (1–3 ft) tall. The lance-shaped leaves are bright green, 15–20 cm (6–8 inches) long, with a prominent longitudinal rib, enclosing conical clusters of small yellow-green flowers marked with purple speckles. It is propagated from rhizome cuttings, planted on rich, well drained loam. It requires a tropical climate with both a heavy rainy season and a hot dry season. Plants shoot in ten days and are harvested after nine to ten months (Figs. 1a and 1b). Ginger is used

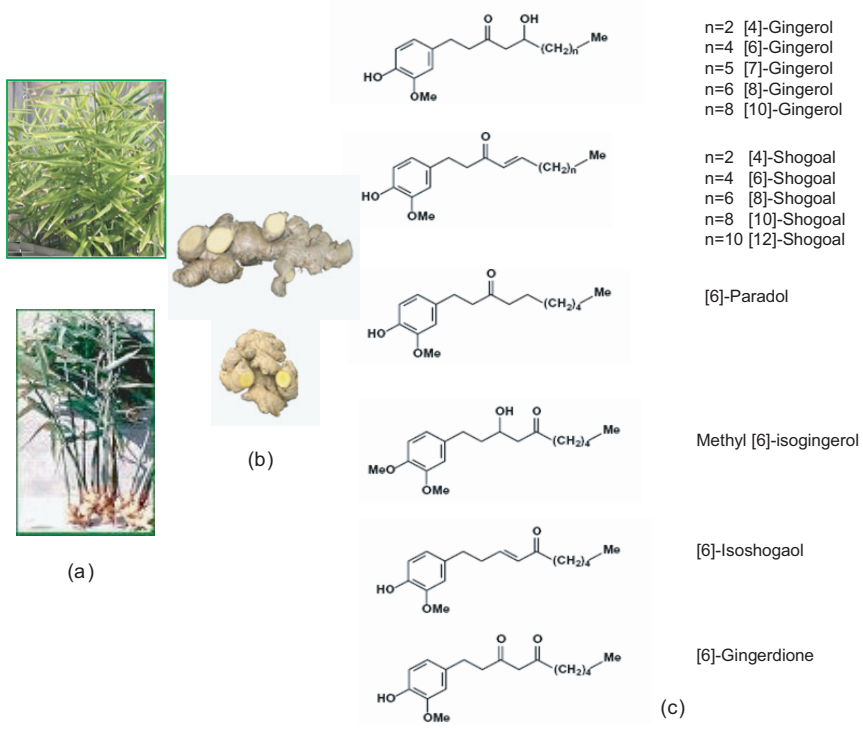

Fig. 1. **(a)** Ginger plant, **(b)** ginger rhizome and **(c)** chemical structure of major chemical constituents of ginger.

extensively in traditional Chinese medicine to treat headaches, nausea, febrile conditions and colds; and in Ayurvedic and Western herbal medical practices for the treatment of arthritis, rheumatic disorders and muscular discomfort.[4,5] In South Africa, the fresh (or dried) rhizomes of ginger are used medicinally as stomachics and tonics to treat indigestion or dyspepsia, flatulence and nausea.[6] The dried rhizomes of ginger are also used traditionally in other parts of the world for a variety of human ailments, including the treatment, management and/or control of diarrhea, dysentery, fever, cough, ulcers, boils and wounds. Other reported pharmacological effects of ginger rhizomes include antimicrobial, analgesic, antipyretic, antiemetic, antiulcer, anxiolytic, cardiotonic, antihypertensive, hypoglycemic, antihyperlipidemic, anti-inflammatory and immunostimulant properties[5,7] (Fig. 2). Besides these well-recognized effects of ginger and its constituents on laboratory animals, cell culture and clinical studies reveal that certain constituents of ginger play a pivotal role in the etiology and prevention of various types of human cancer[8] (Fig. 2). This chapter encompasses comprehensive knowledge of the

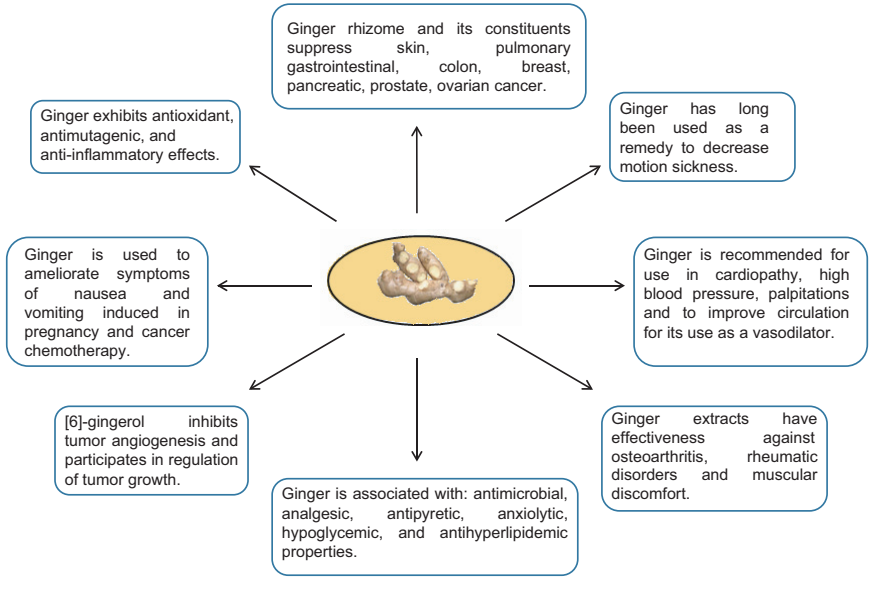

Fig. 2. Biological properties of ginger and its active constituents.

target molecules and the biological and therapeutic uses of ginger and its active constituents in the treatment of cancer and other diseased conditions.

CHEMICAL CONSTITUENTS

The constituents of ginger are numerous and vary depending on the place of origin and whether the rhizomes are fresh or dry. Though the pungency of fresh ginger is primarily due to the presence of gingerols, which are a homologous series of phenolic ketones, the sensory perception of ginger in the mouth and the nose are contributed by two distinct groups of chemicals:

(i) *Volatile oils:* The volatile oil components in ginger consist mainly of sesquiterpene hydrocarbons, predominantly zingeberene (35%), curcumene (18%) and farnesene (10%), with smaller amounts of bisabolene and b-sesquiphellandrene. A smaller percentage of at least 40 different monoterpenoid hydrocarbons are present with 1,8-cineole, linalool, borneol, neral, and geraniol being the most abundant (see Shukla and Singh[8] and references cited therein). Many of these volatile oil constituents contribute to the distinct aroma and taste of ginger. Diarylheptanoids have been reported as components of both fresh and dry ginger (see Jolad *et al*.[9,10] and references cited therein).

(ii) *Nonvolatile pungent compounds:* Biologically active constituents include the nonvolatile pungent principles such as the gingerols, shogaols, paradols and zingerone that produce a ‘‘hot’’ sensation in the mouth. The gingerols, a series of chemical homologs differentiated by the length of their unbranched alkyl chains, were identified as the major active components in the fresh rhizome (see Shukla and Singh[8] and references cited therein). The major gingerol is [6]-gingerol, while [8]- and [10]-gingerol occur in smaller quantities. The gingerols are thermally unstable and are converted under high temperature to [6]-, [8]- and [10]-shogaol, another homologous series and the dehydrated form of the gingerols (after *shoga*, the Japanese word for ginger). Paradol is similar to gingerol and is formed

on hydrogenation of shogaol. The major constituents of ginger are shown in Fig. 1c.

Jolad *et al.* examined organically-grown fresh ginger and identified [4]-, [7]-, [8]- and [10]-gingerol in addition to [6]-gingerol, as well as methyl [4]-gingerol and methyl [8]-gingerol. [4]-, [6]-, [8]-, [10]- and [12]-shogaol were characterized, as methyl [4]-, methyl [6]- and methyl [8]-shogaol.[9] Paradols are 5-deoxygingerols. [6]-paradol, along with [7]-, [8]-, [9]-, [10]-, [11]- and [13]-paradols, have been detected in fresh ginger, as was methyl [6]-paradol, methyl [8]-paradol, methyl [6]-isogingerol and [6]-isoshogaol. Jolad *et al.* also examined commercially processed dry ginger using the same techniques that they had utilized in their earlier study.[9] They detected [6]-, [8]-, [10]- and [12]-gingerdiones, which had not previously been reported in fresh ginger.[10] The concentrations of gingerols in dry ginger were reduced slightly in comparison to fresh ginger, whereas the concentrations of shogaols increased (Fig. 1c).

(iii) *Other constituents:* In addition to the extractable oleoresins, ginger contains many fats, waxes, carbohydrates, vitamins and minerals. Ginger rhizomes also contain a potent proteolytic enzyme called zingibain.

MOLECULAR TARGETS

Nuclear Factor-kappa B

A number of studies have shown that ginger and its active constituents exert their anticancer effects through the suppression of nuclear factor-kappa B (NF-κB) (see Aggarwal and Shishodia[1] and references cited therein). In an *in vitro* study, [6]-gingerol enhances TRAIL-induced viability reduction by inhibiting TRAIL-induced NF-κB activation while [6]-shogaol alone reduces viability by damaging microtubules.[11] Prior treatment of [6]-gingerol inhibits translocation of NF-κB from cytosol to nucleus via suppression of IkappaBalpha (I-κBα) phosphorylation (ser-32) in *in vivo* and *in vitro* assays. It also reduced UVB-induced expression and transactivation of COX-2.[12,13] Zerumbone's inhibition of expression of NF-κB-regulated genes also correlated

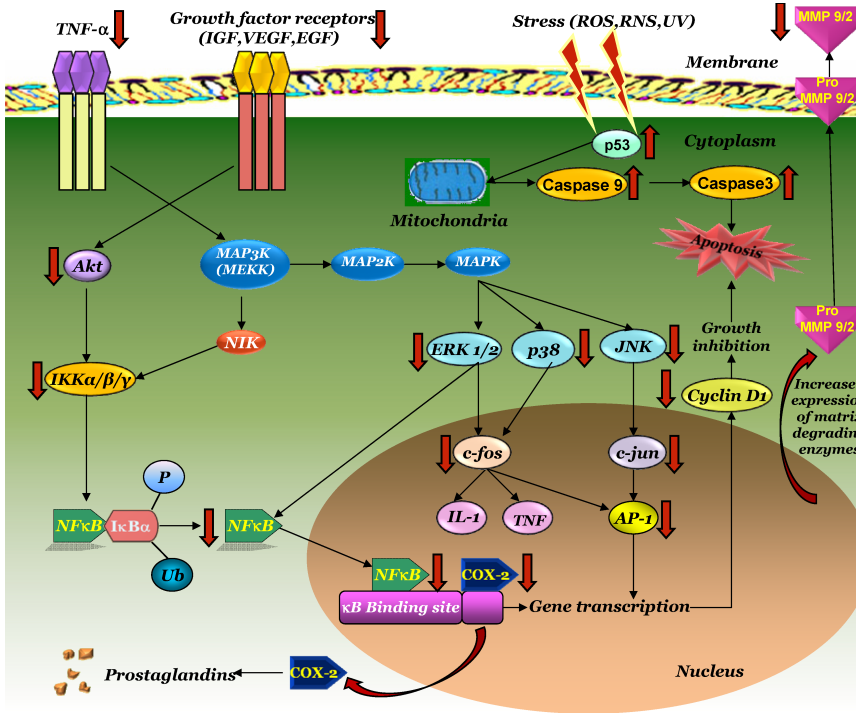

Fig. 3. Molecular targets of ginger and its active constituents against various forms of cancers and other chronic diseases.

with the suppression of tumor necrosis factor (TNF)-induced invasion activity[14] (Fig. 3).

Activator Protein-1

Activator protein-1 (AP-1) activation is linked to growth regulation, cell transformation, inflammation, and innate immune response. These oncogenic properties of AP-1 are primarily dictated by the dimer composition of the AP-1 family proteins and their post-transcriptional and translational modifications (see Aggarwal and Shishodia[1] and references cited therein). [6]-gingerol inhibited epidermal growth factor (EGF)-induced AP-1 transactivation by blocking EGF-induced AP-1 DNA binding activity in a concentration-dependent manner, and in contrast, [6]-paradol had

no effect on AP-1 activation and appears to act through induction of apoptosis[15] (Fig. 3).

Growth Factor Signaling and Angiogenesis

Tumor angiogenesis is the proliferation of a network of blood vessels that penetrates into cancerous growths, supplying nutrients and oxygen and removing waste products. Potent cell proliferation signals generated by various angiogenic growth factor receptors such as the EGF receptor, insulin-like growth factor (IGF-1) receptor, and vascular endothelial growth factor (VEGF)-receptor networks constitute the basis for receptor-driven tumorigenicity in the progression of several cancers (see Aggarwal and Shishodia[1] and references cited therein). Ginger treatment resulted in inhibition of NF-κB activation as well as diminished secretion of VEGF and IL-8 in ovarian cancer cells[16] and, conversely, stimulation of AP-1 by 12-O-tetradecanoylphorbol 13-acetate (TPA) substituted for EGF in the induction of epithelial-mesenchymal transition by transforming growth factor-beta1 (TGF-beta1) in cells containing normal Ras.[17] [6]-gingerol inhibits angiogenesis by inhibiting both the VEGF- and bFGF-induced proliferation of human endothelial cells and causes cell-cycle arrest in the G1 phase. It has also been shown to block capillary-like tube formation by endothelial cells in response to VEGF, and strongly inhibited sprouting of endothelial cells in the rat aorta and formation of new blood vessels in the mouse cornea in response to VEGF[12,13] (Fig. 3).

Cell-Cycle Arrest and Apoptosis

Major control switches of the cell cycle are the cyclins and the cyclin-dependent kinases (Cdk). Cyclin D1, a component subunit of Cdk4 and Cdk6, is a rate-limiting factor in progression of cells through the first gap (G1) phase of the cell cycle (see Aggarwal and Shishodia[1] and references cited therein). [6]-gingerol stimulates apoptosis through upregulation of NAG-1 and G(1) cell-cycle arrest through downregulation of cyclin D1.[18] Apoptosis helps to establish a natural balance between cell death and cell renewal in mature animals by destroying excess, damaged or abnormal cells. Our laboratory and others have demonstrated the modulation of

[6]-gingerol on apoptosis related proteins both *in vitro* and *in vivo*, via upregulation of p53, Bax, caspase-9 and caspase-3 and downregulation of antiapoptotic proteins, Bcl-2 and survivin expression.[13,19,20] In 2005, Wei *et al.* reported significant cytotoxic and apoptotic activities against human promyelocytic leukemia cells of several ginger constituents, including some diarylheptanoids and gingerol-related compounds.[21] They showed that the following structural features contribute significantly to the enhancement of activity: (i) acetoxyl groups at the 3- and/or 5-positions of the side chain, (ii) the appropriate longer alkyl side chain length, (iii) the ortho-diphenoxyl functionality on the aromatic ring, and (iv) the a,b-unsaturated ketone moiety in the side chain (Fig. 3).

Chemokines and Metastasis

Chemokines are small chemotactic cytokines that direct migration of leukocytes, activate inflammatory responses, and participate in regulation of tumor growth. [6]-gingerol has been shown to inhibit cell adhesion, invasion, motility and activities of matrix metalloprotein (MMP)-2 and -9 in MDA-MB-231 human breast cancer cell lines.[22] Studies document that the ginger (*Zingiber officinale* and *Alpinia galanga*) extract (GE) inhibits lipopolysaccharide (LPS), cytokine, chemokines, and amyloid Abeta peptide-induced expression of the proinflammatory genes TNF-alpha, IL-1beta, COX-2, monocyte chemoattractant protein-1 (MCP-1), and interferon-gamma inducible protein-10 (IP-10) in these microglial-like cells[5] and IL-2 production by T-cells in response to allostimulation and RANTES, MCP-1 (pro-inflammatory chemokines) production in LPS-stimulated macrophages and indirectly inhibits T-cell activation[23] (Fig. 3).

Tumor Necrosis Factor

Tumor necrosis factor (TNF), initially discovered as a result of its antitumor activity, has now been shown to mediate tumor initiation, promotion and metastasis. Almost all cell types, when exposed to TNF, activate NF-κB, leading to the expression of inflammatory genes. Because of the critical role of TNF in mediating tumorigenesis, agents that can suppress

TNF activity have potential for therapy in TNF-linked diseases (see Aggarwal and Shishodia[1] and references cited therein). [6]-gingerol is reported as an effective inhibitor of TNF-α induced c-Jun-NH(2)-terminal kinase signaling activation.[24] NF-κB inhibition by zerumbone has been reported to be correlated with sequential suppression of the downstream regulators along with inhibition of the NF-κB-dependent reporter gene expression activated by TNF.[14] Ginger itself has been shown to have no inhibitory effect on the growth of influenza virus but could exert its effect via macrophage activation leading to production of TNF-α[25] (Fig. 3).

Cyclooxygenase-2

Cyclooxygenases (COXs) are prostaglandin-H-synthases, which convert arachidonic acid released by membrane phospholipids into prostaglandins. Depending upon the stimulus and the cell type, several transcription factors including AP-1, NFIL-6, NF-κB can stimulate COX-2 transcription (see Aggarwal and Shishodia[1] and references cited therein). Gingerols have been shown to inhibit LPS-induced COX-2 expression and thus are also capable of inhibiting PGE(2) production.[26] Ginger constituents, [8]-paradol and [8]-shogaol, as well as two synthetic analogs, 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-decane and 5-hydroxy-1-(4-hydroxy-3-ethoxyphenyl)-dodecane, showed strong inhibitory effects on COX-2 enzyme activity due to the presence of (i) lipophilicity of the alkyl side chain, (ii) a substitution pattern of hydroxy and carbonyl groups on the side chain, and (iii) a substitution pattern of hydroxy and methoxy groups on the aromatic moiety[27] (Fig. 3).

Lipoxygenase

Lipoxygenases (LOXs) are the enzymes responsible for generating leukotrienes (LT) from arachidonic acid. Members of the gingerol family of pungent compounds can act as dual inhibitors of arachidonic acid metabolism by the inhibition of human neutrophil 5-lipoxygenase activity, which could account in part for the anti-inflammatory and analgesic properties of compounds within this group (see Aggarwal and Shishodia[1] and references cited therein). [6]-gingerol should be active against arachidonate

5-LOX, an enzyme of LT biosynthesis. This was verified by testing its inhibitory effects on 5-lipoxygenase prepared from rat basophilic leukemia RBL-1 cells,[28] which distinguishes ginger from nonsteroidal anti-inflammatory drugs[5] (Fig. 3).

Inducible Nitric Oxide Synthase

Inducible nitric oxide synthase (iNOS) is responsible for the release of the gaseous free radical nitric oxide (NO) during the formation of L-citrulline from L-arginine. Excessive and prolonged iNOS-mediated NO generation has been linked with inflammation and tumorigenesis (see Aggarwal and Shishodia[1] and references cited therein). The inhibitory effect of a stable [6]-gingerol metabolite RAC-[6]-dihydroparadol ([6]-DHP) and a closely related gingerol analog RAC-2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-dodecan-3-one [a capsaicin/gingerol (Capsarol) analog referred to as ZTX42] on NO production was observed in murine macrophages by partially inhibiting iNOS enzymatic activity and reducing iNOS protein production, via attenuation of NF-κB-mediated iNOS gene expression, providing a rationale for the anti-inflammatory activity reported for this class of compound.[29] [6]-gingerol has also shown inhibition of NO production and significant reduction of inducible iNOS in LPS-stimulated J774.1 cells[30] (Fig. 3).

Vanilloid Receptor Subtype-1

The discovery of gingerols as potent vanilloid receptor subtype-1 (VR1) agonists serves to explain the traditional and recent use of ginger for pain relief in rheumatic and inflammatory conditions.[3,31] [6]-gingerol and [8]-gingerol evoked capsaicin-like intracellular Ca^{2+} transients and ion currents in cultured DRG neurons. These effects of gingerols were blocked by capsazepine, the VR1 receptor antagonist. The potency of gingerols increased with increasing size of the side chain and with the overall hydrophobicity in the series.[4] Therefore, further exploring the functional groups on the side chain of gingerol structures would allow for development of more desirable pharmacological compounds acting via the VR1 receptor for controlling pain (Fig. 3).

PHARMACOLOGICAL PROPERTIES

Ginger has been extensively studied for its pharmacological activities.[32] It has been used safely for thousands of years in cooking and medicinally in folk and home remedies. The National Center for Complementary and Alternative Medicine (NCCAM) has evaluated the results of the available studies on ginger, rating the reports from "suggestive" (short-term use of ginger for safe relief from pregnancy related nausea and vomiting), to "mixed" (when used for nausea caused by motion sickness, chemotherapy, or surgery), to "unclear" (for treating rheumatoid arthritis, osteoarthritis, or joint and muscle pain).[33] Ginger is also well known all over the world especially for its use in disorders of the gastrointestinal tract such as constipation, dyspepsia, diarrhea, nausea and vomiting[3] (Fig. 2).

Antimicrobial Effects

In assessments of ethnobotanical uses of plants, ginger was found to significantly inhibit a wide range of bacteria and fungi pathogenic to humans.[34,35] Significant antifungal activities were evident with extracts from members of the Zingiberaceae, in particular those of *A. galanga*, *C. zedoaria*, *Z. purpureum* and *Z. officinale*. Of specific interest are those extracts that inhibited several fungi that are resistant to commonly used antifungals. As species belonging to the Zingiberaceae family are generally regarded as safe for human consumption, they are excellent candidates for development as antifungal phytomedicines.[34] *In vitro* studies have shown that active constituents of ginger inhibit multiplication of the colon bacteria that ferment undigested carbohydrates causing flatulence. Ginger also inhibits the growth of *Escherichia coli*, *Proteus sp*., *Staphylococci*, *Streptococci* and *Salmonella*.[35] GE has an antimicrobial action at levels equivalent to 2000 mg/ml of the spice. Fresh ginger juice showed an inhibitory action against *A. niger*, *S. cerevisiae*, *Mycoderma* sp. and *L. acidophilus* at 4%, 10%, 12% and 14%, respectively, at ambient temperatures.[36]

Analgesic and Hypoglycemic Effects

Based on earlier studies[3,32] it is speculated that [6]-gingerol is most probably responsible for the analgesic and hypoglycemic effects of GE.

GE has been shown to produce significant analgesic effects against thermally- and chemically-induced nociceptive pain in mice. The plant extract (50–800 mg/kg p.o.) also significantly inhibited fresh egg albumin-induced acute inflammation, and caused dose-related significant hypoglycemia in normal (normoglycemic) and diabetic rats.[37] On the basis of these beneficial effects, ginger rhizomes can be recommended for use as a natural supplementary herbal remedy in patients suffering from painful, arthritic and other inflammatory conditions, and/or diabetes mellitus.

Cardioprotective Effects

Ginger is also recommended by traditional healers in South Asia for use in cardiopathy, to treat high blood pressure and palpitations, and to improve the circulation with its use as a vasodilator.[32] Ghayur and Gilani reported that the methanolic extract of fresh ginger exhibits hypotensive, endothelium-independent vasodilator and cardiosuppressant properties through its specific inhibitory action at the voltage-dependent calcium channels.[7] The aqueous extract of fresh ginger has been reported to lower blood pressure through cholinergic and calcium channel blocking properties and possesses a combination of cardio-suppressant and cardio-stimulant actions sensitive to blockade of Ca^{2+} release from the cardiac sarcoplasmic reticulum evident only in the presence of cholinergic receptor blockade in experimental animals. Apart from the crude extract, some of the known pungent principles of ginger, namely [6]-gingerol, [8]-ginegrol, [10]-gingerol and [6]-shogaol, have also been tested on the vascular tissues (due to the limited quantity, they were only screened in aorta) and were found to exhibit a vasodilator effect through a combination of NO-releasing and calcium antagonist mechanisms.[38] [6]-gingerol was found to exhibit strong inhibition against lipid peroxidation in low-density lipoprotein induced by 2,2′-azobis(2-amidinopropane) hydrochloride and hemin and as well as against lipid peroxidation of erythrocyte membranes in a tert-butylhydroperoxide (tBHP)/hemin oxidation system, and so might have potential applications in pro-oxidant state-related cardiovascular disorders.[39]

Antioxidant and Anti-inflamatory Effects

Since tumor promotion is closely linked to inflammation and oxidative stress, a compound that exhibits anti-inflammatory and/or antioxidant properties could act as an anticarcinogenic agent. This has clearly been demonstrated by inhibition of phospholipid peroxidation induced by the $FeCl_3$-ascorbate system and its inhibitory effect on the xanthine oxidase system, which is responsible for the generation of reactive oxygen species such as superoxide anions. Another study reported concentration-dependent (0.5–10 μM) inhibition by [6]-gingerol of arachidonic acid induced platelet aggregation and formation of thromboxane B2 and prostaglandin D2 (see Shukla and Singh[8] and references cited therein). In one study, the oxidative stress induced by malathion (a pesticide) in rats was overcome by introducing ginger into the rats' diets.[40] The antioxidant activity of ginger was shown to be as effective as vitamin C in lowering lipid peroxidation in rats by influencing the enzymatic blood level of superoxide dismutase, catalase, and glutathione peroxidase.[41] A strong positive effect of ginger on plasma lipid composition, which may be important for the prevention of atherosclerotic events, was also demonstrated in apolipoprotein E-deficient mice (i.e. mice that are prone to develop atherosclerosis), showing a significant reduction in basal concentration of LDL-associated lipid peroxides in mice that consumed ginger in their drinking water.[42]

IN VITRO STUDIES

Studies have suggested that these compounds suppress the proliferation of human cancer cells and exert inhibitory effects on the viability of human HL-60 (promyelocytic leukemia) through the induction of apoptosis cells. Exposure of Jurkat human T-cell leukemia cells to various ginger constituents galanals A and B (isolated from the flower buds of Japanese ginger) resulted in apoptosis mediated through the mitochondrial pathway accompanied by a downregulation of antiapoptotic protein Bcl-2 and an enhancement of proapoptotic protein Bax expression. The rhizome of ginger has been reported to possess antitumor promotional potential as determined by abrogation of activation induced

by a phorbol-ester promoter, 12-O-hexadecanoylphorbol-13-acetate (HPA) induced Epstein–Barr virus (EBA) activation in Raji cells in short-term *in vitro* assay. In another short-term *in vitro* assay involving Epstein–Barr virus early antigen (EBV-EA) activation in Raji cells promoted by phorbol ester, TPA and ginger extract exhibited potent anti-EBV-EA activity. The rhizome extract, which exhibited EBV activation inhibitory activity, had no cytotoxic effect in Raji cells (see Shukla and Singh[8] and references cited therein). Also, studies have shown that the putative chemopreventive effect of ginger varies according to the specific type of cancer.

Skin Cancer

In vitro, pretreatment with [6]-gingerol reduced UVB-induced intracellular reactive oxygen species levels, activation of caspase-3, -8, -9, COX-2 and Fas expression. Translocation of NF-κB from cytosol to nucleus in human keratinocyte (HaCaT) cells was inhibited by [6]-gingerol via suppression of I-κBα phosphorylation (ser-32)[12,13] as well as EGF-induced cell transformation and AP-1 activation in JB6 cells.[15] The inhibition of the AP-1 transcriptional complex by [6]-gingerol, in HaCaT cell lines has also been reported.[17] In another study, it was observed that at low concentrations (up to 25 μM), [6]-paradol induced apoptosis in JB6 cells, but concentrations of 50 μM or greater resulted in apparent necrotic cell death.[43] [6]-gingerol can circumvent the resistance of mutant p53-expressing cells towards chemotherapy by inducing apoptotic cell death while it exerts a cytostatic effect on wild type p53-expressing cells by inducing temporal growth arrest.[20]

Pulmonary Cancer

[6]-gingerol inhibits angiogenesis, apparent from the reduction in the number of lung metastases, in mice receiving i.v. injection of B16F10 melanoma cells.[13] *Keishi-ka-kei-to*, a traditional Chinese herbal medicine composed of a mixture of crude extracts from five medicinal plants including Zingiberis rhizome, inhibits pulmonary metastasis in mice bearing B16F10 melanoma cells through the stimulation of CD8+ T-cells.[44]

Wang *et al.* showed that b-elemene, a novel anticancer drug extracted from the ginger plant, induced caspase-3, -7 and -9 activities, decreased Bcl-2 expression, caused cytochrome *c* release and increased the levels of cleaved caspase-9 and poly (ADP-ribose) polymerase in non-small-cell lung cancer cells.[45] In A549 human lung cancer cells, a novel phenylbutenoid dimer (+/−)-trans-3-(3,4-dimethoxyphenyl)-4-[(E)-3,4-dimethoxystyryl]-cyclohex-1-ene, isolated from *Zingiber cassumunar*, has suppressed cell-cycle progression by increasing p21 expression and downregulating cyclins and CDKs.[46]

Breast Cancer

[6]-gingerol inhibits cell adhesion, invasion, motility and activities of MMP-2 and MMP-9 in the MDA-MB-231 human breast cancer cell line, showing a concentration-dependent decrease in cell migration and motility. The activities of MMP-2 or MMP-9 in MDA-MB-231 cells were decreased by treatment with [6]-gingerol in a dose-dependent manner.[22] 1′-acetoxychavicol acetate (ACA), a tropical ginger compound, exhibits potential anticancer effects against breast carcinoma cells by inducing apoptosis in human breast carcinoma-derived MCF-7 and MDA-MB-231 cells.[47]

Gastrointestinal Cancer

Helicobacter pylori is the primary etiological agent associated with peptic ulcer disease and the development of gastric and colon cancer. The anti-*H. pylori* effects of ginger and its constituents were tested *in vitro* by Mahady *et al.*[48] It was found that gingerol inhibited the growth of *H. pylori* CagA+ strains and this activity may contribute to its chemopreventive effects against colon cancer. [6]-gingerol facilitated TRAIL-induced apoptosis by increasing TRAIL-induced caspase-3/7 activation in gastric cancer cells.[11] [6]-gingerol stimulates apoptosis through upregulation of NAG-1 and G(1) cell-cycle arrest through downregulation of cyclin D1 in human colorectal cancer cells involving mechanisms of protein degradation as well as beta-catenin, PKCepsilon, and GSK-3beta pathways.[18]

Pancreatic Cancer

[6]-gingerol inhibited cell growth through cell-cycle arrest at the G1 phase in two human pancreatic cancer cell lines, HPAC expressing wild-type p53 and BxPC-3 expressing mutated p53. [6]-gingerol can circumvent the resistance of mutant p53-expressing cells towards chemotherapy by inducing apoptotic cell death while it exerts a cytostatic effect on wild type p53-expressing cells by inducing temporal growth arrest. p53 expression was also found to decrease through [6]-gingerol treatment in both cell lines, suggesting that the induction of cyclin-dependent kinase inhibitor, p21cip1, was p53-independent. [6]-gingerol induced mostly apoptotic death in the mutant p53-expressing cells, while no signs of early apoptosis were detected in wild type p53-expressing cells and this was related to the increased phosphorylation of AKT.[20]

Renal Cancer

A recent study examined the early signaling effects of [6]-gingerol in Madin–Darby canine kidney (MDCK) cells. It was found that [6]-gingerol caused a slow and sustained rise of [Ca^{2+}] ions in a concentration-dependent manner. [6]-gingerol induced a significant rise in [Ca^{2+}] ions in MDCK renal tubular cells by stimulating both extracellular Ca^{2+} influx and thapsigargin (an endoplasmic reticulum Ca^{2+} pump inhibitor) sensitive intracellular Ca^{2+} release via as yet unidentified mechanisms.[49]

Prostate Cancer

A recent study from our laboratory demonstrated the modulation of [6]-gingerol on testosterone-induced alterations of apoptosis-related proteins in *in vitro*, androgen-sensitive LNCaP cells via upregulation of p53, Bax, caspase-9 and caspase-3 and downregulation of testosterone-induced anti-apoptotic proteins, Bcl-2 and survivin expression.[19] A study showed that mitogen-activated protein kinase phosphatase-5 (MKP5) overexpression decreased cytokine-induced NF-κB activation, COX-2, IL-6 and IL-8 in normal prostatic epithelial cells, suggesting potent anti-inflammatory activity of MKP5. [6]-gingerol has been shown to upregulate MKP5 in

normal prostate epithelial cells. Moreover, studies have found that prostate cancer (PCa) cell lines DU 145, PC-3, LNCaP and LAPC-4 retained the ability to upregulate MKP5 following [6]-gingerol exposure, suggesting utility of its chemopreventive actions by decreasing prostatic inflammation.[50]

Ovarian Cancer

The ginger component [6]-gingerol has been shown to exert anti-inflammatory effects through mediation of NF-κB. NF-κB can be constitutively activated in epithelial ovarian cancer cells and may contribute towards increased transcription and translation of angiogenic factors. Ginger inhibited growth by the inhibition of NF-κB activation and modulated secretion of angiogenic factors VEGF and IL-8 in ovarian cancer cells.[16] In a recent study conducted on five compounds, namely [4]-, [6]-, [8]- and [10]-gingerols and [6]-shogaol isolated from the methanolic extract of the dried rhizomes of ginger, [6]-shogaol exhibited the most potent cytotoxicity against human A549, SK-OV-3, SK-MEL-2, and HCT15 tumor cells. [6]-shogaol inhibited proliferation of the transgenic mouse ovarian cancer cell lines C1 [genotype: p53(–/–), c-myc, K-ras] and C2 [genotype: p53(–/–), c-myc, Akt], with ED(50) values of 0.58 μM (C1) and 10.7 μM (C2).[51]

ANIMAL STUDIES

Skin Cancer

Ginger and its major pungent principle constituent [6]-gingerol have been shown to suppress promotion of mouse skin carcinogenesis in laboratory animals (see Shukla and Singh[8] and references cited therein). The inhibitory activity of ginger extracts in tumor initiation and promotion is due to its pungent vanillyl ketones, including [6]-gingerol and [6]-paradol. It has been reported that [6]-gingerol inhibited TPA-induced skin tumor promotion in addition to the inhibition of TNF-α production and epidermal ornithine decarboxylase activity in ICR mice. Another study revealed that topical application of the ethanol extract of ginger resulted in suppression of TPA-mediated induction of ornithine decarboxylase and its mRNA

expression in SENCAR mouse skin. Pre-application of ginger extract to mouse skin afforded significant inhibition of TPA caused epidermal edema (56%) and hyperplasia (44%). Topical application of [6]-gingerol inhibited TPA-induced COX-2 expression along with suppressed NF-κB DNA binding activity in mouse skin (see Shukla and Singh[8] and references cited therein). Recently, topical application of [6]-gingerol (30 μM) prior to UVB irradiation (5 kJ/m^2) of hairless mice has been reported to inhibit the induction of COX-2 mRNA and protein, as well as NF-κB translocation by blocking the p38 MAP kinase-NF-κB signaling pathway.[12,13]

Gastrointestinal Cancer

Ginger has commonly been traditionally used in disorders of the gastrointestinal tract, as a stomachic, laxative, sialogogue, gastric emptying enhancer, appetizer, antiemetic, and antidyspepsic, and at the same time as an antidiarrheal and anticolic agent.[6] Ginger is often prescribed in Chinese and Japanese medicine for a variety of gastrointestinal disorders. In the US, ginger is frequently promoted as a digestive aid and treatment for abdominal pain, indigestion and ulcers. Although human clinical trials have not yet been conducted, several animal studies support some of these claims (see Shukla and Singh[8] and references cited therein). In one study, [6]-gingerol, when given to male F344 rats in the diet at a concentration of 0.02% for 3 wks, significantly reduced the multiplicity of azoxymethane-induced intestinal carcinogenesis. However, it is unclear which stage of carcinogenesis was suppressed by [6]-gingerol in this experiment because the carcinogen was administered during the gingerol treatment. Several observations made in the past are suggestive of the antiulcerogenic effect of ginger and its constituents. Studies on the cytoprotective and gastric antiulcer properties of ginger have been carried out in albino rats using cytodestructive effects produced by 80% ethanol, 0.6 M HCl, 0.2 M NaOH and 25% NaCl as well as those induced by non-steroidal anti-inflammatory drugs. The results of this study demonstrated that the extract (500 mg/kg, orally) exerted highly significant cytoprotective effects on gastric lesions. It was reported that crude acetone extract of ginger, isolated zingiberene (the main terpenoid from acetone extract), as well as [6]-gingerol significantly inhibited gastric lesions induced by HCl

and ethanol in rats. The orally-administered acetone extract at 1,000 mg/kg body weight (bw), zingiberene at 100 mg/kg bw and [6]-gingerol at 100 mg/kg bw significantly inhibited gastric lesions by 97.5%, 53.6% and 54.5%, respectively. These results are suggestive of zingiberene and [6]-gingerol being the important constituents in stomachic medications containing ginger. Similarly, Chinese investigators utilized various experimental gastric ulcer models with orally-administered dry and roasted ginger decoctions and found that roasted ginger has an obvious inhibitory tendency against stomach damage. These protective effects on the gastric mucosa seem to involve an increased mucosal resistance or potentiating of some defensive factor or mechanism against noxious chemicals. Clearly, more work is necessary to further substantiate these animal studies and to clarify which ginger constituents are active and by what mechanisms.

Colon Cancer

Ginger and its components have been used traditionally for the treatment of gastrointestinal ailments such as motion sickness, dyspepsia and hyperemesis gravidarum, and are also reported to have colon cancer chemopreventive activity. Gingerol also inhibited the growth of human colorectal cancer cells (see Shukla and Singh[8] and references cited therein). These reports claim that the results strongly suggest that ginger compounds may be effective chemopreventive and/or chemotherapeutic agents for colorectal carcinomas. The efficacy of ginger was found to be significant in a combined set of experiments where mice were fed with ginger before and after tumor cells were injected and in a second set where mice were fed with ginger only after their tumors had grown to a certain size. Another study looked at its modifying potential on the process of colon carcinogenesis induced by 1,2-dimethylhydrazine (DMH) in male Wistar rats using aberrant crypt foci assay. The results showed that dietary intake of ginger does not significantly change the proliferative or apoptosis indexes of colonic crypt cells. The effect of ginger on the initiation and postinitiation stages of DMH-induced colon carcinogenesis in male Wistar rats was studied and the results showed a lower incidence of tumors. It was further concluded by researchers from the same group that ginger supplementation suppressed colon carcinogenesis

by reducing lipid peroxidation and significantly enhanced the enzymatic and nonenzymatic antioxidant levels.

Breast Cancer

Despite the advent of new and aggressive therapeutics, breast cancer remains a leading killer among women; hence, there is an urgent need for the prevention of this disease. Dietary habits may have a role in breast cancer risk and prevention. The effects of chronic treatment with hot water extract of ginger rhizome on spontaneous mammary tumorigenesis have been examined in SHN mice. In mice given free access to extract of ginger (0.125%) in drinking water, the development of mammary tumors was significantly inhibited.[52] Hormone-induced persistent changes in gene expression of RbAp46 and a novel gene that specifies a noncoding RNA (designated G.B7) have been shown in the involuted mammary gland regulated in the glands of estrogen (E)- and progesterone (P)-treated Wistar–Furth rats 28 days after steroid hormone treatment compared with age-matched virgins leading to permanent changes in cell fate that determine the subsequent proliferative response of the gland.[53]

Prostate Cancer

Prostate cancer is the leading cause of cancer-related deaths in American men, responsible for over 29,000 deaths in 2007. In one of the findings from our laboratory we reported the modulatory effects of [6]-gingerol on testosterone-induced alterations on apoptosis-related proteins in the *in vivo* ventral prostate of Swiss albino mice. [6]-gingerol upregulated the testosterone depleted levels of p53 and upregulated its downstream regulator Bax and further activated caspase-9 and caspase-3. We also demonstrated the downregulation of testosterone-induced antiapoptotic proteins, Bcl-2 and survivin expression by [6]-gingerol in mouse ventral prostate.[8]

CLINICAL TRIALS

Ginger has been evaluated as a treatment for various conditions, including motion sickness, nausea and vomiting, and arthritis (Fig. 2).

Motion Sickness

Ginger has long been used as a remedy to decrease nausea and vomiting associated with several conditions. A follow-up study showed that 1 g of ginger was effective at reducing the subjective severity of sea sickness in naval cadets on the high seas, although the results were not statistically significant.[54] A randomized, double-blind, placebo-controlled study was performed to assess the effects of ginger extract on motion sickness and gastric slow-wave dysrhythmias induced by circular vection.[55] Volunteers with a history of motion sickness were pretreated with ginger (1,000 mg and 2,000 mg). Individuals then underwent circular vection during which nausea, tachygastria, and vasopressin were assessed. Ginger improved each of the above parameters, significantly prolonging the latency period before nausea onset and shortening the recovery time after vection cessation. Other research has shown no benefit of ginger for motion sickness.[54]

Nausea and Vomiting

Pregnancy-induced

Ginger is used to ameliorate symptoms of nausea in pregnancy. It is perhaps most popular for its antinausea and antiemetic effects in pregnancy. Up to 85% of pregnant women experience nausea in early pregnancy, and some 50% of those with nausea experience vomiting as well.[56] In an extensive review of studies using ginger as an agent to fight morning sickness, the authors concluded that "ginger … may be beneficial."[57] In one study of 70 pregnant women, participants received either 250 mg of freshly prepared ginger powder or a placebo. Results indicated a significant reduction in nausea and number of vomiting episodes.[58] A randomized, controlled equivalence trial involving 291 women less than 16 wks pregnant was undertaken at a teaching hospital in Australia. Women took 1.05 g of ginger or 75 mg of vitamin B6 daily for 3 wks and found that ginger was equivalent to vitamin B6 in reducing nausea, retching and vomiting, averaged over time.[59] In a double-blind, randomized, controlled trial (RCT), 170 pregnant women with symptoms of nausea and vomiting were randomly allocated into group A receiving one capsule of ginger twice daily (one capsule contained 0.5 g of ginger powder) ($n = 85$)

and group B receiving an identical capsule of 50 mg dimenhydrinate twice daily ($n = 85$). The vomiting episodes of group A were found to be greater than group B during the first and second day of the treatment with a statistically significant difference. There was a statistically significant difference in the side effect of drowsiness after treatment with group B greater (77.64%) than group A (5.88%). Thus, ginger was shown to be as effective as dimenhydrinate in the treatment of nausea and vomiting during pregnancy and had fewer side effects.[60]

Postoperative

In a study by Phillips *et al.* ginger also significantly reduced nausea and vomiting when compared to placebo and metoclopramide.[61] In a recent double-blind study, ginger failed to demonstrate an antiemetic effect following laparoscopic surgery.[62] Preoperative doses of 100 or 200 mg were administered and followed by repeated 100 or 200 mg doses. An earlier double-blind study on the effect of ginger on postoperative nausea also failed to demonstrate beneficial effects compared to placebo.[63] In a double-blind RCT, 120 patients who underwent major gynecologic surgery were randomized into group A receiving two capsules of ginger taken one hour before the procedure (one capsule contained 0.5 g of ginger powder) ($n = 60$) and group B receiving the placebo ($n = 60$). A statistically significant difference in nausea between group A (48.3%) and group B (66.7%) was observed.[64]

Chemotherapy induced

Cancer chemotherapy can cause severe nausea, vomiting and abdominal discomfort, which can limit therapy. In relation to chemotherapy-induced nausea and vomiting in one study, the addition of ginger to the standard antiemetic regimen had no advantage in reducing nausea or vomiting in the acute phase of cisplatin (Platinol)-induced emesis.[54] A systematic review of six RCTs analyzing ginger for clinical nausea and vomiting found insufficient data to draw firm conclusions.[54] To explore the use of protein meals with ginger for the treatment of the delayed nausea of chemotherapy, a recent study was designed for 28 patients with cancer

receiving chemotherapy for the first time. The results showed that high protein meals with ginger reduced the delayed nausea of chemotherapy and reduced the use of antiemetic medications.[65]

Arthritis

Studies evaluating the effectiveness of ginger in patients with osteoarthritis have had mixed results. One study showed ginger extract to have a statistically significant effect on reducing symptoms of osteoarthritis of the knee.[54] In a another crossover study the effect of ginger in osteoarthritis was significant only in the first period of treatment (i.e. before crossover).[54] In a randomized, placebo-controlled, double-blind crossover study, ginger extract was compared to ibuprofen in patients with osteoarthritis of the hip and knee. While ibuprofen showed a significant reduction in pain, ginger extract was not significantly different from a placebo when the study was terminated.[66] It appears that use of ginger for osteoarthritis or as an anti-inflammatory is not supported by human clinical trials. More studies are recommended using different doses and treatment durations to assess the efficacy of ginger extract for these conditions.

Vascular Conditions

Ginger is recommended by the traditional healers in South Asia for use in cardiopathy, to treat high blood pressure and palpitations, and to improve the circulation for its use as a vasodilator. Although one study has shown that ginger does not affect the International Normalized Ratio,[15] another study demonstrated a significant increase in fibrinolytic activity after dietary supplementation with 5 g of ginger powder.[54] In a human placebo-controlled study of patients with coronary artery disease, administration of 4 g of powdered ginger daily for three months did not affect blood cholesterol levels.[68] These negative results could be due to an insufficient dose of ginger. Alternatively, the levels of cholesterol-lowering components in ginger may be too low in whole ginger preparations and require isolation and concentration to produce effects on cholesterol blood levels. In a recent study,[67] the synergistic effect of ginger and nifedipine on

antiplatelet aggregation in normal human volunteers and hypertensive patients was reported. The authors showed that the percentage of platelet aggregation induced by collagen, ADP and epinephrine in hypertensive patients was larger than that in normal volunteers. Both aspirin and ginger could potentiate the antiplatelet aggregation effect of nifedipine in normal volunteers and hypertensive patients. These results suggest that ginger and nifedipine exhibit a synergistic effect on antiplatelet aggregation. A combination of 1 g ginger with 10 mg nifedipine per day could be valuable for cardiovascular and cerebrovascular complications due to platelet aggregation.

CONCLUSIONS

Spices and condiments are an integral part of the human diet, particularly in the Orient. Besides their use to impart flavor and color, for food preservation and to enhance palatability, they have been extensively used for their beneficial effects on health. Fortunately, even long-term consumption of these substances is not known to produce any side effects. Ginger has been used extensively in folk medicine to treat common ailments. Scientific evidence in favor of some of these beneficial properties is now emerging, supporting their use for ameliorating certain disorders. The use of ginger for controlling nausea and vomiting in traditional folk medicine is well documented. It is also used during pregnancy to control morning sickness, but conclusive evidence in the scientific literature concerning the safety of this practice is still lacking. Pharmacists are often advised to counsel pregnant women not to use ginger for morning sickness unless their physician has recommended it.

Overall, a significant number of laboratory and clinical studies have provided substantial evidence that ginger and its organic pungent vallinoid compounds are effective inhibitors of the carcinogenic process. The use of this ancient medicine for gastrointestinal problems (stimulation of digestion) has been given scientific approval. Today, medicinal ginger is used mainly for prevention of the symptoms of travel sickness and in cancer chemoprevention. The use of ginger under medical supervision in conjunction with chemotherapy or for occasional nausea or motion sickness may produce some benefits, provided the patient is not pregnant or

on anticoagulant medications. Due to its abundance, low cost and safety in consumption, ginger has been the subject of intensive scientific research over the past two decades, which has shown antiemetic, antibacterial, antifungal, antiatherosclerotic, hypoglycemic, and anticarcinogenic activity. Several mechanisms are likely to account for this protection. The benefits provided by ginger must be viewed as part of the entire diet, since several dietary constituents can influence the degree of protection. Future research should focus on how genetic variability and daily environmental factors influence the therapeutic benefits attributed to ginger and its pungent vallinoid components. Further studies on determining the anticancer activity of ginger and its active components should include human intervention trials to investigate its effectiveness against human cancers and other diseases.

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10

Kalonji (Thymoquinone)

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Our objective is to determine the most effective evidence-based approach for the use of thymoquinone (TQ), a product of the herb *Nigella sativa*, also known as *kalonji*, as an anticancer agent in basic and clinical research. The *Nigella sativa* herb has been used for thousands of years in folk medicine based on several observations, but its mechanism of action has remained ill-defined. TQ was first extracted in the 1960s and was defined as the main active constituent of *Nigella sativa* after a series of experiments. Investigating TQ effects against cancer cells has been increasing over the last decade in an effort to explore a potential anti-cancer herbal agent that lacks the toxicity record of most currently used chemotherapies. This chapter summarizes the results of TQ application to different cancer cells in addition to its potential use in selected clinical situations. The molecular biomarkers presented in this review will provide the foundation for future studies of TQ anticancer effects.

INTRODUCTION

Complementary and Alternative Medicine (CAM) refers to the use and practice of therapies or diagnostic techniques that fall outside of conventional biomedicine. It includes mind-body interventions, biologically-based therapies (e.g. herbal medicines), manipulative and body-based

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methods, and energy therapies (e.g. chiropractic manipulation and therapeutic touch). It has been reported that four in ten Americans use some form of CAM[1] and spent about US$27 billion out-of-pocket dollars in 1997, approximately the same amount spent on all medical services in the same year.[2] Herbal agents appear to be ideal candidates for extended research in the cancer field since they have been used for thousands of years with very minimal side effects — if any. Several herbal products have been recently incorporated into cancer research but their exact mechanisms of action remain unclear. Among these products is the herb *Nigella sativa*, which has been traditionally employed as a medicinal remedy for a variety of diseases.

Nigella sativa goes by many different names: *panacea* (meaning "cure all" in old Latin), *habbah sawdaa* or *habbat el baraka* (literally, "seeds of blessing" in Arabic) *kalonji* (Hindi) *hak jung chou* (Chinese). It is a spice that grows in the Mediterranean region and in western Asia. In English, it is commonly referred to as *Nigella sativa* or blackseed, along with black cumin, fennel flower, black caraway, nutmeg flower or Roman coriander. It belongs to the Ranunculaceae (a family of flowering plants) and is unrelated to common cumin (*Cuminum cyminum*). It grows to a height of about 30 cm and has a thin stem, narrow leaflets, and blue flowers (see Fig. 1).

Fig. 1. *Nigella sativa* flower.

The seeds (the part used, known as blackseed) are contained in an inflated capsule formed from the united follicles. They have a pungent, bitter taste coupled with a faint smell of strawberries, and contain a considerable amount of oil. They are used primarily as a spice and food preservative. In folk medicine, they are usually ingested with food or mixed with honey and are used primarily as lactogogues, carminatives, and antihelminthic agents. They have also been used as diuretics, antihypertensives and muscle relaxants, as well as immunity enhancers to fight infectious diseases in immunocompromised people. These observations have recently been supported by various studies. Several beneficial pharmacological effects have been attributed to various crude and purified components of blackseed, including antihistaminic,[3] antihypertensive,[4] hypoglycemic,[5] antifungal,[6] anti-inflammatory[7] and antioxidant activities.[8] Blackseed preparations have also demonstrated significant *in vitro* and *in vivo* antineoplastic activities.[9] In addition, blackseed is reported as being "likely safe" when used orally in amounts found in food.[10] However, in pregnant women blackseed has traditionally been found to lead to abortion by stimulating uterine contractions when used in large quantities, although it has also been shown to inhibit these contractions in rats and guinea pigs.[11] According to recent reports, it can also cause contact dermatitis when used topically.[12,13]

Based on these series of experiments, chemotherapeutic and chemopreventive effects of *Nigella sativa* seeds have been attributed to quinones, mainly thymoquinone (TQ), present in the seed's oil. As first suggested and extracted by El-Dakhakhny[14] in 1965, TQ is the main active constituent of blackseed oil.

CHEMICAL CONSTITUENTS

Nigella sativa; Kalonji

Eight fatty acids (99.5%) and 32 compounds (86.7%) have been identified in *Nigella sativa* fixed and volatile oils, respectively. The main fatty acids of the fixed oil are linoleic acid (55.6%), oleic acid (23.4%), and palmitic acid (12.5%). The major compounds of the volatile oil are trans-anethole (38.3%), p-cymene (14.8%), limonene (4.3%), and carvone (4.0%); thymoquinone represented 0.6%[15] (see Table 1).

Table 1. Constituents of *Nigella Sativa* seeds. Modified from Muhammad *et al.* (2002). *Pak. J. Med. Res.* **41**(2).

Component	Concentration (%)	Details
Fixed oil	32–40	Saturated fatty acids: Palmitic, stearic and myristic acid Unsaturated fatty acids: Arachidonic, eicosadienoic, linoleic, linolenic, oleic acid
Volatile oil	0.4–0.45	Nigellone, thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, α- and β-pinene, d-limonene, d-citronellol, p-cymene
Proteins	16–19.9	Arginine, glutamic acid, leucine, lysine, methionine, tyrosine, proline, threonine
Minerals	1.79–3.74	Calcium, phosphorous, potassium, sodium, iron
Carbohydrates	33.90	
Fiber	5.50	
Water	6	

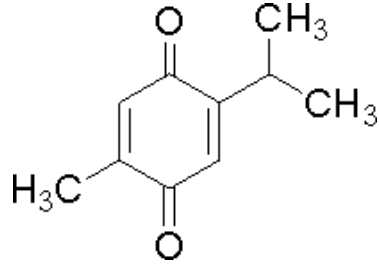

Fig. 2. Thymoquinone structure.

Thymoquinone

Thymoquinone, 2-isopropyl-5-methyl-1,4-benzoquinone, has the molecular formula $C_{10}H_{12}O_2$, a melting point of 44–47°C, a boiling point of 230–232°C and a molecular weight of 164.2 (see Fig. 2).

CHEMOPREVENTIVE POTENTIALS

Thymoquinone as an Antioxidant Agent

In several recent reports TQ has been found to possess antioxidant properties through different mechanisms (Fig. 3). TQ inhibited the production of

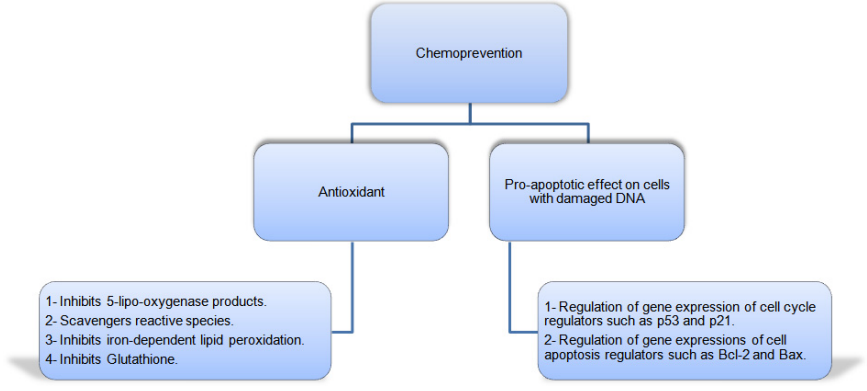

Fig. 3. Important chemopreventive potentials of thymoquinone.

5-hydroxyeicosatetraenoic acid and 5-lipoxygenase products,[16] both of which are required for the viability of colon cancer cells. TQ was shown to work as a scavenger of various reactive oxygen species such as superoxide anion radicals and hydroxyl radicals.[17–19] In addition, TQ was able to produce significant reductions in hepatic superoxide dismutase, catalase and glutathione peroxidase.[17–19] It was shown to efficiently inhibit iron-dependent microsomal lipid peroxidation in rats with doxorubicin-induced hyperlipidemic nephropathy.[20] TQ was also observed to decrease oxidative stress by inducing glutathione in experimental allergic encephalomyelitis in female Lewis rats.[21]

Multiple epidemiological studies have shown that a high intake of anti oxidant-rich foods is inversely related to cancer risk.[22] Recent experimental and clinical studies have implicated oxidative stress in the development and progression of different cancer types.[23,24] Antioxidants work to inhibit the action of free radicals, which are unstable oxygen molecules in the body caused by aging, smoking or other environmental factors. These free radicals attempt to stabilize themselves by "stealing" electrons from other cells, causing a chemical chain reaction and leading to cell mutation and eventually cancer. The ability to scavenge free radicals can give TQ a very important role as a cancer-preventive substance as well as a chemopreventive agent that has the potential of being highly tolerable and non-toxic to normal cells. Clinical trials are warranted to test this hypothesis.

The present findings suggest that TQ may play a role in inhibition of carcinogenesis by modulating lipid peroxidation and antioxidant status, a role that involves a chemopreventive potential of TQ against several cancer types (see Fig. 3).

Thymoquinone as an Anti-Inflammatory Agent

TQ has been described as a potent inhibitor of leukotrienes formation in human blood cells.[25] This inhibitory effect was dose- and time-dependent and was exerted on both 5-lipoxygenase and leukotrien C4 synthase activity. In another study,[26] rats pre-treated with oral TQ showed complete protection against acetic acid-induced colitis compared to a sulfasalazine (500 mg/kg) control group. TQ was also found to suppress the production of nitric oxide by macrophages,[27] an effect useful in ameliorating inflammatory and autoimmune conditions.

Inflammation produces pro-inflammatory cytokines and diverse reactive oxygen and nitrogen species that can cause predisposition to cancer.[28] Examples include inflammatory bowel diseases, either Crohn's disease or ulcerative colitis,[29–32] gastric *Helicobacter pylori* infection[33] and an association between leuktrien D and colorectal adenocarcinoma.[34] A strategy to modify this process may lead to a delay in cancer progression and may improve patient morbidity and mortality as well.

Arachidonic acid is another example of a major precursor of several classes of signal molecules and alteration of its metabolism is involved in human carcinogenesis. 5-lipoxygenase (5-LOX) converts arachidonic acid to hydroxyeicosatetraenoic acids or leukotrienes, which are able to enhance proliferation, increase survival, and suppress apoptosis of human cells. It was reported that 5-LOX protein expression is increased in esophageal cancer and that 5-LOX inhibitors can induce esophageal cancer cells to undergo apoptosis.[35] The potential role of TQ in suppressing inflammation through inhibition of leukotrienes synthetase and 5-lipoxygenase is currently a very active area of research. The clinical impact of these observations is crucial in order to initiate chemoprevention programs for patients

with lifelong chronic inflammatory conditions that eventually lead to cancer.

NF-κB is a ubiquitous transcription factor consisting of p50, p65, and I-κBα that resides in the cytoplasm and is activated in response to various inflammatory stimuli, environmental pollutants, pro-oxidants, carcinogens, stress, and growth factors.[36] On activation, NF-κB translocates from the cytoplasm to the nucleus, binds DNA, and causes gene transcription. Numerous kinases have been linked with activation of NF-κB, including I-κBα kinase (IKK). Notably, a more recent study of human chronic myeloid leukemia cells (KBM-5) found that TQ suppressed tumor necrosis factor-induced NF-κB activation in a dose- and time-dependent manner and inhibited NF-κB activation induced by various carcinogens and inflammatory stimuli.[37] To determine the specificity of the NF-κB band, the nuclear extracts from TNF-activated cells were incubated with antibodies to the p50 (NF-κB) and the p65 (RelA) subunit of NF-κB; the resulting bands were shifted to higher molecular masses, suggesting that the TNF-activated complex consisted of p50 and p65. In addition, the suppression of NF-κB activation correlated with sequential inhibition of the activation of I-κBα kinase, I-κBα phosphorylation, I-κBα degradation, p65 phosphorylation, p65 nuclear translocation, and NF-κB-dependent reporter gene expression. TQ specifically suppressed the direct binding of nuclear p65 and recombinant p65 to the DNA, and this binding was reversed by DTT.

Thus, targeting these proteins may represent an attractive approach to developing novel chemopreventive agents. The use of agents derived from herbal products would therefore represent an acceptable approach because of their tolerability and ability to spare normal cells[38] (see Fig. 3).

CHEMOTHERAPEUTIC POTENTIALS

Effect of TQ on Proliferation/ Viability and Cell Cycle Control Regulators

In a recent study, we observed that TQ inhibited DNA synthesis, proliferation, and viability of cancerous (LNCaP, C4-B, DU145, and PC-3)

but not non-cancerous (BPH-1) prostate epithelial cells by downregulating androgen receptor (AR) and E2F-1.[39] In LNCaP cells, this was associated with a dramatic increase in p21Cip1, p27Kip1, and BAX. TQ blunted progression of synchronized LNCaP cells from G1 to S phase, with a concomitant decrease in AR and E2F-1 as well as the E2F-1-regulated proteins necessary for cell cycle progression. In a xenograft prostate tumor model, TQ inhibited growth of C4-2B-derived tumors in nude mice. This *in vivo* suppression of tumor growth, as with C4-2B cell growth in culture, was associated with a dramatic decrease in AR, E2F-1, and cyclin A as determined by western blot of tissue extracts. Tissue immunohistochemical staining confirmed a marked reduction in E2F-1 and showed induction of apoptosis on terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labeling assay.

Most recently, our group also reported the effect of TQ on pancreatic tumor cells (HPAC, BxPC-3, Panc-1 and MDA Panc-28, COLO 357, L3.6pl).[40] Cells were treated with TQ (0–50 μM) for 48 hrs to examine antiproliferative and proapoptotic effects. With the exception of Panc-1, most pancreatic cancer cells were found to be sensitive to TQ alone as deduced from reduced viability and undergoing apoptosis in the range between 30–50 μM. Panc-1 cells were seen to be sensitive to TQ concentration as low as 5 μM. TQ pre-treatment also significantly ($p < 0.01$) enhanced the antiproliferative and apoptotic effect of gemcitabine as well as oxaliplatin in these cells relative to untreated controls and with a single drug regimen. Additionally, constitutively active NF-κB and several of its downstream effector genes related to metastasis and invasion were downregulated by TQ, suggestive of an important molecular target in reversing chemoresistance as well as invasion and metastasis of pancreatic cancer.

Moreover, TQ effects were observed in an *in vitro* study[41] using four cancer cell lines: canine osteosarcoma (COS31) and its cisplatin-resistant variant (COS31/rCDDP), human breast adenocarcinoma (MCF7), human ovarian adenocarcinoma (BG-1) and Madin–Darby canine (MDCK). TQ inhibited proliferation of all cell lines in a concentration-dependent manner as determined by MTT assay. MDCK cells (normal kidney cells) were the most resistant to the inhibitory effects of TQ

(IC50 of 101 μM). This observation demonstrates the viability of TQ as a chemotherapy agent that lacks the toxicity record of most of the currently used agents in addition to potentially improving their therapeutic index if used concurrently. Thus, investigating TQ's ability to spare normal cells is a cornerstone of future studies on TQ's anticancer effects (see Fig. 4).

In addition, TQ was found to affect cell-cycle regulatory protein expression. Cell cycle checkpoints allow the cell to correct possible defects and avoid progression to cancer.[42,43] There are two major checkpoints to detect DNA damage; one at the G_1-S transition that prevents the cell from replicating damaged DNA, and one at the G_2-M transition that prevents chromosome segregation if the chromosome is not intact (see Fig. 4).

TQ (30 μM for 48 hrs) was shown to induce G_1 cell-cycle arrest when added to papilloma cells, which correlated with a sharp increase in the expression of the cyclin-dependent kinase inhibitor p16 and a decrease in cyclin D1 protein expression.[44] In future work, studying other cell-cycle regulatory proteins involved in this setting, like Cdk-4, would be very helpful to further define TQ targets. Similarly, as determined by flow cytometric analysis of DNA content by PI staining, TQ induced G_1 cell-cycle arrest of COS31 cells[41] and human colon cancer cells (HCT-116)[45] using TQ (100 μM for 48 hrs), an effect that started after 24 hrs using TQ (50 μM for COS31 cells and 60 μM for HCT-116 cells). G_1 arrest was associated with upregulation of p21 in HCT-116 cells, which was suggested as the principal transcriptional target of p53 in the context of the G_1 checkpoint.[46] The resulting high levels of p21

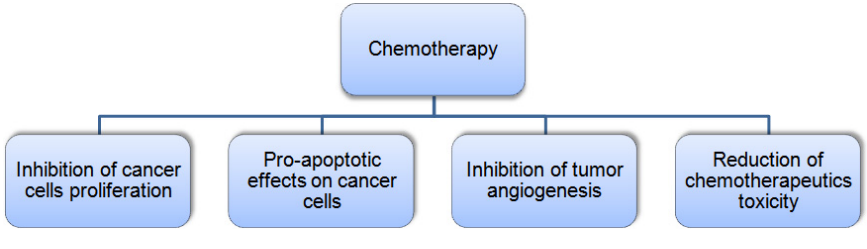

Fig. 4. Chemotherapeutic effects of TQ.

block cdk2 activity and possibly cdk4 and cdk6 activities, leading to G_1 arrest. There was also upregulation of p53, whose postulated role in cancer development has been supported by multiple observations. P53-knockout mice invariably develop spontaneous tumors within the first six months of life.[46] It was also revealed that the deletion of only the p53 gene, out of approximately 30–40,000 mouse genes, caused tumors in 100% of the animals, again highlighting the p53 protective role against cancer.

Although virtually all human tumors deregulate either the pRB or p53 pathway, and oftentimes both pathways simultaneously,[46,47] the unique effect of TQ on p53 justifies future studies to dissect its molecular targets in different cancer cells.

Moreover, TQ induced growth inhibition in spindle carcinoma cells by inducing G_2/M cell-cycle arrest, which was associated with an increase in p53 and a decrease in cyclin B1 protein. It is worth noting that p53 can regulate the G_2/M transition through either the induction of p21 and 14-3-3σ, a protein that normally sequesters cyclin B1-Cdc-2 complexes in the cytoplasm, or through the induction of apoptosis.[48–51] These results justify further research to investigate the effect on p21, Cdc-2 and Cdc-25 in order to further dissect the mechanism of action involved with the G_2-M transition.

Together, these findings indicate the effect of TQ on cell viability and proliferation, in addition to different phases of the cell cycle, highlighting the importance of further studies to define more cell-cycle regulators involved in these actions. TQ may then prove to be a molecule for targeted therapy since cell-cycle proteins have been suggested as targets for therapeutic exploitation. Moreover, many therapeutic approaches currently use compounds that normally trigger checkpoint responses. However, it is too early to judge the efficacy of TQ beyond its success using *in vitro* models.

Effect of TQ on Apoptosis

p53 has been shown to downregulate the DNA damage sensor CHEK1 at the transcriptional level via direct DNA binding.[52] CHEK1 responds

to DNA damage by initiating cell-cycle arrest, thus providing time for the cell to repair the damage and to evade apoptosis before resuming the cell cycle. Thus targeting CHEK 1 may play a role in enhancing the cytotoxicity of DNA-damaging agents. By comparing the gene expression profiles of HCT116 p53+/+ with p53−/− cells after thymoquinone treatment, *in vitro* and in xenografts, a recent study has shown that the sensitivity of these cells to thymoquinone-induced apoptosis was due to transcriptional repression of CHEK1 by p53 in HCT116 p53+/+ cells.[53]

Another study of HCT-116 cells showed that TQ triggered apoptosis in a dose- and time-dependent manner, starting at a concentration of 100 μM after 12 hrs of incubation.[45] This was associated with a 2.5–4.5-fold increase in p53 and p21 mRNA expression, a marked increase in p53 and p21 protein levels, and a significant decrease in Bcl-2 protein levels. Co-incubation with pifithrin-α, a p53 inhibitor, restored Bcl-2, p53 and p21 levels to the untreated control levels and inhibited TQ effects. These data suggested the role of TQ in regulating cell-cycle regulators involved in apoptosis, in addition to downregulating Bcl-2, an antiapoptotic protein. This was supported by similar effects on primary mouse keratinocytes, papilloma (SP-1) and spindle (I7) carcinoma cells.[44] At longer times of incubation (48 hrs), TQ induced apoptosis in both cell lines by increasing the ratio of Bax/Bcl-2 protein expression and decreasing Bcl-xL protein. TQ was shown to increase p53 expression while concomitantly decreasing cyclin B1 protein. It is crucial to understand the effects on p53 expression that is normally induced in response to a variety of cellular stresses, including DNA damage and oncogene activation, resulting in apoptosis. In fact, the best-known example of inactivation of apoptosis is by mutation of p53. Therefore, a selective strategy to raise or restore p53 of tumor cells can lead to cell-cycle arrest, to allow for DNA repair before replication.[54]

In another study,[41] using a DNA fragmentation test, TQ was shown to induce apoptosis of COS31 cells in a time- and concentration-dependent manner and reached maximum effect at 100 μM after 24 hrs. Results were verified by fluorescence microscopy and flow cytometric analysis.

In addition, TQ was also observed to initiate apoptosis via p53-independent pathways through activation of caspases 8, 9 and 3 in p53-null myeloblastic leukemia HL-60 cells.[55] Caspase-8 activity was the highest at one hour following 100 μM of TQ treatment, while caspase-3 activity was the highest after six hours. TQ increased Bax/Bcl-2 ratios through upregulating Bax (proapoptotic) and downregulating Bcl-2 (anti-apoptotic) proteins, which explains its effects on caspases.

From many of the aforementioned observations, one can conclude that TQ caused cell-cycle arrest at relatively smaller doses, compared to the doses required to inhibit proliferation and viability leading to cell death. This can be explained by the fact that several of the proteins that control proliferation — p53, pRB, cyclins, CDKs, CKIs, Bcl-2, c-Myc, E2F-1, Akt/PKA, PKC, and NF-κB — are also involved in signaling pathways leading to cell death.[56,57] Thus, studying TQ effects on these proteins in future studies would be a central to concluding its exact mechanisms of action involved in apoptosis. However, current observations clearly demonstrate that TQ plays a role in inducing apoptosis through modulating multiple targets. This ability appears promising in cancer research, given the current challenge in targeting apoptosis regulators because of the development of resistance and/or the variance in the level of expression of these targets. For instance, therapy to decrease transcriptional activation of Bcl-2 levels has been suggested as a strategy to treat many cancer types that overexpress Bcl-2.[57] Moreover, over-expression of some of these proteins was observed in pre-cancerous lesions, such as Bcl-2 protein, found to be an early event in gastric tumorigenesis, before gastric dysplastic changes occur.[58] In animal models, TQ has been shown to suppress tumor necrosis factor-α (TNF-α) production in murine septic peritonitis.[59]

Effect of TQ on Angiogenesis

Endothelial cells play a major role in each step of tumor angiogenesis, including endothelial cell migration, proliferation, invasion, adhesion, and tube formation. Among the endothelial cell signaling pathways that regulate endothelial cell migration, proliferation, growth, and survival, the two major pathways are phosphatidylinositol 3-kinase-AKT and

Raf-MEK-extracellular signal-regulated kinase (ERK) pathways.[60] AKT signaling stimulates the production of hypoxia-inducible factor-α transcription factors and thereby mediates secretion of vascular endothelial growth factor (VEGF) and other growth factors, which are important proangiogenic factors. TQ was shown to effectively inhibit angiogenesis *in vitro* and *in vivo* and prevented tumor growth in a xenograft mouse model through the activation of both AKT and ERK (VEGF dependent or VEGF independent) in endothelial cells. However, thymoquinone had no inhibitory effects on VEGFR2 activation in a specific VEGFR2 assay. Other than VEGF, there are many other proangiogenic growth factors, such as fibroblast growth factors, placental growth factor, and platelet-derived growth factor.[61] Almost all of these proangiogenic growth factors regulate angiogenesis through AKT and ERK signaling pathways. Therefore, the study concluded that thymoquinone may inhibit angiogenesis/tumor angiogenesis by suppressing AKT/ERK signaling pathways but not directly inhibit VEGFR2 activation. A similar mechanism of action was suggested most recently in a study which reported that thymoquinone can inhibit human umbilical vein endothelial cell (HUVEC) migration, invasion, proliferation, and tube formation by decreasing AKT/ERK activation.[62] To confirm the inhibitory effect of thymoquinone on angiogenesis, the researchers performed an aortic ring assay. Thymoquinone inhibited microvessel growth *in vitro* from 50 to 100 nmol/L after four days of incubation, suggesting that thymoquinone inhibits angiogenesis *in vitro*. To confirm the antiangiogenesis effects of thymoquinone *in vivo*, Matrigel plug assays were done with different concentrations. 1 μmol/L thymoquinone significantly inhibited VEGF-induced angiogenesis, whereas 10 μmol/L thymoquinone almost completely abolished angiogenesis in the Matrigel plug assays, indicating that thymoquinone effectively inhibited angiogenesis *in vivo*. Thymoquinone effects on VEGFR2 activation was investigated with a VEGFR2-specific activation assay. Thymoquinone exhibited very little inhibitory effects on VEGFR2, confirming that thymoquinone was not a direct VEGFR2 inhibitor.

In another study, the antiangiogenic effects of TQ were assessed by cell proliferation and migration assays.[63] TQ (100 μM) significantly

decreased the proliferation of human breast (MCF-7), colon (Caco-2) and prostate (DU-145) cancer cells and also prevented their migration. TQ also inhibited HIF-1α expression and decreased HIF-1α DNA binding activity in all cancer cells, in addition to reducing VEGF and cathepsin D secretion to less than 50% and 40%, respectively, in normal human lung fibroblast cells. In contrast, TQ at 200 μM did not affect normal cell proliferation.

Because tumor growth and metastasis are angiogenesis-dependent, several clinical trials have recently suggested that antiangiogenic therapy is another attractive target for therapeutic intervention. The onset of angiogenesis involves an alteration in the balance between pro-angiogenic and antiangiogenic molecules in the local tissue microenvironment.[64–66] Extending future TQ studies to involve these proteins is an initial step towards understanding its antiangiogenic ability and the impacts related to this finding (see Fig. 4).

ADDITIONAL OBSERVATIONS OF THYMOQUINONE ANTICANCER EFFECTS

Effect on Resistant Variants of Cancer Cells

TQ was four- to five-fold more cytotoxic to COS31/rCDDP, the resistant variant of COS31 cells, than COS31 cells (IC50 of 7.7 μM versus 34.8 μM), as demonstrated by MTT proliferation assay.[41] COS31/rCDDP cells overexpress glutathione-S-transferase (GST) detoxification system, which may explain their resistance to cisplatin. Furthermore, previous studies proposed that the cytotoxicity of quinones was related to their interaction with GSTs.[67–69] Thus, the sensitivity of COS31/rCDDP cells to TQ may be also related to overexpression of GSTs.

Interestingly, several studies reported an association between many types of cancer with GST polymorphism, which may explain the initial drug resistance and/or the subsequent disease relapse.[70–76] In the same context, TQ may hold promise in these clinical situations because of its peculiar ability to be activated by GSTs. Validation of this hypothesis will require further investigations via both *in vitro* and *in vivo* studies.

Reducing Side Effects of Other Chemotherapies

Cisplatin (Cis): Oral TQ reduced cisplatin-induced nephrotoxicity in rats and mice,[77] as indicated by significant reductions in serum urea and creatinine and significant improvement in polyuria, kidney weight, creatinine clearance and histopathological examination. In addition, it was also found to potentiate the antitumor activity of cisplatin after it was applied to Ehrlich ascites carcinoma bearing mice. In addition, TQ reduced cisplatin-induced bone marrow toxicity, anemia and leukopenia in mice.

Doxorubicin (DOX): Oral TQ ameliorated DOX-induced nephrotoxicity in rats, as indicated by lower proteinuria, albuminuria, and urinary excretion of N-acetyl-beta-D-glucosaminidase, in addition to lower serum levels of urea, total cholesterol and triglycerides. Furthermore, oral TQ reduced DOX-induced cardiotoxicity in rats[78] as demonstrated by significantly lower serum level of lactate dehydrogenase and creatine phosphokinase. It was indicated that mechanisms involved in this cardioprotection are superoxide radical scavenging in addition to inhibiting lipid peroxidation. In another mice study,[79] oral TQ exhibited a similar cardioprotective effect as indicated by the same parameters in addition to a histopathological examination of the heart tissue. In the same study, an *in vivo* experiment on mouse Ehrlich ascites carcinoma tumor showed that TQ did not interfere with the antitumor activity of DOX.

Ifosfamide (IFO): Oral TQ significantly reduced IFO-induced nephrotoxicity in rats[80] as indicated by lower serum creatinine and urea in addition to lower phosphaturia and glucosuria. TQ also prevented IFO-induced renal glutathione depletion and lipid peroxide accumulation. The same group also demonstrated that mice treated with ifosfamide in combination with oral TQ demonstrated an enhanced antitumor activity in addition to less body weight loss and mortality rate compared to IFO alone.

Unfortunately, more than 50% of all cancer patients either do not respond to initial therapy or experience relapse after an initial response to treatment and ultimately die from progressive metastatic disease. Thus, the ongoing commitment to the design and discovery of new chemosensitizing agents that can improve the therapeutic index of current chemotherapies

is critically important.[81] One approach to achieving this target is through decreasing the dose-limiting toxicities as demonstrated by TQ effects in the aforementioned *in vivo* studies. Further *in vivo* studies are warranted to test this hypothesis.

POTENTIAL BENEFITS OF ANTICANCER HERBAL EXTRACTS

There has been an increasing interest in anticancer herbal extracts over the last decade, which has been supported by encouraging research studies. In the United States and most Western countries, CAM therapies are often defined as interventions neither taught in medical schools nor available in hospital-based practices. Dietary examples include herbal products/botanicals, amino acids, minerals and vitamins. Prevalence of herbal product use has been investigated in several studies. Fifty-four of 100 adults with mixed types of cancer reported using herbal products.[82] A study of 480 women with breast cancer found that 20% had used CAM. Another study of 343 adults with mixed types of cancer found a 33% CAM rate.[83] Among males, 60% of 50 men with prostate cancer used herbal products.[84]

The safety and efficacy of many CAM approaches are not well studied and most of them lack evidence-based medicine support. In addition, most medical insurance companies do not cover CAM. In 1998, the United States Congress elevated the status of the Office of Alternative Medicine to a National Institutes of Health center: the National Center for Complementary and Alternative Medicine (NCCAM). The budget of NCCAM has been progressively increasing every year toward its mission to "support rigorous research on CAM, to train researchers in CAM, and to disseminate information to the public and professionals on which CAM modalities work, which do not, and why."

CONCLUSION

Current cancer research has begun to use herbal medicine extracts *in vitro* and *in vivo*. However, the mechanisms of actions remain unclear. The anticancer effects of *Nigella sativa* seeds is probably due to TQ. This assumption

is based on a series of *in vitro* and *in vivo* experiments. However, several studies have used the whole *Nigella sativa* oil, both *in vitro* and *in vivo*, and demonstrated strong anticancer effects. More attention paid towards studying other components of *Nigella sativa* could yield potentially useful anticancer components of this herb, which appears to possess various mechanisms of action against cancer cells.

From a literature search, TQ appears to be a promising agent that may have multiple applications. Previous research has indicated that the mechanisms underlying chemopreventive potentials of TQ may be combinations of antioxidant and anti-inflammatory effects. In contrast, chemotherapeutic potentials are related to the influence on cell-cycle control and checkpoints, induction of apoptosis, and inhibition of angiogenesis. In addition, reducing side effects of standard chemotherapy regimens promises an effective role for TQ in potentiating their anticancer effects and widening their safety range, hence allowing an escalation of the dose.

Perhaps the best hope for having a significant impact on cancer treatment will be to use increasing knowledge concerning growth and cell-cycle control, checkpoints, and immortalization to develop effective strategies that bolster known intrinsic defenses against cancer. In the past decade, the primary strategy for discovering new anticancer drugs has shifted from targeting cytotoxic compounds to seeking agents that block the key molecular pathways that lead to cancer.

Indeed, there is a need for effective cancer therapies that do not have similar toxicity records associated with current chemotherapy regimens. Molecularly targeted therapies hold the promise of accurately defining the groups of patients in whom they will be effective. In addition, natural agents are advantageous for application to humans because of their combined mild mechanism and their ability to spare normal cells.

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11

Kokum (Garcinol)

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Kokum, a spice derived from fruit of the *Garcinia indica* tree, is traditionally used in Ayurvedic medicine to facilitate digestion and to treat sores, dermatitis, diarrhea, dysentery, and ear infection. One of the major active components of kokum is garcinol, a polyisoprenylated benzophenone active against bacteria, viruses, gastric ulcers, and cancers. Garcinol's antiproliferative, antibacterial, antioxidant and anti-inflammatory effects result from its modulation of numerous cell-signaling intermediates. This chapter discusses the sources, chemical components, mechanism of action, and disease targets of the kokum spice.

INTRODUCTION

The genus *Garcinia* of the Clusiaceae family includes around 200 species, of which *Garcinia indica* is the most common. *Garcinia indica* is also known as *Brindonia indica*, *Stalagmitis purpurea*, *Garcinia purpurea*, *Garcinia microstigma*, *Stalagmitis indica*, *Garcinia celebica*, and *Oxycarpus indica*. *Garcinia indica*, primarily of Indian origin, is known by many names: bindin, biran, bhirand, bhinda, kokum, katambi, panarpuli, ratamba, and amsol. In the English language, it is commonly known as mangosteen, wild mangosteen, or red mango. The extract and rind of *Garcinia cambogia* is used as a curry condiment in India.

The *Garcinia indica* seed contains 23–26% oil, which is used in confectionery, medicines, and cosmetics. It is used in curries and other dishes

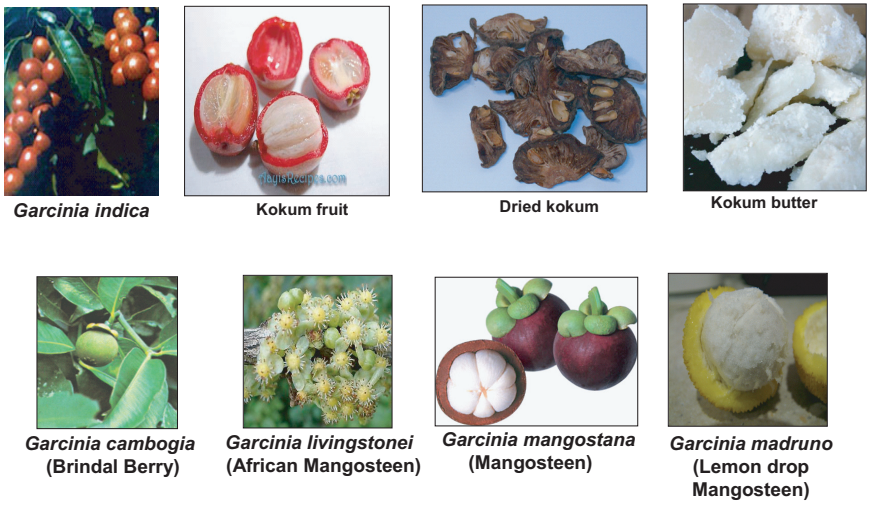

Fig. 1. Forms of the kokum fruit of *Garcinia indica* and related *Garcinia* fruits. The different names of *Garcinia indica* in various languages: English — Goa butter, kokum butter, kokum; French — cocum; German — kokam; Hindi — kokam, kokam-ka-tel, vishambil; Italian — cocum; Kannada — dhupadamara, murgala, murginahuli-mara, devana huli; Malayalam — punampuli; Marathi — amsole, bhirand, chirand, katambi, kokam, ratamba; Sanskrit — amlabija, amlapura, amlashaka, amlavetasa, amlavriksha, amlavrikshaka, atyamala, bijamla, chudamala, chukra, chukramla, chukraphala, phalamlah, phalamlaka, puramla, raktachudah, raktapuraka, rasamla, sakamlah, shalamla, shreshthamla, tintidika, tittidiphala, vrikshamla, vrksamla, vrksamlah, vruksamlah; Spanish — cocum; Tamil — murgal, murgal-mara; Tibetan — da tri ga, da tri gi.

as a slightly bitter spice, a souring agent, and as a substitute for tamarind. Recently, industries have started extracting hydroxycitric acid (HCA) from the rind of the fruit.

In traditional medicine, such as Ayurveda, kokum is prescribed for edema, rheumatism, delayed menstruation, constipation and other bowel complaints, and intestinal parasites. The extract of *Garcinia cambogia* is used as an herbal appetite suppressant and weight-loss supplement.

CHEMICAL CONSTITUENTS

The active compounds isolated from *Garcinia indica* include garcinol, xanthochymol, isoxanthochymol, and HCA (Fig. 2). Garcinol (also

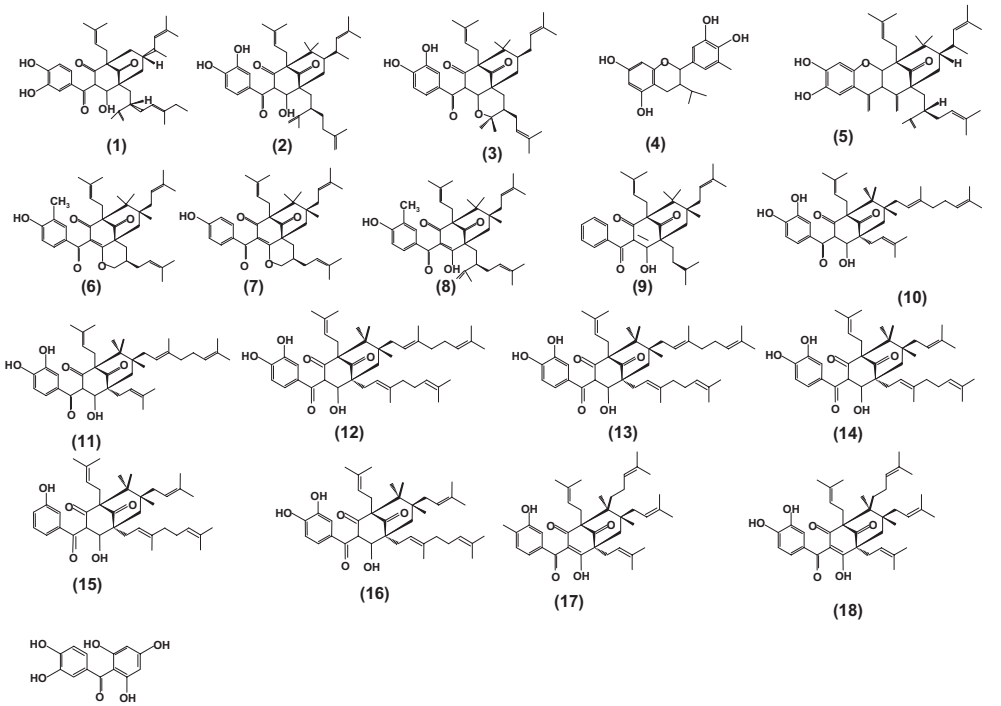

Fig. 2. The major compounds of *Garcinia indica*: (1) garcinol; (2) xanthochymol; (3) isoxanthochymol; and (4) hydroxycitric acid. Derivatives of garcinol: (5) Garcim-1; (6) isogarcinol-13-O-methylether; (7) isogarcinol; (8) garcinol-13-O-methylether; (9) Clusianone; (10) (+)-oblongifolin A(2); (11) (+)-oblongifolin B(3); (12) (+)-oblongifolin C(4); (13) (+)-oblongifolin D(5); (14) guttiferone-M; (15) guttiferone-N; (16) guttiferone-I; (17) guttiferone-J; (18) guttiferone-M; (19) macurin.

called cambogiol), a polyisoprenylated benzophenone, is one of the major constituents of the extract of the rind of *Garcinia indica*.[1] The structure of garcinol includes both a phenolic hydroxyl group and a β-diketone moiety. The major oxidative products of garcinol include garcim-1, garcim-2, and cambogin. Derivatives of garcinol include xanthochymol, isoxanthochymol, gambogic acid, mangostin, isogarcinol, isogarcinol 13-O-methylether, garcinol 13-O-methylether, clusianone, macurin, (+)-oblongifolin A, (+)-oblongifolin B, (+)-oblongifolin C, (+)-oblongifolin D, guttiferone-M, guttiferone-N, guttiferone-I, guttiferone-J, and guttiferone-K.

The fruit rind of *Garcinia indica* contains 10–30% HCA (1,2-dihydroxypropane-1,2,3-tricarboxylic acid),[2] an appetite suppressant. HCA suppressed food intake[3,4] and decreased body weight gain in experimental animals.[5] Rats fed a lipogenic diet showed a dose-dependent reduction in food intake, body weight, epididymal fat, and the level of triglyceride in serum. Additional research has shown that HCA promotes weight loss and reduces serum triglycerides and cholesterol levels in humans fed a high-carbohydrate diet.[5] HCA works by binding to the ATP-citrate lyase to reduce the production of acetyl coenzyme A, which reduces the body's production of fat and cholesterol. It also increases the ability of the liver and muscles to synthesize and store glycogen, thereby suppressing appetite. The theory behind HCA weight-loss formulations is that HCA inhibits an enzyme called *citrate lyase* that helps turn excess carbohydrates into fat. By inhibiting this enzyme, it is believed the body instead boosts carbohydrate oxidation, or simply put, burns the extra carbohydrates. Several HCA-containing products are available commercially as weight-control agents.

MOLECULAR TARGETS

Garcinol exhibits antiproliferative, antioxidant, and anti-inflammatory effects by modulating cell-signaling pathways, enzymes, and molecular targets such as epigenetic regulators, protein kinases, transcription factors, inflammatory biomarkers, and growth regulators (Fig. 3). Through microarray analysis, garcinol modulates many gene products.[6]

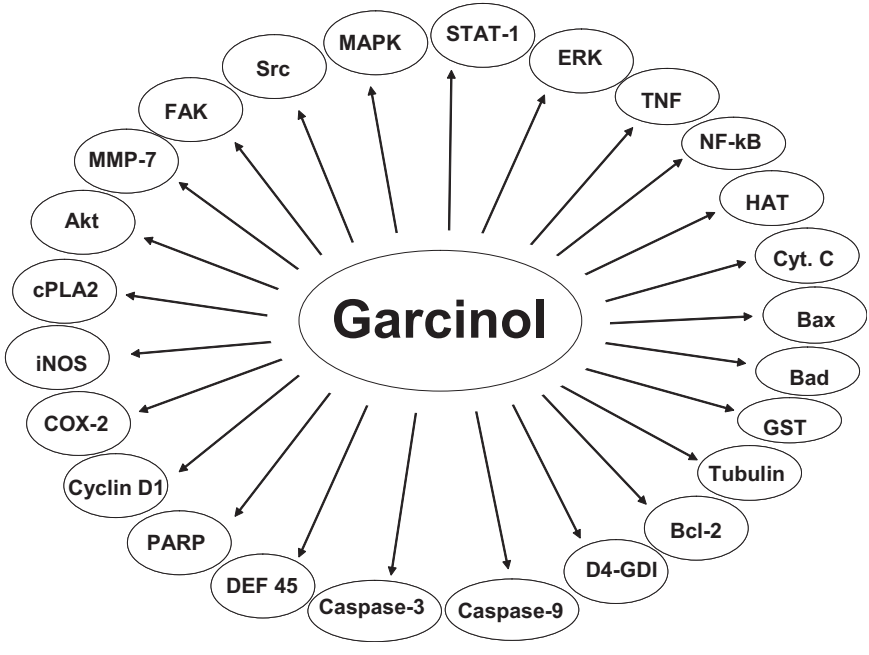

Fig. 3. Molecular targets of garcinol.

Garcinol Suppresses the Production of Reactive Oxygen Species (ROS)

ROS have been linked with various cell-signaling pathways. Garcinol, suppresses the production of ROS induced by lipopolysaccharide (LPS).[7,8] Its antioxidant activity is greater than that of tocopherol.[9,10] Garcinol also suppresses the iron-induced oxidation of low-density lipoproteins.[11]

Garcinol Suppresses the Proliferation of Tumor Cells

Agents that suppress the proliferation of tumor cells can potentially be used to treat cancer. Garcinol may be such an agent. Research has shown that garcinol suppresses the proliferation of human colon cancer cells HT-29 and HCT-116[12] and human hepatocellular carcinoma cells MH1C1 and HepG2[13] and that it induces apoptosis in human leukemia HL-60

cells.[14,15] In most cells, the apoptotic effects of garcinol are accompanied by a decrease in the level of the anticellular Bcl-2 protein, cleavage of BID, loss of mitochondrial membrane potential, release of cytochrome *c*, and sequential activation of caspase-9 and caspase-3. However, further research is needed to determine effective doses of garcinol against various cancers because *in vitro* studies have shown that at high doses garcinol inhibits the growth of intestinal cells, but at low doses it stimulates their growth.

Garcinol Inhibits Signaling of Nuclear Factor-Kappa B (NF-κB)

The transcription factor NF-κB is one of the major mediators of inflammation and is linked with many diseases including cancer, diabetes, arthritis, and neurological disorders. Therefore, an agent that can suppress NF-κB activation has potential for clinical use against various chronic illnesses. Garcinol suppression of NF-κB activation induced by LPS[7,16] leads to the suppression of NF-κB-regulated products cyclooxygenase type 2 (COX-2) and inducible nitric oxide synthase (iNOS). These actions give it great potential as a broad-spectrum clinical agent.

Garcinol Inhibits Phosphatidylinositol 3′-Kinase/Protein Kinase B (PI3K/Akt)

Serine/threonine-specific protein kinase B, commonly designated Akt, is a central regulator of widely divergent cellular processes, including proliferation, differentiation, migration, survival, and metabolism. Akt is activated by a variety of stimuli, through growth factor receptors, in a PI3K-dependent manner. Frequently in human cancer, normal signaling along the Akt/PKB/phospatase and tensin homolog (PTEN) pathway is disrupted. Akt plays important roles in development, progression, and resistance to chemotherapy in cells. Blocking Akt signaling can mediate apoptosis and inhibit the growth of tumor cells *in vitro*.[17] Garcinol inhibits Akt activation, which leads to inhibition of tumor cell proliferation and survival.[8,12]

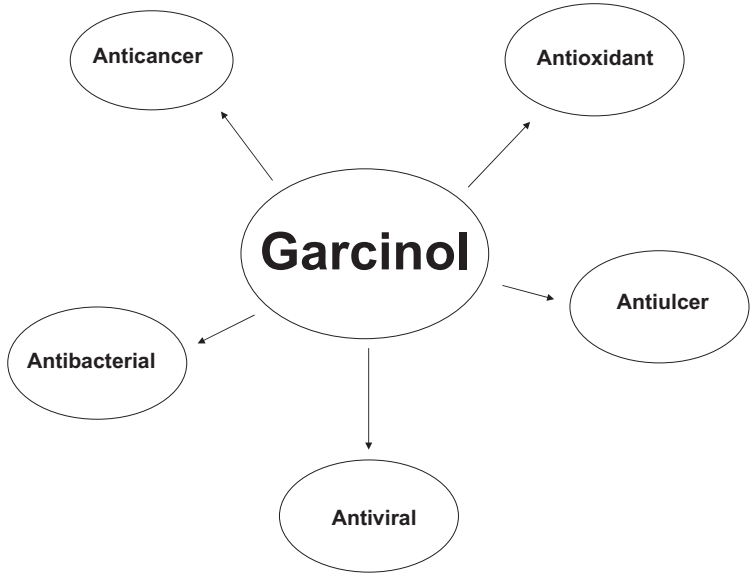

Fig. 4. Biological activities of garcinol.

Garcinol Inhibits Mitogen-Activated Protein Kinase (MAPK)

MAPKs are evolutionarily conserved enzymes that play a key role in the inflammatory stimuli and environmental stresses that lead to the activation of three independent pathways: p44/42 MAPK extracellular signal-regulated kinases 1 and 2 (ERK1/ERK2), c-Jun N-terminal kinase, and p38 MAPK. *In vitro* studies of the mouse Raw 264.7 cell line have shown that garcinol inhibits LPS-induced activation of the p38 MAPK pathway.[7] Moreover, this phytochemical also inhibited ERK in HT-29 cells.[8]

Garcinol Inhibits Src

The Src family of proteins consists of eight non-receptor tyrosine kinases characterized by a common structure. Src kinases are involved in signal transduction pathways that are triggered by a variety of surface receptors, including receptors for tyrosine kinases, integrins, and antigens, as well as receptors coupled with the G-protein. As a consequence of changes

observed in protein expression and kinase activity in cancer cells, the Src family has been implicated in the development of cancer. This prompted the design of specific inhibitors, the most common of which are adenine mimetics, to treat solid tumors and leukemia clinically. In addition, some of the Src kinases expressed in hematopoietic cells play pivotal roles in lymphocyte maturation and activation. This finding has encouraged the development of safe and effective Src-specific inhibitors that are currently in clinical trials as immunosuppressants for the treatment of immunological disorders. Separate research showing that garcinol inhibits Src in HT-29 cells[8] suggests that garcinol may also have clinical potential against cancers and immunological disorders in which Src plays a pivotal role.

Garcinol Inhibits Signal Transducer and Activator of Transcription-1 (STAT-1)

Proteins in the STAT family are among the best studied of the latent cytoplasmic signal-dependent transcription factors. Recently, STAT-1 was implicated in modulating pro- and anti-apoptotic genes after stress was induced.[18] *In vitro* studies of the mouse Raw 264.7 cell line suggest that garcinol modulates the metabolism of arachidonic acid by blocking the phosphorylation of cPLA2 and that it decreases the amount of iNOS in protein by inhibiting STAT-1 activation.[16]

Garcinol Inhibits iNOS

iNOS is expressed in a variety of cell types, particularly inflammatory cells, in response to diverse proinflammatory stimuli.[19] iNOS, which may be induced by bacterial LPS or its derivative lipid A, is expressed by a variety of solid tumors and generates high levels of nitric oxide inside tumor cells.[20] *In vitro* studies have shown that garcinol inhibits LPS- and interferon-gamma-induced iNOS in Raw 264.7 cells[7,16,21] and LPS- and interleukin-1beta (IL-1β)-induced iNOS in astrocytes.[8]

Garcinol Inhibits COX-2

Overexpression of COX-2 is associated with many cancers and is linked with tumor cell proliferation and suppression of apoptosis.[22] Therefore, COX-2

inhibitors have great potential in the treatment of cancers and inflammatory conditions, as evidenced by the U.S. Food and Drug Administration's approval of celecoxib, a known COX-2 inhibitor, for the treatment of various inflammatory conditions. Garcinol, too, has been shown to inhibit COX-2 activation induced by LPS in Raw 264.7 cells[7,16] and astrocytes.[8] Further, garcinol inhibits the induction of colonic aberrant crypt foci (ACF) by azoxymethane (AOM)[21] and, by downregulating COX-2 expression, the induction of oral carcinogenesis by 4-nitroquinoline 1-oxide (4-NQO) in rats.[23]

Garcinol Inhibits Matrix Metalloproteinase 7 (MMP-7)

Also known as matrilysin, MMP-7 is a "minimal domain" MMP that exhibits proteolytic activity against components of the extracellular matrix. MMP-7 is frequently overexpressed in human cancer tissues and is associated with cancer progression. Therefore, MMP-7 inhibitors have great potential in the treatment of cancer.[24] Studies have shown that garcinol inhibits the expression of MMP-7 in HT-29 cells,[8] further supporting the idea that garcinol may be effective against at least some colon cancers in humans.

Garcinol Inhibits Tubulin

Microtubules are a major component of the cytoskeleton. They are important in many cellular events and play a crucial role in cell division. As such, microtubules are a highly attractive target for anticancer-drug design. Tubulin-binding agents, also called antimicrotubule or microtubule-targeted agents, are widely used chemotherapeutic drugs with a proven clinical efficacy against breast, lung, ovarian, prostate, and hematologic malignancies, as well as childhood cancers.[25] Research has shown that garcinol and its derivatives belong to this class of agents because they inhibit microtubule assembly and prevent cell division.

Garcinol Inhibits Expression of Cyclin D1

The sequential transcriptional activation of cyclins, the regulatory subunits of cell-cycle-specific kinases, is thought to regulate progress through

the cell cycle.[26] Thus, cyclins are potential oncogenes, and overexpression of cyclin D1 or amplification at its genomic locus, 11q13, is commonly seen in breast cancer, head and neck cancer, non-small-cell lung cancer, and mantle cell lymphoma.[27] Experimental studies using rats have shown that garcinol inhibits cyclin D1 in 4-NQO-induced tongue carcinogenesis[23] and has great potential in the treatment of cancer.

Garcinol Inhibits the Expression of Bcl-2

The Bcl-2 gene family consists of at least 25 genes that are proapoptotic or antiapoptotic and share at least one of the four characteristic BH domains. The antiapoptotic protein Bcl-2, which displays sequence homology in all four domains (i.e. BH1–BH4), promotes cell survival.[28] Increased expression of the Bcl-2 protein commonly occurs in human malignancies and is associated with disease maintenance and progression, resistance to chemotherapy, and poor clinical outcome. Antisense oligonucleotides targeting Bcl-2 have been shown to facilitate apoptosis in various tumor types.[29–34] Therefore, Bcl-2 inhibitors have great potential in the treatment of cancer. *In vitro* studies have shown that garcinol inhibits Bcl-2 expression in HL-60[14] and HT-29 cells.[8]

Garcinol Inhibits DNA Fragmentation Factor (DFF) 45

One of the hallmarks of the terminal stages of apoptosis is internucleosomal DNA breakdown, with DNA fragmentation bearing functional significance.[35] Recently, it was suggested that apoptotic DNA fragmentation and associated nuclear changes are largely attributable to a caspase-activated DNAse (CAD) also known as DFF40. In nonapoptotic cells, CAD remains inactive because it is bound to its natural inhibitor, which is expressed as two isoforms, DFF45 and DFF35.[36] DFF45, which acts as a molecular chaperone for the DNAse to ensure its correct folding, is a caspase-3 substrate that must be cleaved to be inactivated; DFF45 allows DFF40 to execute nuclear internucleosomal DNA fragmentation.[36] Because activation of DFF40 (i.e. CAD) downstream of the caspase cascade is responsible for internucleosomal DNA fragmentation during apoptosis, it was deduced that DFF45 inhibits this process.[37,38]

Consequently, the expression of DFF45 may be involved in impaired apoptosis in neoplastic tissues. This means that DFF45 inhibitors have great potential in the treatment of cancer. *In vitro* studies have shown that garcinol induces DFF45 cleavage in HL-60 cells[14] restoring apoptosis.

Garcinol Induces Cleavage of Poly(ADP-ribose) Polymerases (PARPs)

PARPs are cell-signaling enzymes present in eukaryotes and are involved in poly(ADP-ribosylation) of DNA-binding proteins. Pharmacological degradation of PARP-1 may enhance the activity of antitumor drugs by inhibiting necrosis and activating apoptosis. *In vitro* studies have shown that garcinol induces PARP degradation and enhances apoptosis in HL-60 and HT-29 cells.

Garcinol Inhibits CBP/p300 Histone Acetyltransferase (HAT) and Histone Deacetylase (HDAC)

The process of histone acetylation and deacetylation in eukaryotic cells alters chromatin structure and thereby modulates gene expression. HATs and HDACs are classes of enzymes that effect histone acetylation. These enzymes can also acetylate and deacetylate several nonhistone substrates, which can have functional consequences. Altered HAT and HDAC activities can lead to several diseases, ranging from cancer to neurodegenerative disorders. Therefore, HAT and HDAC inhibitors are being developed as therapeutic agents. Garcinol inhibits HAT and HDAC activity,[6,39–41] and thus is a potential agent in the treatment of cancer and neurodegenerative diseases.

Garcinol Inhibits the Activation of Focal Adhesion Kinase (FAK)

FAK is a 119- to 121-kDa nonreceptor protein kinase widely expressed in various tissues and cell types. Several studies have shown that FAK plays an important role in integrin signaling. Once activated, whether by integrin or non-integrin stimuli, FAK binds to and activates

several other molecules, such as Src, Src adaptor protein p130Cas, the growth factor receptor-bound protein 2 (Grb2), PI3K, and paxillin, and thus promotes signaling transduction. In a recent study, FAK was held responsible for cancer cells' uninhibited proliferation, protection from apoptosis, invasion, migration, adhesion, and spread, as well as tumor angiogenesis.[42] Other studies have shown that garcinol modulates the tyrosine phosphorylation of FAK and subsequently induce apoptosis by downregulating Src, ERK, and Akt signaling in HT-29 cells.[8]

Garcinol Inhibits Tumor Necrosis Factor-alpha (TNF-α)

TNF-α is a vital member of the multifunctional superfamily of tumor necrosis factors (TNFs) and plays important roles in immunity and cellular remodeling, as well as apoptosis and cell survival.[26] Because TNF-α is a key player in inflammation and cancer, several efforts are underway to develop therapeutic TNF-α antagonists. Two such antagonists are from the *Garcinia* species. At a dose of 5 μM, both garcinol and cambogin inhibited the release of TNF-α by LPS-activated macrophages,[7] suggesting another mechanism for their antitumor activity.

Garcinol Inhibits Cytosolic Phospholipase A2 (PLA2)

The PLA2 enzyme catalyzes the hydrolysis of cellular phospholipids at the sn-2 position, which liberates arachidonic acid and lysophospholipid. This generates a family of proinflammatory eicosanoids and platelet-activating factors. PLA2 catalysis is the rate-limiting step in the production of proinflammatory eicosanoids and free radicals. These peroxides and ROS, in turn, activate the PLA2 enzyme and further attenuate the inflammatory process. Thus, a single molecule, such as an antioxidant, that can simultaneously scavenge these free radicals and inhibit the PLA2 enzyme is of great therapeutic interest.[43] Recent results suggest that garcinol does this because it modulates arachidonic acid metabolism by blocking the phosphorylation of cytosolic PLA2.[16]

Garcinol Induces Glutathione S-transferase (GST)

Human GSTs have enzymatic and nonenzymatic functions and are involved in many important cellular processes, such as phase II metabolism, stress response, cell proliferation, apoptosis, oncogenesis, tumor progression, and drug resistance.[44] Experimental studies have shown that feeding rats with garcinol increased the level of GST in AOM-induced colonic ACF.[21]

Garcinol Inhibits Cytochrome *c*

Cytochrome *c*, an intermediate in apoptosis, is released by the mitochondria in response to proapoptotic stimuli. Studies have shown that isogarcinol, a derivative of garcinol, induces the expression of cytochrome *c* in HL-60 cells.

Garcinol Induces BID

Proapoptotic BID activates the multi-domain Bcl-2 family members Bcl-2-associated X protein (Bax) and Bcl-2 homologous antagonist killer (Bak). Activation of either Bax or Bak produces an allosteric conformational change and releases cytochrome *c*.[45] This means that compounds that can induce BID could be very useful in the treatment of cancer. Garcinol is such an inducer because it activates BID and induces apoptosis in cancer cells.[8]

Garcinol Induces BAD

BAD is proapoptotic and proliferative, suggesting that the cell cycle functions of the multi-domain Bcl-2 family members.[45] BAD antagonizes both the cell cycle and antiapoptotic functions of Bcl-2 and Bcl-xL through BH3 binding. Overexpression of the BH3-only molecule BAD renders the cell unable to arrest in G0 and persistently activates cdk2.[45] Studies have shown that garcinol activates BAD and induces apoptosis in HL-60 cells.[14]

Garcinol Induces Cleavage of the D4-GDP Dissociate Inhibitor (D4-GDI)

D4-GDI, also known as Rho-GDI 2, is cleaved by caspase-3 during drug-induced apoptosis. The cleavage of D4-GDI occurs simultaneously with the activation of caspase-3 but precedes DNA fragmentation and the morphologic changes associated with apoptotic cell death.[46] *In vitro* studies have shown that garcinol induces cleavage of D4-GDI in HL-60 cells and induces apoptosis.[14]

Garcinol Induces the Activation of Caspase-3 and Caspase-9

Caspases play a central role in mediating various apoptotic responses. *In vitro* research of garcinol has shown that it induces the activation of caspase-3 and caspase-9 in HL-60 cells and induces apoptosis.[14,15]

BIOLOGICAL ACTIVITIES OF GARCINOL

Research conducted over the past few years has shown that garcinol and its derivatives have potent biological activities (Fig. 3). *In vitro* studies in particular have demonstrated garcinol's antioxidant, antibacterial, antiviral, and antitumor properties.

Antibacterial Activity of Garcinol and Its Derivatives

Garcinol is a natural antibacterial agent against *Staphylococcus aureus* and *Helicobacter pylori*. At a minimum inhibitory concentration of 16 μg/ml, garcinol suppressed the proliferation of methicillin-resistant *S. aureus*. Garcinol showed remarkable activity at a minimum inhibitory concentration of 16 μg/ml, while isogarcinol and the biphenyl derivative exhibited weaker activity at minimum inhibitory concentrations of 32 μg/ml and 64 μg/ml, respectively.[47] Nearly 50% of the world's population is infected with *H. pylori*. The *Helicobacter pylori* Antimicrobial Resistance Monitoring Program (HARP), a prospective, multicenter U.S. network that tracks national incidence rates of *H. pylori*, has reported clarithromycin resistance in 12.9% of isolates. *In vitro* studies have shown

that garcinol inhibits *H. pylori* in the presence and absence of clarithromycin and Protykin®.[48]

Antioxidant Activity of Garcinol

ROS play a critical role in cancer, arteriosclerosis, and ulceration, and the intake of antioxidants has been very attractive in the prevention of these diseases. *In vitro* studies have shown that garcinol scavenges the superoxide anion in the hypoxanthine/xanthine oxidase system, the hydroxyl radical in the Fenton reaction system, and ROS in the H_2O_2/NaOH/DMSO system.[10]

Antiviral Activity of Garcinol and Its Derivatives

Garcinol and its derivatives isogarcinol, isogarcinol 13-O-methylether, garcinol 13-O-methylether, clusianone, and macurin inhibited 12-O-tetradecanoyl-phorbol-13-acetate and thus prevented the induction of the Epstein–Barr virus early antigen in Raji cells.[49]

Anticarcinogenic Activity of Garcinol and Its Derivatives

In vitro studies have shown that garcinol and its derivatives are potent anticarcinogenic agents. MTT assay showed that garcinol and its derivatives cambogin, garcim-1, and garcim-2 inhibit the proliferation of HT-29 and HCT-116 cells, INT-407 human immortalized intestinal cells, and IEC-6 rat immortalized intestinal cells in a dose-dependent manner.[12] Moreover, they also showed that the inhibition of cell proliferation is due to the downregulation of pAkt and survivin.[12] Garcinol also inhibited BCl-2 and MMP-7 in HT-29 cells and induced apoptosis in these cells by modulating tyrosine phosphorylation of FAK and downregulating Src, ERK and Akt.[8] Garcinol and its derivatives isogarcinol and xanthochymol inhibited the growth of human leukemia cells NB4, K562 and U937 in a dose-dependent manner by inducing the release of cytochrome *c* and the activation of caspase-3.[15] Moreover, garcinol induced apoptosis in HL-60 cells by inducing the release of cytochrome *c* and activation of caspase-3.[14] Gambogic acid, another garcinol derivative, has shown similar

antitumorigenic activity against a wide range of tumor cell lines, including HeLa,[50] human hepatoma SMMC-7721,[51] human gastric carcinoma MGC-803[52] and BGC-823,[53] human lung carcinoma SPC-A1,[54] human myeloid leukemia KBM-5,[55] breast carcinoma MCF-7,[56] and Jurkat T cells.[57]

IN VIVO ACTIVITIES OF GARCINOL

The effects of garcinol have been examined in several animal models, including models for stress- and indomethacin-induced gastric ulceration, AOM-induced colonic ACF, and 4-NQO-induced oral carcinogenesis in rats. The results of all these analyses suggest a therapeutic role for garcinol.

Antiulceration Activity of Garcinol

Gastric ulceration is a worldwide health problem. *In vivo* studies have shown that garcinol (200 mg/kg body weight) inhibited gastric ulceration in rats that, 30 mins before, had been stressed by being immersed in 23°C water or given indomethacin on an empty stomach.[9] Further, these studies showed that garcinol was more protective than cetraxate at inhibiting gastric ulceration resulting from stress and indomethacin toxicity.[9]

Garcinol Inhibits Colonic ACF

Garcinol's ability to inhibit ACF induced by AOM was tested in rats given weekly subcutaneous injections of 15 mg/kg body weight AOM during the middle three weeks of a five-week diet that included 0.01% or 0.05% garcinol.[21] Rats whose diets included garcinol had significantly fewer ACF than control rats: 72 ± 15 ACF/rat for those getting 0.01% garcinol and 58 ± 8 ACF/rat for those getting 0.05% garcinol versus 97 ± 15 ACF/rat for those getting no garcinol, or 26% and 40% reductions, respectively, in the number of ACF. Rats whose diets included garcinol also had lower proliferating cell nuclear antigen index in ACF and increased levels of GST and quinone reductase in their livers.[21]

Garcinol Inhibits Oral Tongue Carcinogenesis

Dietary garcinol significantly decreased the incidence and multiplicity of 4-NQO-induced tongue neoplasms and/or preneoplasms as compared to the control diet. Dietary administration of garcinol also significantly reduced the BrdU-labeling index and cyclin D1-positive cell ratio, suggesting reduction in cell proliferation activity in the tongue by garcinol. The COX-2 expression in the tongue lesions was also suppressed by feeding with garcinol. These results indicated that dietary administration of garcinol inhibited 4-NQO-induced tongue carcinogenesis through suppression of increased cell proliferation activity in the target tissues and/or COX-2 expression in the tongue lesions.[23]

CLINICAL STUDIES

To date there are no clinical studies concerning garcinol.

CONCLUSIONS

The spice derived from kokum, the fruit of *Garcinia indica*, is used in Indian cuisines and Ayurvedic medicine. The main component isolated from kokum is garcinol, which demonstrates antioxidant, antimicrobial, antiulceration, and anticarcinogenic properties. Although garcinol is a potent, biologically active compound, only a limited number of studies have been carried out in animals and none have been done in humans. Because of its diverse range of biological activities *in vitro*, more *in vivo* and clinical studies are warranted to establish its true usefulness as a clinical therapeutic agent in a variety of human diseases. Until such time, the kokum-based recipes below will aid in readers' self-investigation of the benefits of this spice. Further recipes can be found through online searches for "kokum recipes" and in Indian cookbooks, particularly those concerning the cuisine of southern India.

Kokum-Based Receipes

Sol Kadi

Serves: 4

Cooking time: Approximately 4 min
Serving suggestions: Serve chilled in tall glasses before, during or after meals as an appetizer or digestive. Or, instead of drinking it, the consistency can be adjusted and the sol kadi can be served over boiled rice.

Ingredients

2 cups plain yogurt
4 cups water
5 dried kokum rinds
2 teaspoons garlic, chopped and crushed (about 2 cloves)
2 green chilies, chopped and crushed with salt
2 teaspoons mustard seeds
2 sprigs curry leaves
2 tablespoons oil
Salt and sugar to taste
Coriander leaves, finely chopped, for garnish (optional)

Method

Boil the dried kokum rinds with some water for about 4 min. Strain the red water and discard the rinds. Set aside to cool.

For sol kadi, combine the yogurt, water, crushed garlic and chiles, and 2 tablespoons of the kokum juice in a mixing bowl. Stir until all ingredients are blended and strain. Return strained contents to mixing bowl and stir in salt and sugar to taste.

Heat the oil in a small cooking pan, add the mustard seeds, and remove from heat. Add the curry leaves, and stir. Pour this seasoning over the sol kadi.

Pour into chilled glasses. Garnish each with some of the finely chopped coriander leaves (optional).

Kokum Tambli (Yogurt- and Coconut-Based Curry)

Serves: 3–4

Ingredients

¼ cup kokum
¼ cup coconut (fresh), coarsely chopped
¼ inch ginger, coarsely chopped
½ teaspoon cumin seeds
1 cup fresh plain yogurt
½ teaspoon ground black pepper
Salt to taste

Method

Soak dried kokum rinds in about 1 cup of warm water for 10–20 min until they are soft enough that the juice may be easily squeezed from them. Over a mixing bowl, hand squeeze as much juice as possible from each kokum rind; discard rinds. Leave the dark red kokum juice in the bowl.

With mortar and pestle, grind fresh coconut, ginger and cumin seeds into a smooth paste, adding drops of water, a little at a time to maintain a smooth consistency. Mix this ground coconut paste with kokum juice, yogurt, and ground black pepper. Add a little water if tambli is too thick. Adjust the seasoning to taste, adding salt if desired. Refrigerate until ready to serve.

Serve chilled with a bowl of steaming rice.

Kokum Potato

Ingredients

8–10 small potatoes, boiled and cubed
4–5 kokum
1 teaspoon unrefined sugar (gur or jaggery)
4–5 garlic cloves
½ teaspoon red chili powder
⅓ teaspoon cumin seeds
2–3 teaspoons oil
Salt to taste

Method

With mortar and pestle, grind kokum, sugar, cumin seeds, red chili powder, and garlic. Heat oil in a skillet or wok, fry the masala for a few minutes or until it separates from the oil. Add boiled, cubed potatoes and fry for 4–5 min. Serve with rice.

Kokum Shrimp

Ingredients

15–20 jumbo shrimp, peeled, deveined, and washed
1 teaspoon garlic, finely chopped (about 1 clove)
¾ teaspoon ground coriander
Pinch ground turmeric
¾ teaspoon red chili powder
½ cup water
2–3 dried kokum rinds
Oil
Salt

Method

In small bowl, mix chopped garlic, coriander, chili powder, turmeric, and salt.

Heat oil in a skillet or wok and fry the garlic and spices for 1 min. Add shrimp and fry for 2–3 min. Add water and then add kokum rinds. Cook until the shrimps are completely done; they should be opaque. Serve hot.

Goan Fish Curry

Ingredients

500 g fish fillets
1 teaspoon ground turmeric

1 teaspoon salt
2 teaspoons lemon juice or white vinegar
3 red chiles, coarsely chopped
1 teaspoon cumin seeds
1 teaspoon coriander seeds
1 teaspoon ground black pepper
6 cloves, garlic, coarsely chopped
1 inch ginger root, coarsely chopped
100 g grated coconut
1 quart water
1 large onion, coarsely chopped
2 tablespoons vegetable oil
5 dried kokum rinds or 2 tablespoons tamarind juice or lime juice
3 tomatoes, diced
3 green chiles, finely chopped

Method

Wash the fish fillets in cold water; pat dry. In a bowl large enough to accommodate the fish, mix the turmeric, salt, and lemon juice (or vinegar). Add the fish, coating each side with marinade. Cover with plastic wrap and put in the refrigerator to marinate for a few hours.

With mortar and pestle, grind the red chiles, cumin and coriander seeds, and pepper into a fine powder. Add the ginger root, garlic, and coconut and grind all together to make a smooth paste.

Add 1 quart water to a saucepan. Bring the water to boil while you heat the oil in a small frying pan to sauté the chopped onion until it is golden brown. Add the spice paste and cook for 10 min. Gently pour this mixture into the boiling water. Lower heat and simmer for 20 min. Add the fish fillets and kokum rinds and cook on a low heat for 20 min, stirring occasionally. Just before the fish are cooked (they should be opaque), add tomatoes and green chiles. Serve hot.

Table 1. Genes differentially expressed with treatment with garcinol in HeLa cells.

Genes down-regulated

Apoptosis

- BCL2-like 2
- BCL-6 interacting corepressor
- Bifunctional apoptosis regulator
- Death-associated protein kinase 1
- Fas apoptotic inhibitory molecule 2
- p53-induced protein PIGPC1

Cell cycle

- ATP-binding protein associated with cell differentiation
- Candidate tumor suppressor protein
- Cyclin A1
- Cyclin-dependent kinase 5
- HIR histone cell cycle regulation defective homolog A (*S. cerevisiae*)
- Myeloid cell nuclear differentiation antigen
- NIMA (never in mitosis gene a)-related kinase 3
- p21(CDKN1A)-activated kinase 6
- BRCA1-associated protein

Oncogene

- Cervical cancer 1 proto-oncogene
- Hepatocellularcarcinoma-associated antigen HCA557a
- Melanoma-associated gene
- Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, *Drosophila*)
- Ovarian carcinoma immunoreactive antigen
- Pim-2 oncogene
- Pituitary tumor-transforming 1-interacting protein
- Promyelocytic leukemia
- RAB22A, member RAS oncogene family
- T-cell leukemia/lymphoma 1A
- Tumor protein p63
- Tumor protein, translationally-controlled 1
- vav 3 oncogene
- v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)
- v-rel reticuloendotheliosis viral oncogene homolog B, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3 (avian)
- v-ski sarcoma viral oncogene homolog (avian)

(*Continued*)

Table 1. (*Continued*)

Transcription factors

- Bromodomain adjacent to zinc finger domain, 1A
- Bromodomain and PHD finger containing, 1
- Cofactor required for Sp1 transcriptional activation, subunit 2, 150 kDa
- DNA-directed RNA polymerase II polypeptide J-related gene
- E1B-55 kDa-associated protein 5
- E74-like factor 4 (ETS domain transcription factor)
- General transcription factor II, I
- General transcription factor IIIA
- Glucocorticoid receptor DNA-binding factor 1
- High-mobility group nucleosome-binding domain 1
- High-mobility group protein 2-like 1
- MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C)
- Nuclear factor (erythroid-derived 2)-like 1
- Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)
- Nuclear receptor subfamily 4, group A, member 3
- Putative DNA/chromatin-binding motif
- Sp2 transcription factor
- Special AT-rich sequence-binding protein 1 (binds to nuclear matrix/scaffold-associating DNAs)
- Sterile-motif and leucine zipper containing kinase AZK
- TAF12 RNA polymerase II, TATA box-binding protein (TBP)-associated factor, 20 kDa
- TAF6 RNA polymerase II, TATA box-binding protein (TBP)-associated factor, 80 kDa
- Transcription factor 4
- Transcription factor 7-like 2 (T-cell specific, HMG-box)
- Transcription factor B2, mitochondrial
- Zinc finger protein 3 (A8-51)
- Zinc finger protein 317
- Zinc finger protein 335
- Zinc finger protein 36, C3H type, homolog (mouse)

Genes up-regulated

Apoptosis

- Caspase 4, apoptosis-related cysteine protease
- CED-6 protein

(*Continued*)

Table 1. (*Continued*)

Cell cycle
Anaphase-promoting complex 1 (meiotic checkpoint regulator)
BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)
G1 to S phase transition 1
Cell division cycle 34
Oncogene
v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog
RAB9A, member RAS oncogene family

For references: Balasubramanyam *et al.*[6]

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Capsaicin — A Hot Spice in the Chemoprevention of Cancer

Joydeb Kumar Kundu and Young-Joon Surh*

Capsaicin (*trans*-8-methyl-*N*-vanillyl-6- nonenamide), a homovanillic acid derivative, is the major pungent ingredient of the capsicum fruits including green or red chili peppers (*Capsicum frutescence* L. or *Capsicum annum* L.), which are widely consumed as spices and condiments. Besides its use as a food additive, capsaicin has a long history of medicinal use to alleviate peripheral painful conditions such as rheumatoid arthritis, post-herpectic neuralgia, diabetic neuropathy, etc. Although capsaicin constitutes a 'hot' subject in neuroscience, the compound has also been extensively studied for its chemopreventive potential. While some early studies reported that capsaicin may act as a carcinogen or a co-carcinogen, others have demonstrated its chemopreventive effects. Molecular mechanisms underlying chemoprevention with capsaicin include inhibition of carcinogen activation, attenuation of lipid peroxidation and oxidative DNA damage, inhibition of tumor cell proliferation, induction of apoptosis in cancerous or premalignant cells, suppression of inflammation, and blockade of angiogenesis and metastasis. This chapter focuses on the mechanistic aspects of cancer chemopreventive effects of capsaicin.

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[a]American Cancer Society (2008). *Cancer Facts & Figures 2008*. Available from: www.cancer.org/downloads/STT/2008CAFFfinalsecured.pdf, p. 57.

INTRODUCTION

Despite remarkable progress in understanding the molecular basis of carcinogenesis and formulating numerous therapeutic modalities, the number of cancer-related deaths is expected to double in the next 50 years.[1] According to statistics published by the American Cancer Society, 7.6 million people around the world have died of cancer in 2007 alone, and this figure is expected to be 17.5 million by the year 2050.[a] It is notable that many types of cancer are preventable, and there is now a paradigm shift from chemotherapy to chemoprevention for the management of cancer. Chemoprevention refers to the use of either natural or synthetic substances or their combination to block, reverse or retard the process of carcinogenesis.[2] The concept of chemoprevention stems from the proposition made by Lee W. Wattenberg in the 1960s[3] and is currently being considered as one of the most practical and attractive strategies to prevent cancer. Interestingly, a wide variety of chemopreventive phytochemicals have been indentified in our regular plant-based diet.[4] Spices represent also an important reservoir of chemopreventive phytochemicals. Some phenolic substances present in commonly used spices have been shown to possess substantial antioxidant, anti-inflammatory and chemopreventive properties.[5]

One of the most widely consumed spices is hot chili pepper, which belongs to the plant genus Capsicum (family: Solanaceae). The primary pungent principle present in chili peppers (*Capsicum frutescence* L.) or hot red peppers (*Capsicum annum* L.) is capsaicin (*trans*-8-methyl-N-vanillyl-6-nonenamide), a homovanillic acid derivative.[6] Capsaicin has been recognized for its selective effects on small primary sensory neurons of C fiber types.[7] While administration of capsaicin to peripheral nerve endings initially produces neurogenic inflammation and evokes pain, repeated application of the compound makes sensory neurons refractory to further capsaicin challenges, perhaps due to depletion of the neurotransmitter of painful impulses known as Substance P[8] and the release of an anti-inflammatory neuropeptide somatostatin.[9] Capsaicin-induced desensitization of afferent fibers provides a rationale for its use as an experimental tool for studying pain mechanisms as well as a pharmacotherapy for various painful conditions, such as rheumatoid arthritis, post-herpectic neuralgia,

and diabetic neuropathy.[8] The versatile effects of capsaicin are mediated through its cognate receptor named vanilloid receptor-1 (VR1)/transient receptor potential of vanilloid-1 (TRPV1).[7,10] Prolonged exposure of this receptor to capsaicin leads to injury and the death of neuronal pain fibers, thereby blocking transmission of pain stimulus.[11]

While the role of capsaicin in the modulation of pain signaling has positioned this alkaloid as a "hot" topic in neuroscience, the compound has also been extensively studied for its carcinogenic and anticarcinogenic potential. Early studies with capsaicin have resulted in conflicting data regarding its effects on experimental mutagenesis and carcinogenesis. While some researchers have reported that capsaicin may act as a carcinogen or a co-carcinogen, others have demonstrated the chemopreventive potential of the compound.[8] This chapter focuses on the mechanistic aspects of the cancer chemopreventive effects of capsaicin (Fig. 1).

EFFECTS ON EXPERIMENTAL MUTAGENESIS

Dietary factors play a critical role in both the etiology and prevention of many human ailments including cancer. Because of its extensive use as a condiment, hot chili pepper or its active principle capsaicin has been subjected to extensive assessment for any adverse or harmful effects. Thus, capsaicin and chili extracts have been tested for mutagenicity in both bacterial and mammalian cells in culture. While several studies have reported capsaicin to be mutagenic in the presence or absence of an external metabolic activation system, others have failed to provide evidence for its genotoxic potential.[8] Some other groups of investigators have instead reported the antimutagenic activity of capsaicin.[8] Surh *et al.*[12] examined the effects of purified capsaicin on vinyl carbamate (VC)- or N-nitrosodimethylamine (NDMA)-induced mutagenesis in *Salmonella typhimurium* TA100. According to this study, capsaicin (0.42 mM) attenuated the bacterial mutagenicity of VC and NDMA by 50% and 42%, respectively, which was partly ascribed to its inhibition of cytochrome P450 2E1 (CYP 2E1) responsible for the metabolic activation of the aforementioned carcinogens.[12] Subsequently, Chanda *et al.*[13] demonstrated that pure synthetic *trans*-capsaicin was not mutagenic to *S. typhimurium* or *Escherichia coli*, but was weakly mutagenic in mouse lymphoma L5178Y cells in the pres-

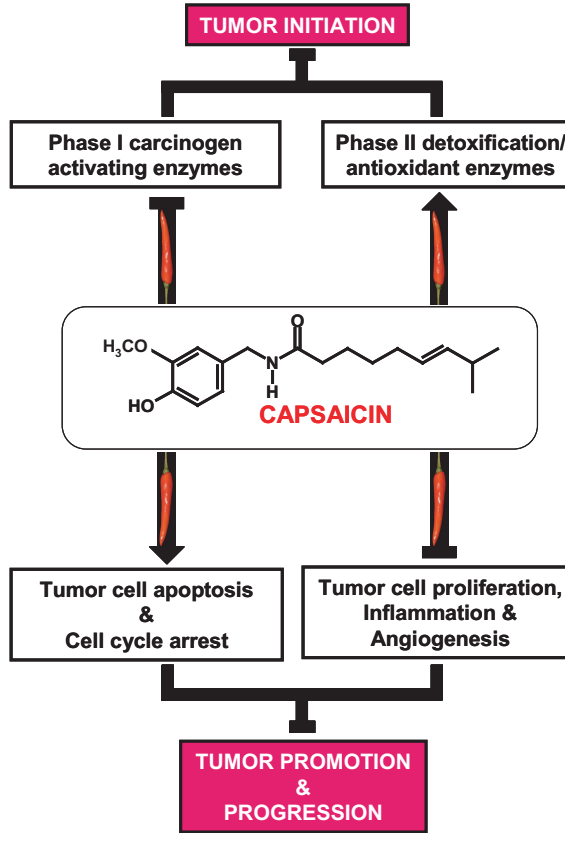

Fig. 1. Schematic representation of the biochemical mechanism underlying the chemopreventive effects of capsaicin in hot chili pepper. For further details, refer to Table 1.

ence of S9 mix. Interestingly, nontoxic concentrations (0.25 and 0.5 μmole/plate) of capsaicin exerted a dose-dependent inhibition of the mutagenicity of heterocyclic amines, such as 2-amino-6-methyldipyrido[1,2-a:3′,2′-d] imidazole (Glu-P-1) and 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP), when they were metabolically activated by rat, hamster and human liver S9, and also that of 3-amino-1-methyl-5H-pyrido[4,3-b] indole (Trp-P-2) when activated by rat and hamster liver S9.[14] In contrast, capsaicin enhanced the mutagenicity of Trp-P-2 in the TA98 strain when incubated with human liver S9.[14]

Although capsaicin did not induce mutagenesis in male germ cells *in vivo*, intraperitoneal administration of the compound in Swiss mice resulted in the formation of micronuclei in polychromatic erythrocytes and inhibition of DNA synthesis in the testes.[15] In addition, mice injected intraperitoneally with capsaicin (1.94 mg/kg body weight) exhibited increased frequencies of sister chromatid exchanges and a significant increase in the number of micronucleated normochromic erythrocytes and the ratio of polychromatic/normochromatic erythrocytes after 32 days of treatment, suggesting the genotoxic potential of capsaicin.[16] In contrast, a maximal tolerated dose of *trans*-capsaicin neither caused micronuclei formation in bone marrow cells nor induced chromosomal aberrations in blood lymphocytes of CD-1 mice.[13] Moreover, capsaicin significantly inhibited cyclophosphamide-induced chromosomal aberrations and DNA strand breakages in mice.[17] Proudlock and colleagues[18] reported the absence of genotoxic activity of high-purity capsaicin in bacterial mutagenicity and chromosome aberration tests. The same study also revealed that subcutaneous administration of high-purity capsaicin failed to cause genotoxicity in rat bone marrow micronucleus tests, suggesting that the previously reported genotoxic activity of capsaicin was probably due to mutagenic impurities present in commercial grades of capsaicin.[18]

EFFECTS ON CARCINOGEN-INDUCED TUMOR FORMATION IN EXPERIMENTAL ANIMALS

In line with some early studies demonstrating its mutagenic potential, capsaicin was subjected to extensive investigations with regard to its possible carcinogenicity in different experimental models. Although capsaicin has been reported to act as a carcinogen or a co-carcinogen in laboratory animals[19,20] as well as in humans,[21] other studies have demonstrated that the compound has preventive effects as evidenced by its inhibition of experimentally induced carcinogenesis.[22–24]

While the carcinogenic or co-carcinogenic potential of capsaicin was under controversy, Jang *et al*. reported the antitumor promoting effects of capsaicin. According to this study, NIH (GP) mice were treated with benzo[a]pyrene (BP) or 7,12-dimethylbenz[a]anthracene (DMBA) within 24 h of birth and subsequently fed with either vehicle or capsaicin

(0.01% in diet) for 6 weeks after weaning. Treatment with capsaicin in this study significantly lowered the incidence of BP-induced pulmonary adenomas and the multiplicity of DMBA-induced lung tumors.[22] In a rat multiorgan carcinogenesis model, dietary administration of capsaicin (0.01%) also significantly inhibited the formation of carcinogen-induced glutathione-S-transferase-positive (GST-P^+) hepatic foci and pulmonary adenomas.[25] A later study demonstrated that intragastric administration of capsaicin failed to inhibit tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumor formation in female A/J mice,[26] suggesting that the route of administration may affect the chemopreventive potential of capsaicin.

The antitumor initiating effects of capsaicin against chemically-induced mouse skin carcinogenesis have also been reported. Pretreatment of female ICR mice topically with capsaicin lowered the multiplicity of VC-induced skin tumors partly by inhibiting CYP 2E1, an enzyme responsible for the metabolic activation of VC.[12] When capsaicin was given prior to the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA), following initiation with DMBA, there was a significant reduction in skin tumor formation.[23] Repeated topical application of capsaicin did not cause significant enhancement of papilloma formation in DMBA-initiated mouse skin,[27] suggesting that the compound lacks tumor-promoting effects. Likewise, prolonged dietary administration of capsaicinoids, a mixture of 64.5% capsaicin and 32.5% dihydrocapsaicin, showed no carcinogenic effects in B6C3F1 mice.[28] In a *v*-Ha-Ras-transgenic (Tg.Ac) mouse model, topical application of TPA resulted in a 50% induction of multiple skin papillomas, while dermal application of *trans*-capsaicin to Tg.Ac mice for 26 weeks did not produce any preneoplastic or neoplastic skin lesions, suggesting that the compound lacks oncogenic potential.[29]

Hot spicy foods are generally suspected to damage the mucous membranes of the gastrointestinal tract, thereby causing gastric cancer. A relatively low dose of capsaicin (0.1 μg/kg body weight) was found to be gastroprotective against diverse noxious stimuli, such as HCl, ethanol and aspirin.[30] Intragastric administration of capsaicin either at the initiation or the promotion phase reduced the incidence of azoxymethane (AOM)-induced aberrant crypt foci and colonic adenocarcinoma,[31] as well as 4-nitroquinoline-1-oxide (4-NQO)-induced tongue carcinogenesis[24] in male F344 rats.

MOLECULAR MECHANISMS OF CHEMOPREVENTION WITH CAPSAICIN

As discussed in previous sections, capsaicin exhibits both carcinogenic and anticarcinogenic properties. Such discrepancies may be explained in terms of the dose and the purity of capsaicin used in each study as well as the specific responsiveness of various tissues or organs to capsaicin. The carcinogenic potential of capsaicin, as reported in early studies, may be due to the presence of possible impurities mixed with capsaicin during its isolation from hot chili peppers. More recent studies using pure *trans*-capsaicin have reported chemopreventive activities of the compound in different experimental models. The following sections will focus on the molecular insights into the mechanisms of chemoprevention with capsaicin (Table 1).

Inhibition of Metabolic Activation of Carcinogens

Many carcinogens are inactive *per se* and are activated largely by phase I xenobiotic metabolizing enzymes, especially those that belong to the members of the CYP superfamily. The phase I metabolic end-products are often highly reactive and are usually eliminated through a second phase of metabolic reactions, commonly known as phase II detoxification. Shutting off the detoxification pathway may lead to the accumulation of metabolically activated carcinogens, which can directly attack the target cell DNA, thereby contributing to tumor initiation.

Capsaicin has been shown to modulate CYP-dependent monooxygenase activities, thereby blocking metabolic activation of carcinogens and other deleterious xenobiotics.[12,32] For example, capsaicin diminished the activity of rat epidermal arylhydrocarbon hydroxylase, which is linked to CYP1A isoform, blocking the metabolic activation and subsequent DNA binding of BP in human and murine keratinocytes.[32] Capsaicin inhibited the mutagencity of NNK in *S. typhimurium* TA1535 by blocking metabolic activation of the carcinogen.[33,34] Similarly, the α-hydroxylation of NNK was blocked by hepatic and pulmonary microsomes from golden Syrian hamsters treated with capsaicin.[34] Furthermore,

Table 1. Molecular targets of chemoprevention with capsaicin.

Molecular Mechanisms	Targets/Effects	Experimental Model	Refs.
Inhibition of carcinogen activation			
	↓Arylhydrocarbon hydrolase activity; ↓BP metabolism; ↓ [^{3}H]-BP DNA binding	Neonatal rat epidermal microsomes, Human keratinocytes, Balb/c mice	32
	↓CYP 2E1 activity; ↓*p*-nitrophenol hydroxylation; ↓NDMA-N-demethylation; ↓VC-induced mouse skin carcinogenesis	VC- or NDMA-treated ICR mice	12
	↓CYP2A2, 3A1, 2C11, 2B1, 2B2, and 2C6; decreased metabolic activation of NNK	Hamster and rat liver microsomes	96
	↓α-carbon hydroxylation of NNK; ↓methylation of NNK	Liver and lung microsomes from Syrian Golden hamster	34
Anti-oxidant effects			
	↓Lipid peroxidation; ↓myeloperoxidase activity	Ethanol-treated rat gastric mucosa	39
	↓BP-induced lipid peroxidation; restored BP-depleted activities of SOD, CAT, GPx, GR, GST, G6PD	Lung tissue or lung mitochondria of BP-treated mice	40, 41
	↑GST and QR activity	F344 rats	31
	↑Expression and activity of HO-1; ↑Nrf2 activation	HepG2 cells	43

(*Continued*)

Table 1. (*Continued*)

Molecular Mechanisms	Targets/Effects	Experimental Model	Refs.
Inhibition of cell proliferation			
	G0-G1 arrest; ↓expression of cyclin E; ↓expression of Cdk4 and Cdk6; ↓E2F level; ↑p16 expression	CE 81T/VGH cells	45
	G0-G1 phase arrest; ↓expression of pRb and cyclin D1	Human leukemic cells	46
	G1 arrest; ↓STAT3 phosphorylation; ↓STAT3 DNA binding; ↓expression of survivin; ↓expression of cyclin D1; ↓expression of cSrc and phosphorylation of JAK1; ↓growth of myeloma cells xenograft	Human multiple myeloma cells	47
Induction of apoptosis			
	↑ROS and Ca^{+2}, ↑levels of p53 and p21; ↓mitochondrial membrane potential; ↑Bax; ↓Bcl-2; ↑cytochrome *c* release; ↑caspase-3 activity	CE 81T/VGH cells	45
	↑ROS; ↑expression of p53, p21 and Bax; ↑phosphorylation of p53 (serine-15)	Human leukemic cells	46

(*Continued*)

Table 1. (*Continued*)

Molecular Mechanisms	Targets/Effects	Experimental Model	Refs.
	↓Expression of Bcl-2 and Bcl-xl; ↑caspase-3 activation; ↑apoptotic effect of thalidomide	Human multiple myeloma cells	47
	↑iNOS expression; ↑peroxynitrite level; ↑nitrotyrosine containing proteins; ↓expression of MnSOD and Cu/Zn SOD	C6 glioma cells	64
	↑ROS and Ca^{2+}; ↓mitochondrial membrane potential; ↑caspase-3 activation; ↑phosphorylation of ERK and JNK; ↑ceramide accumulation; ↑expression of Par-4; ↑expression of p16 and eIF2α; ↑expression GADD153/CHOP and ATF4	PC3 cells	52, 65, 66
	↑Phosphorylation of p38 MAP kinase and JNK	H-*ras*-transformed MCF-10A cells	54
	↑Phosphorylation of AMPK and acetyl CoA carboxylase	HT-29 cells	67
	↓Bcl-2 expression; ↓Bcl-2/Bax ratio; ↑caspase-3 activity	SK-Hep-1 cells	69
	↑Expression of p53, p21 and Bax	LnCaP, DU-145, PC3 cells	51
	↑Phosphorylation of p53 (serine-15)	Human leukemic cells	46
	Activation of PPAR-γ	HT-29 cells	68

(*Continued*)

Table 1. (*Continued*)

Molecular Mechanisms	Targets/Effects	Experimental Model	Refs.
	VR1/TRPV1-dependent apoptosis; ↑Ca^{2+} influx; ↑p38 MAP kinase; ↓mitochondrial membrane potential; ↑caspase-3 activation	Glioma cells	10
Anti-inflammatory effects			
	↓Expression of COX-2 and iNOS; ↓production of PGE_2 and NO; ↓phosphorylation of ERK, p38 MAP kinase and JNK; ↓NF-κB and AP-1 DNA binding	LPS-stimulated Raw 264.7 macrophages	82
	↓PGE_2 production; ↓DNA binding of NF-κB and AP-1	LPS-stimulated macrophages	83
	↓NF-κB activation; ↓p65 nuclear migration	TNF-α-treated ML-1a cells	6
	↓NF-κB and AP-1 DNA binding	TPA-treated mouse skin, TPA-stimulated HL-60 cells	85 88
Inhibition of angiogenesis			
	↓Constitutive expression of VEGF	Human multiple myeloma cells	47
	↓VEGF-induced cell proliferation; ↓DNA synthesis; ↓capillary-like tube formation	Human malignant melanoma cells	90

capsaicin attenuated the activity of CYP2E1, thereby inhibiting VC- or NDMA-induced mutagenesis in the *S. typhimurium* TA100 tester strain.[12]

Antioxidant Effects — Attenuation of Oxidative DNA Damage and Tissue Injury

Oxidative stress contributes to tumorigenesis either directly by damaging critical biomolecules such as DNA, proteins and membrane lipids, or indirectly by modulating intracellular signal transduction pathways. Interestingly, our body is endowed with various antioxidant enzymes, which help to maintain the cellular redox status by facilitating the elimination or inactivation of reactive oxygen species (ROS) or other reactive species. Several studies have reported that capsaicin possesses antioxidant activity,[35,36] which may account for its chemopreventive effects.

Capsaicin ameliorated the peroxidative changes in rat hepatic and pulmonary tissues induced by various noxious stimuli, such as chloroform, carbon tetrachloride and dichloromethane.[37] The ultraviolet radiation-induced lipid peroxidation in liposomal membrane was also attenuated by capsaicin.[38] Intragastric administration of capsaicin inhibited lipid peroxidation and the myeloperoxidase activity in ethanol-induced gastric mucosal lesions in Sprague–Dawley rats.[39] Anandakumar *et al.*[40,41] have recently demonstrated that intraperitoneal administration of capsaicin protects against lipid peroxidation and restores the BP-depleted activities of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PD), and GST in pulmonary tissues and lung mitochondria of Swiss albino mice.[40,42]

Phase II detoxification/antioxidant enzymes guard against excessive accumulation of ROS and facilitate elimination of metabolically activated carcinogens. Capsaicin given by gavage elevated the levels of antioxidant enzymes, such as GST and quinone reductase (QR), in liver, tongue and colon of F344 rats.[24,31] The expression of heme oxygenase-1 (HO-1), at both protein and mRNA levels, in human hepatoma HepG2 cells was markedly elevated by treatment with capsaicin.[43] Mechanistically, a mild pro-oxidant effect of capsaicin was involved in capsaicin-induced

HO-1 expression.[43] Capsaicin, being a quinone analog, induced ROS generation in HepG2 cells by downregulating the expression and the activity of NAD(P)H:quinone oxidoreductase-1, thereby resulting in the activation of a redox-sensitive transcription factor nuclear factor erythroid related factor-2 (Nrf2), a key transcription factor responsible for HO-1 expression.[43]

Antiproliferative Effects

Capsaicin exhibited chemopreventive effects partly by blocking proliferation of various cancer cells in culture. Treatment of cultured human malignant melanoma cells with capsaicin resulted in the inhibition of their proliferation, which was associated with downregulation of constitutive or interleukin (IL)-1β- or tumor necrosis factor-α (TNF-α)-induced activation of nuclear factor-kappa B (NF-κB) and reduced production of a pro-inflammatory cytokine IL-8.[44] Capsaicin arrested the growth of human esophagus epidermoid carcinoma (CE 81T/VGH) cells[45] and human leukemic cells[46] at the G_0-G_1 phase of the cell cycle. Interestingly, capsaicin did not arrest the growth of normal bone marrow mononuclear cells,[46] suggesting a selective growth inhibitory effect of the compound in tumor cells. Capsaicin also inhibited proliferation of human multiple myeloma cells as corroborated by accumulation of these cells at the G_1 phase.[47] According to this study, capsaicin inhibited the constitutive as well as IL-6-induced activation of signal transducer and activator of transcription-3 (STAT3) through blockade of Janus kinase-1 and c-Src. The antiproliferative effect of capsaicin in these cells was partly ascribed to its inhibition of STAT3-regulated expression of cyclin D1 and survivin. A more recent study revealed that capsaicin inhibited proliferation of immortalized endometriotic cells, and hence could be a potential therapeutic choice for the treatment of endometriosis,[48] which is a predisposing factor for endometrial adenocarcinomas.[49]

The growth of B16 mouse melanoma cells, transplanted in C57BL/6 mice, was significantly inhibited by intratumoral administration of capsaicin along with *tert*-butylhydroperoxide.[50] The compound also attenuated the growth of human promyelocytic leukemia (NB4) cells inoculated subcutaneously into NOD/SCID mice.[46] Capsaicin, when given orally[51] or

subcutaneously,[52] suppressed the growth of human prostate cancer xenograft in nude mice. Likewise, intraperitoneal administration of capsaicin inhibited the growth of human multiple myeloma xenograft tumors in male athymic nu/nu mice.[47]

Induction of Tumor Cell Apoptosis

Capsaicin has been shown to induce apoptotic cell death preferentially in cancerous or transformed cells, while barely affecting the growth of normal cells.[53,54] Initially, capsaicin was reported to induce apoptosis by inhibiting the plasma membrane NADH-oxidoreductase, an enzyme that is constitutively activated in tumors and transformed cells.[50,55–57] Alternatively, the induction of apoptosis in human cutaneous squamous cell carcinomas by capsaicin was mediated through suppression of mitochondrial respiration, especially at complex I, resulting in the disruption of mitochondrial membrane permeability transition.[58] While several investigators reported that capsaicin induced apoptosis in different cancerous or transformed cells[46,59] by generating intracellular ROS, others have found that the proapoptotic activity of the compound was correlated with its ability to reduce the constitutive production of ROS.[60–62] The biochemical mechanisms underlying capsaicin-induced generation of ROS appear to interfere with the coenzyme Q binding site, which redirects the normal electron flow in the complex, thereby producing excess endogenous ROS.[63] Capsaicin treatment caused apoptosis of human esophagus epidermoid carcinoma (CE 81T/VGH) cells through generation of ROS, release of intracellular Ca^{2+}, and activation of caspase-3. Treatment of cells with an intracellular Ca^{2+} chelator abrogated capsaicin-induced apoptosis.[45] Besides generation of superoxides, an elevated level of peroxynitrite was also implicated in capsaicin-induced apoptosis. Thus, cultured C6 glioma cells in the presence of capsaicin showed increased expression of iNOS, generation of superoxide and release of nitrite and nitrate in culture medium. Pretreatment with ebselen, a peroxynitrite inhibitor, effectively inhibited capsaicin-induced apoptosis in these cells.[64]

The perturbation of the plasma or mitochondrial membrane or disruption of the mitochondrial respiratory system by capsaicin leads to

generation of a pro-oxidant state that may cause dissipation of the mitochondrial transmembrane potential ($\Delta\Psi m$), which is a prerequisite for the induction of apoptosis. Capsaicin induced apoptosis in human prostate cancer (PC3) cells by a mechanism involving generation of ROS, dissipation of the mitochondrial inner transmembrane potential and activation of caspase-3.[52] In addition, increased phosphorylation of extracellular signal-regulated protein kinase (ERK) and c-Jun-N-terminal kinase (JNK), elevated expression of prostate apoptosis response-4 (par-4) and intracellular accumulation of ceramide were associated with capsaicin-induced PC3 cell death.[65] A recent microarray analysis revealed that capsaicin induced expression of the growth arrest and DNA damage-inducible gene 153 (GADD153)/CHOP, an endoplasmic reticulum stress-induced gene, in PC3 cells. Blockade of GADD153/CHOP expression by RNA interference reduced capsaicin-induced PC3 cell death.[66] H-*Ras*-transformed human mammary epithelial cells treated with capsaicin underwent apoptosis via activation of JNK and p38 mitogen-activated protein (MAP) kinase, and deactivation of ERK.[54] Kim and colleagues recently reported that capsaicin induced apoptosis in human colon cancer (HT-29) cells through activation of adenosine monophosphate-activated protein kinase, an enzyme that is usually activated during ATP-depleting metabolic stress including oxidative stress.[67] In addition, apoptosis induced in capsaicin-treated HT-29 cells was likely to be mediated via peroxisome proliferator activated receptor (PPAR)-γ as capsaicin-induced cell death was inhibited by bisphenol A diglycidyl ether, a specific PPAR-γ antagonist.[68]

Capsaicin-induced apoptosis has been shown to be blocked by overexpression of Bcl-2 in human B-cell and mouse myeloid cell lines.[57] The induction of apoptosis in human hepatocellular carcinoma (SK-Hep-1) cells by capsaicin was associated with a decrease in the ratio of Bcl-2/Bax and an increase in caspase-3 activation.[69] Likewise, the induction of apoptosis in human gastric adenocarcinoma[70] and murine B16-F10 melanoma[71] cells by capsaicin was associated with a downregulation of Bcl-2 expression. Capsaicin activated caspases and diminished the expression of STAT3-regulated genes, such as Bcl-2 and Bcl-xL, thereby inducing apoptosis in human multiple myeloma cells.[47]

Capsaicin-induced apoptosis in cultured human gastric cancer (SNU-1) cells accompanied upregulation of p53 and c-Myc.[72] The induction of

apoptosis in androgen receptor (AR)-positive (LNCaP) and -negative (PC3, DU-145) prostate cancer cells by capsaicin was associated with an increase in p53, p21 and Bax.[51] Capsaicin caused human leukemic cell death partly by phosphorylating p53 at serine 15 residue, and the inactivation of p53 by antisense oligonucleotide significantly attenuated capsaicin-induced cell cycle arrest and apoptosis.[46] The compound also increased mitochondrial membrane permeability and induced apoptosis in gastric cancer cells through activation of Bax and p53 in a JNK-dependent manner.[53]

As mentioned at the beginning of this chapter (under Introduction), some physiologic and toxicologic functions of capsaicin are mediated by the VR1/TRPV1. Amantini *et al.*[10] demonstrated a VR1/TRPV1-dependent apoptosis of glioma cells by capsaicin. According to this study, capsaicin-induced glioma U373 cell death involved Ca^{2+} influx, activation of p38 MAP kinase, dissipation of the mitochondrial transmembrane potential, and caspase-3 activation. Marked inhibition of these apoptotic events by capsazepine suggests a functional role for VR1/TRPV1 in capsaicin-induced apoptosis. In contrast, capsazepine failed to inhibit capsaicin-induced apoptosis in human colon cancer (HT-29),[68] human glioblastoma (A172),[62] and HepG2[73] cells, indicative of a VR1/TRPV1-independent mechanism underlying capsaicin-induced apoptosis in these cells. Overexpression of TRPV6, another vanilloid receptor subtype with a calcium ion-selective channel protein, sensitized cells to capsaicin-induced apoptosis, while knockdown of TRPV6 in cancer cells suppressed this action.[53] Thus, TRPV6 also plays a functional role in capsaicin-induced apoptosis.[53]

Anti-Inflammatory Activity

Since inflammation plays a multifaceted role in different stages of carcinogenesis, substances with pronounced anti-inflammatory properties appear to be potential candidates for cancer chemoprevention. Although topical application of capsaicin can initially induce ear edema in mice[74,75] and neurogenic inflammation in human skin,[76] repeated administration of the compound suppressed the subsequent inflammatory response.[74,77] The resolution of neurogenic inflammation by repeated administration of capsaicin was attributed to depletion of substance P, a pro-inflammatory mediator,

from sensory nerve terminals. Thus, topical application of capsaicin suppressed the substance P-mediated flare response in human skin.[78] Since substance P acts as a promoter of gastric carcinogenesis initiated by N-methyl-N′-nitro-N-nitrosoguanidine (MNNG),[79] the depletion of substance P by capsaicin may have contributed to protection against MNNG-induced gastric cancer.

Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), two representative pro-inflammatory enzymes, have been implicated in carcinogenesis.[80] Intragastric administration of capsaicin attenuated the expression of COX-2 in ethanol-induced rat gastric mucosal lesion.[39] Capsaicin inhibited lipopolysaccharide (LPS)- or TPA-induced expression of COX-2 protein and its mRNA transcript, and suppressed the production of prostaglandin E_2 (PGE_2) in murine macrophage Raw 264.7 cells (reviewed in Ref. 81). In addition, capsaicin diminished the expression of iNOS and the production of nitric oxide (NO) in Raw 264.7 cells stimulated with LPS plus interferon-γ (IFN- γ). The expression of COX-2 and iNOS is regulated by signaling mediated via upstream MAP kinases and their downstream transcription factors, such as NF-κB and activator protein-1 (AP-1).[81] Capsaicin blocked LPS-induced activation of upstream kinases, such as ERK, JNK and I-κB kinase (IKK), and decreased the DNA binding activity of NF-κB and AP-1 in Raw 264.7 cells.[82] Although capsaicin treatment inhibited the expression of iNOS, it did not affect the expression of COX-2 protein and its mRNA transcript in murine peritoneal macrophage cells stimulated with LPS.[83] However, capsaicin attenuated LPS-induced PGE_2 production in these cells, indicative of its inhibition of COX-2 activity. This study also revealed that capsaicin suppressed LPS-induced NF-κB activation by blocking I-κBα degradation.[83] Since murine macrophages did not express VR1/TRPV1, the inhibitory effects of capsaicin on LPS-induced COX-2 and iNOS expression appeared to involve a VR1/TRPV1-independent mechanism.[82,83] In contrast, treatment with capsaicin of human keratinocytes (HaCaT), expressing VR1/TRPV1 resulted in elevated expression of COX-2 and IL-8, and increased production of PGE_2, which were blocked by co-treatment with capsazepine.[84] Thus, it appears that capsaicin induces COX-2 expression in cells expressing a functional VR1/TRPV1, while it abrogates induced COX-2 expression in cells lacking this receptor.

The induction of various inflammatory and growth regulatory genes requires the activation of NF-κB and/or AP-1. Capsaicin exerts anti-inflammatory and antiproliferative effects by targeting these transcription factors. Singh *et al.*[6] demonstrated that capsaicin blocked the activation of NF-κB in human myeloid (ML-1a) cells stimulated with TNF-α or TPA. However, the compound failed to inhibit okadaic acid-induced NF-κB activation in these cells. The inhibition of TNF-α-induced NF-κB activity by capsaicin was associated with the blockade of I-κBα degradation and subsequent inhibition of nuclear translocation of p65. Capsaicin treatment also attenuated the TNF-α-dependent promoter activity of I-κBα that contains NF-κB binding sites.[6] Likewise, capsaicin inhibited constitutive or IL-1β-induced activation of NF-κB in malignant melanoma cells.[44] Capsaicin abrogated the activation of NF-κB by blocking its nuclear migration through inhibition of proteasomal degradation of I-κBα in TNF-α-stimulated PC3 cells.[51] Moreover, pretreatment with capsaicin suppressed TPA-induced activation of both NF-κB and AP-1 in mouse skin *in vivo*[85] and in cultured human promyelocytic leukemia (HL-60) cells.[86] Similarly, the DNA binding of NF-κB and AP-1 in human leukemia (K562 and U937) cells treated with TNF-α or TPA was mitigated by capsaicin.[87] While capsaicin-mediated inhibition of TPA-induced NF-κB in HL-60 cells was associated with blockade of degradation of I-κBα and nuclear translocation of p65, methylation of the phenolic hydroxyl group of capsaicin abolished this inhibitory effect, suggesting that the presence of this functional group is essential for capsaicin inhibition of NF-κB.[88]

Effects on Tumor Angiogenesis and Metastasis

Capsaicin downregulated the expression of vascular endothelial growth factor (VEGF), a key molecular switch in tumor angiogenesis, by inhibiting both constitutive and IL-6-induced activation of STAT3 in human multiple myeloma cells.[47] Thus, the growth of these cells xenografted in athymic nu/nu mice was suppressed by capsaicin.[47] In contrast, capsaicin upregulated VEGF production in human malignant melanoma (A375P and A375SM) cells by increasing the DNA binding activity of hypoxia inducible factor-1α (HIF-1α). In addition, treatment of these cells with capsaicin inhibited the activation of NF-κB and reduced proliferation.[89] A subsequent study by Min *et al.* demonstrated that capsaicin inhibited

VEGF-induced proliferation, DNA synthesis, chemotactic motility, and capillary-like tube formation in primary cultured human endothelial cells.[90] The inhibition of VEGF-induced vessel sprouting and vessel formation by capsaicin was also demonstrated in a rat aortic ring assay and a mouse matrigel plug assay, respectively. Moreover, capsaicin suppressed tumor-induced angiogenesis in a chick chorioallantoic membrane assay.[90] Erin and colleagues reported a significant increase in the lung metastasis of orthotopically injected murine mammary carcinoma (4T1) cells in mice, which resulted from sensory denervation with capsaicin at a dose of 125 mg/kg body weight.[91]

Role in Chemosensitization

The development of resistance towards conventional anticancer agents is an emerging setback in cancer therapy. P-glycoprotein (P-gp), a product of multidrug resistant-1 (mdr-1) gene, plays a key role in developing chemoresistance. P-gp reduces the intratumoral bioavailable concentrations of chemotherapeutic agents by actively effluxing drugs from cells. Some chemopreventive phytochemicals have been shown to sensitize chemoresistant cancer cells to undergo apoptosis or growth arrest while minimizing the chemotherapy-induced side effects.[92] Capsaicin holds the promise of an adjuvant therapy to anticancer drugs. Thus, treatment of P-gp overexpressing multidrug-resistant human carcinoma KB-C2 cells with capsaicin led to enhanced accumulation of daunorubicin and rhodamine 123, and increased the cytotoxicity induced by vinblastine.[93] Capsaicin also blocked the efflux of rhodamine 123 from intestinal carcinoma (Caco2) cells by suppressing the P-gp function.[94] The modulation of the P-gp activity in Caco2 cells was shown to be dependent on the concentration and duration of treatment with capsaicin. At concentrations ranging from 10–100 μM, capsaicin inhibited P-gp-mediated efflux transport of [^{3}H]-digoxin, while prolonged incubation of Caco-2 cells with capsaicin (50 and 100 μM) increased the expression and the activity of P-gp in these cells.[95] Hence, caution should be exercised in taking capsaicin for a prolonged period with various anticancer drugs, which are P-gp substrates, to avoid any plausible chemotherapy failure.

CONCLUSION

In the context of the increasing trend of chemotherapy resistance and the alarmingly high incidence of and the mortality from cancer in general, the concept of chemoprevention has been widely accepted as a practical approach to fighting against cancer. Food-derived phytochemicals, especially those from common spices, have been shown to possess substantial cancer chemopreventive properties. Hot chili pepper and its major pungent ingredient capsaicin have long been debated for carcinogenic or anticarcinogenic effects. While several early studies pointed out its carcinogenic or co-carcinogenic potential, recent studies with pure *trans*-capsaicin have demonstrated the anticarcinogenic effects of the compound. Capsaicin exerts chemopreventive effects through multiple mechanisms involving the inactivation of carcinogens, inhibition of cell proliferation, induction of apoptosis, the blockade of angiogenesis, and so on. Since the resolution of neurogenic inflammation by repeated use of capsaicin frequently involves the death of sensory neurons, and capsaicin-induced sensory denervation favors lung metastasis of breast cancer cells, more rigorous studies are required to ascertain the safety of capsaicin before its prolonged use at a high dose for achieving chemoprevention. Although capsaicin had been included in the GRAS (generally recognized as safe) list defined by the US Food and Drug Administration, the link between capsaicin consumption and the risk of carcinogenesis needs further investigation.

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13

Rosemary (Rosmarinic Acid)

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Rosmarinic acid (RosA) is a naturally occurring ester of caffeic acid and 3,4-dihydroxyphenyllactic acid. It is widely distributed in *Labitae* herbs, which include rosemary, sweet basil, and perilla. RosA has a broad range of applications that include use as a food preservative, in cosmetics, and medicinal uses as an antimicrobial and an antioxidant. In addition, it has been reported that RosA has the ability to block complement fixation and inhibit lipoxygenase and cyclooxygenase activity. Recently, the inhibition of T-cell receptor (TCR)-mediated signaling has been identified as one of the many effects of anti-inflammatory drugs. Despite the fact that their points of action are quite different, nonsteroidal anti-inflammatory drugs (NSAIDs) such as tacrolimus, pimecrolimus and hydroxychloroquine have been found to inhibit TCR-induced signaling events, including Ca^{2+} mobilization, activation of p38 mitogen-activated protein kinase (MAPK), and expression of the CD40 ligand. Similarly, RosA was recently found to inhibit the Ca^{2+}-dependent pathways by which TCR-mediated signaling occurs by inhibiting PLC-γ1 and Itk activities. In addition, the results of many studies evaluating the anti-inflammatory and immunomodulatory effects of RosA have suggested that it has many beneficial and health-promoting effects.

INTRODUCTION

Rosmarinic acid (Ros A, also known as α-O-caffeoyl-3,4-dihydrocyphenyl-lactic acid) is a phenolic compound (Fig. 1) found in plants belonging to the Lamiaceae family, which contains many aromatic and medicinal plants that are commonly used in traditional medicine. The most well-known plants that belong to the Lamiaceae family and contain RosA are *Rosmarinus officinalis* (rosemary),[1] *Ocimun basilicum* (basil), *Melissia officinalis* (lemon balm), *Mentha spicata* (mint),[2] and the Eastern medicinal plant, *Perilla frutescens* (perilla) (Fig. 2).[3] RosA is also found in species of other higher plant families, as well as in some fern and hornwort species.[4,5]

The presence of RosA in medicinal plants, herbs and spices is known to have beneficial and health-promoting effects. For example, it has been identified as the primary compound responsible for the antioxidant activity

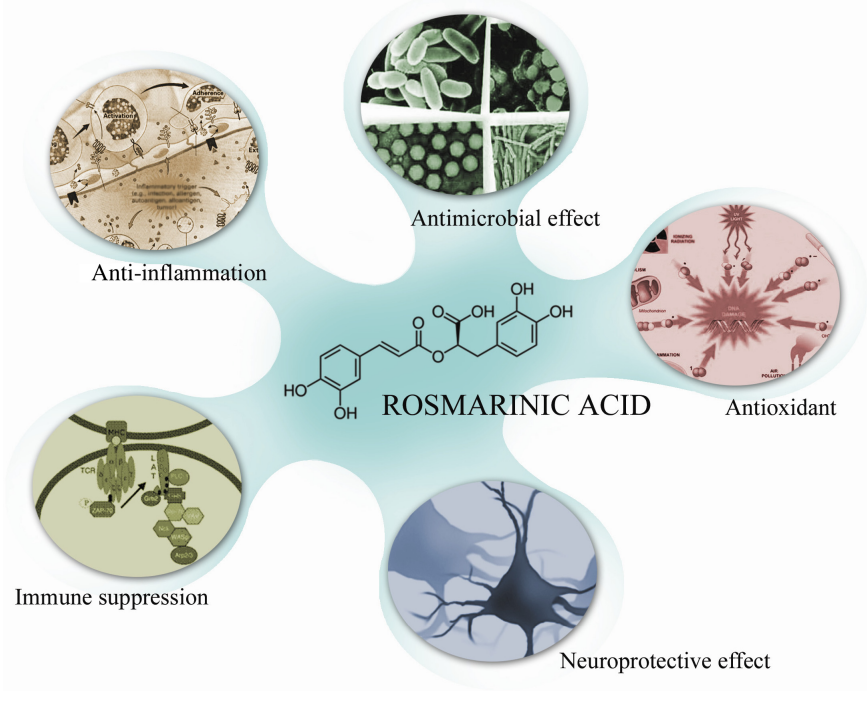

Fig. 1. Function of RosA.

Fig. 2. Lamiaceae family.

of rosemary extract[1] and the antiviral activity of lemon balm, which is used to treat herpes simplex virus.[6] In addition, it has been reported that perilla and its constituent, RosA, have anti-inflammatory and antiallergic activity.[3,7] These plants have been used as traditional medicines in Asian countries to reduce asthma symptoms and to prevent seasonal allergies.

RosA has a broad application spectrum that ranges from use as a food preservative to medicinal use as an antimicrobial and anti-inflammatory substance. The anti-inflammatory properties of RosA are believed to be based on inhibition of lipoxygenases and cyclooxygenases, as well as interference with the histamine release from mast-cells with a complement cascade.[8,9] Inhibition of T-cell antigen receptor-mediated signaling through the inhibition of IL-2 promoter activation has been identified as one of the mechanisms by which the anti-inflammatory activity of rosmarinic acid occurs.[10] Furthermore, rosmarinic acid inhibits the expression of CCL11 and CCR3 by suppressing the IKK-β activity involved in nuclear factor-kappa B (NF-κB) activation signaling in human dermal fibroblasts.[11]

RosA has also been shown to confer anticarcinogenic effects in a murine, two-stage skin carcinogenesis model by inhibiting the inflammatory response and scavenging reactive oxygen radicals.[12] In addition, RosA is known to have protective effects against Alzheimer's amyloid-β peptide (Aβ)-induced neurotoxicity in PC12 cells.[13] Moreover, RosA is known to exert an inhibitory effect against lung injury induced by diesel exhaust particles, which occurs via a reduction in expression of the pro-inflammatory molecule.[14] Finally, RosA is known to exert a suppressive effect against synovitis in a murine collagen induced arthritis model.[15]

CHEMICAL CONSTITUENTS

Single Compounds

Rosmarinic acid was first isolated as a pure compound by Scarpati and Oriente,[16] who named the compound after the plant it was isolated from, *Rosmarinus officinalis*. Before elucidation of its chemical structure, rosmarinic acid and similar compounds were collectively known as ''Labiatengerbstoffe,'' which is a type of tannin that is produced by many species belonging to the family Lamiaceae. RosA is comprised of an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid (α-O-caffeoyl-3,4-dihydroxy-phenyl lactic acid). RosA is biosynthesized from the amino acids L-phenylalanine and L-tyrosine, which are transformed into rosmarinic acid via eight enzymes that include phenylalanine ammonia-lyase and cinnamic acid 4-hydroxylase. RosA is produced in suspension cultures of *Anchusa officinalis* (Boraginaceae) and *Coleus blumei* (Lamiaceae), which results in accumulation of higher amounts of rosmarinic acid than are found in the plant itself.

Derivatives of Rosmarinic Acid

A number of derivatives of rosmarinic acid that contain one or two types of rosmarinic acid combined with other aromatic moieties have been identified in higher plants. The best known rosmarinic acid derivatives may be lithospermic acid, which is a conjugate of rosmarinic acid and caffeic acid, and lithospermic acid B, which is a dimer of rosmarinic acid (Fig. 3).[17] Lithospermic acid is known to have inhibitory activities against adenylate cyclase and to exert an antioxidant effect on low density lipoprotein.[18]

Salvianolic acid derived from rosmarinic acid is isolated from the root of red-rooted salvia (*Salvia mitiorrhiza* Bunge), which is a Chinese herb that is widely used for the prevention and treatment of atherosclerosis-related disorders.[19] Salvianolic acid is a water-soluble polyphenolic antioxidant that has anti-lipid peroxidation activity and protective effects against cerebral and heart ischemia-reperfusion.[20,21] In addition, salvianolic acid has been reported to have inhibitory activity against liver fibrosis and hepatoprotection.[22]

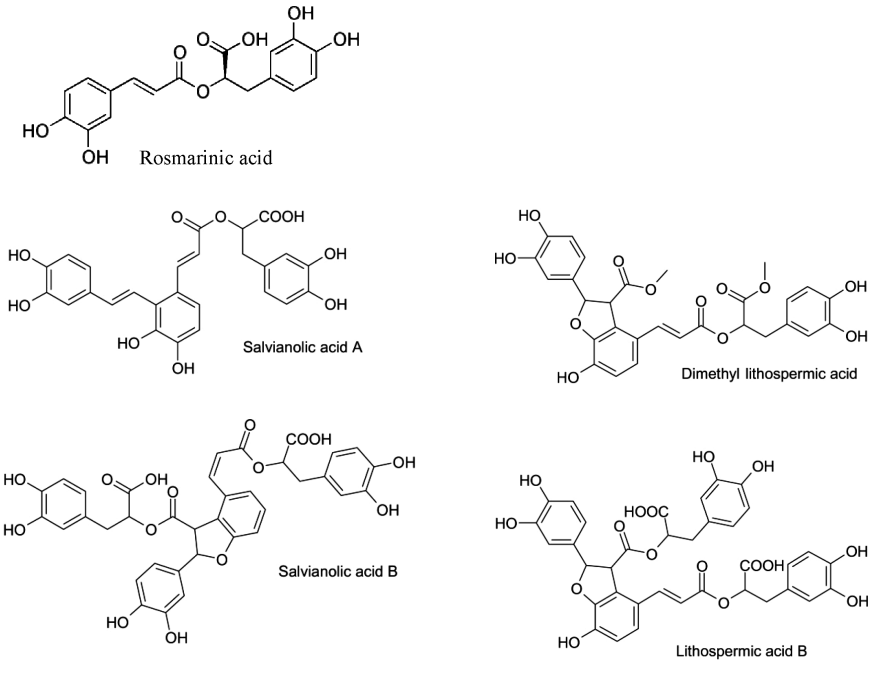

Fig. 3. Rosmarinic acid and its derivatives.

MOLECULAR TARGETS

IKK-β

NF-κB transcription factors play an important role in the balance between cell survival and apoptosis and are involved in the regulation of cell proliferation and the development or differentiation of various types of cells.[23,24] NF-κB transcription factors are rapidly activated in response to various stimuli, which allows quick activation of target genes that encode cytokines, membrane proteins, transcription factors and inhibitors of apoptosis. This rapid response system depends on the sequestration of NF-κB dimers in the cytoplasm, which occurs through interaction with inhibitory I-κB proteins. Degradation of I-κBs liberates NF-κB dimers, which then translocate to the nucleus where they activate the transcription of target genes. Cell stimulation leads to I-κB phosphorylation, thereby creating a recognition signal for ubiquitinating enzymes, which mark the I-κBs for

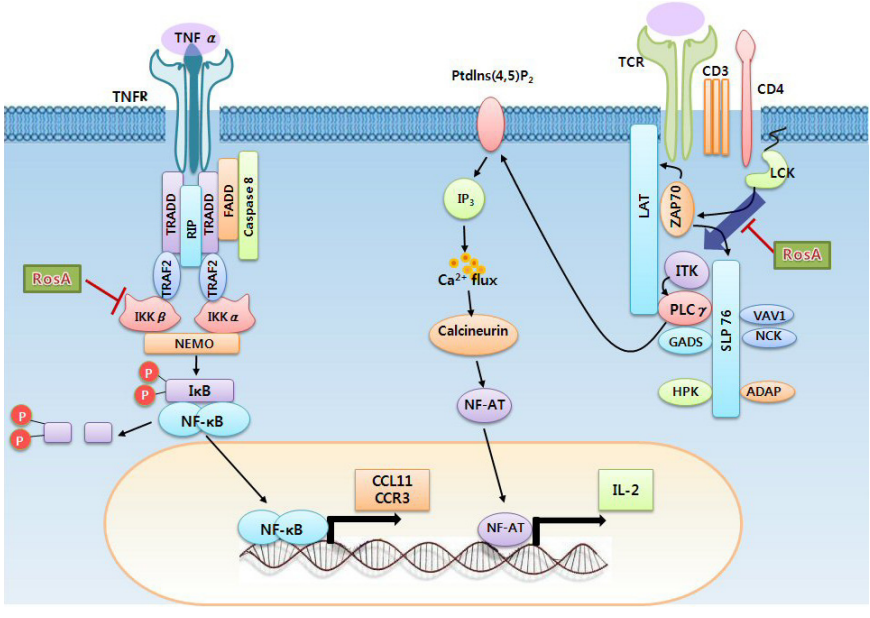

Fig. 4. Molecular targets of RosA.

rapid proteasomal degradation.[25] Phosphorylation is accomplished by protein kinases, which are tightly controlled and represents the primary mode of NF-κB regulation. The most important I-κB kinases are I-κB kinase alpha (IKK-α) and IKK-β.[26] Although these kinases have high similarities and share a common protein complex, IKK-α and IKK-β have largely nonoverlapping functions due to different substrate specificities.

IKK-β is the major IKK catalytic subunit through which NF-κB activation by pro-inflammatory stimuli including TNF-α, IL-1 and Toll-like receptor (TLR) agonists such as lipopolysaccharide (LPS) occurs (Fig. 4). Conversely, IKK-α activity is required for activation of a specific type of NF-κB dimer (p52/RelB) in response to a subset of TNF family members including BAFF (B cell-activating factor), CD40-ligand, and lymphotoxin-α (LT-α)/LT-β heterotrimers. Although the IKK-β-dependent pathway is essential for the activation of innate immunity, the IKK-α-dependent pathway is more important for regulation of adaptive immunity and lymphoid organogenesis.[27]

Although many different signaling molecules involved in the activation or regulation of IKK-β have been identified and evaluated by different cell culture experiments *in vitro*, it is clear that physiological studies conducted *in vivo* using knockout or transgene mouse models are required to fully elucidate the complex signaling network involved. Classical knockout mice with an IKK-β deletion die during the embryonic stage (around E13) due to TNF-α-mediated apoptosis of the hepatocytes.[28] This phenotype is very similar to that of RelA/p65 knockout mice,[29] which further indicates the importance of IKK-β for p65/RelA activation downstream of TNF-α signaling. In addition, conditional knockout models of IKK-β have demonstrated the important role that this kinase plays in protecting macrophages,[30] osteoclasts[31] or gut epithelium[32] from apoptosis triggered by Toll-like receptor (TLR) signals. Cell-type specific differences in the role that IKK-β plays in inflammation have also been observed. While this kinase is essential for inflammatory signaling pathways in many cell types, it has also been reported to play an anti-inflammatory role in keratinocytes.[33] Additionally, conditional IKK-β knockout models have demonstrated that IKK-β is involved in the development of obesity-induced insulin resistance. Specifically, these studies demonstrated that IKK-β-dependent production of inflammatory molecules by myeloid cells is required for the onset of type II diabetes.[34] Although a variety of signaling molecules involved in NF-κB activation via IKK-β have been identified, deciphering the exact pathways through which this activation occurs is an ongoing process.

Taken together, the results of the studies conducted to date indicate that inhibition of IKK-β activity likely contributes to anti-inflammatory and antioncogenic activities, but that the relative roles of the different pathways are difficult to determine. As a result, specific IKK-β inhibitors have been sought and identified by a variety of pharmaceutical companies,[35–37] and several compounds have been found to have very low effective inhibitory concentrations.[36,38,39] RosA has recently generated interest because it has been found to inhibit IKK-β in the TNF-α-induced upregulation of CCL11 and CCR3.[11]

Chemokines are small chemotactic peptides that play a central role in autoimmune, inflammatory, viral, and allergic diseases, as well as in the organization of innate and adaptive immune responses due to their ability

to attract and activate leukocytes.[40,41] Chemokine receptor signaling involves different pathways that sustain cell survival, induce gene expression, and importantly, enable directional cell migration. A subset of chemokines including CCL11, CCL24, CCL26, CCL7, CCL13, CCL17 and CCL22 are highly expressed by the three primary cell types involved in allergic inflammation: eosinophils, basophils and Th2 lymphocytes.

Increased numbers of circulating eosinophils are frequently observed in patients with atopic dermatitis. In addition, although intact eosinophils are rarely observed in lesional atopic skin, eosinophil products such as major basic protein and eosinophil cationic protein are known to be deposited within the skin and present in increased concentrations in the peripheral blood of patients with atopic dermatitis.[42] Enhanced expression of CCL11, which is also known as eotaxin, has been described in cases of lesional atopic dermatitis when compared to nonlesional controls.[43] CCL11 binds to CCR3, which is preferentially expressed on eosinophils.[44] Therefore, the enhanced local production of eotaxin may lead to the recruitment of eosinophils and contribute to the initiation and maintenance of allergic skin inflammation. Analysis of serial sections has identified lymphocytes, mononuclear cells, fibroblasts, and eosinophils as the source of CCL11 in lesional atopic skin.[43] The role of CCR3 in the recruitment of leukocytes was recently investigated in a murine model of allergic skin inflammation using CCR3-deficient mice. The results of this investigation demonstrated that CCR-3 is essential for eosinophil recruitment into the skin at sites of allergic inflammation.[45]

The specificity of the chemokine system provides a unique opportunity to interfere with leukocyte recruitment and function during atopic dermatitis. Because CCR3 is preferentially expressed in cells that play pivotal roles in atopic dermatitis, these cells represent promising therapeutic targets for the attenuation of allergic skin inflammation. Blocking CCR3 inhibits eosinophil activation *in vitro* and *in vivo*, which suggests that CCR3 represents an excellent target for the treatment of allergic diseases. Therefore, many academic and industrial studies have been conducted to identify compounds capable of blocking this receptor. As a result, many antagonists for CCR3 have recently been described.[46] For example, Lee *et al*. reported that rosmarinic acid inhibits the expression of CCL11 and CCR3 in human dermal fibroblasts by suppressing IKK-β

activity during NF-κB activation signaling.[11] Although the exact mechanisms regulating the accumulation of inflammatory cells in atopic skin are not yet fully understood, recent advances in chemokine biology suggest that chemokine networks play an important role in this process.

Itk and PLC-γ

Innate and adaptive immune responses against antigens or pathogens activate multiple signaling pathways that involve surface receptors, kinases, adapters, and scaffolding proteins. These pathways help orchestrate the complex cellular interactions required for proper immune responses. In addition, T-cells participate in various protective immune responses by actively exerting inflammatory and cellular responses and by indirectly regulating the activation and differentiation of other immune cells. T-cell response is initiated by the interaction between the antigen presented in antigen-presenting cells (APC) and the T-cell antigen receptor (TCR) complex.

Stimulation of the TCR leads to rapid activation of tyrosine kinases, which, in turn, phosphorylate a variety of signal-transducing proteins. TCR signaling requires activation of ζ-associated protein-70 (ZAP-70) and Src family tyrosine kinases. Lymphocyte-specific cytoplasmic protein tyrosine kinase (Lck), which has been implicated in the signal transduction of T-cells, is a member of the Src family of protein tyrosine kinases (PTKs: Blk, Fyn, Lyn, Hck and Src). The phosphorylation of immunoreceptor tyrosine-based activation motifs by Lck leads to the recruitment and activation of ZAP-70, which belongs to the Src family PTK.[47–49] TCR engagement triggers phospholipase C-γ1 (PLC-γ1) activation through the Lck-ZAP70-linker of the activated T-cell adaptor protein pathway. PLC-γ1 activity is known to be controlled by both protein tyrosine kinases (PTK) and non-PTK factors.[50] Ca^{2+} is a universal cellular messenger that is precisely controlled in all cell types. Therefore, dynamic changes in Ca^{2+} release or entry signal a wide range of short- and long-term cellular functions, from rapid changes in contraction, secretion and actin reorganization to alteration of gene transcription, cell-cycle progression and apoptosis.[51,52] Evidence for multiple PLC-γ functions is mounting. For example, PLC-γ is known to generate the second-messenger molecules,

Ins(1,4,5)P_3 and DAG, which are both involved in receptor-mediated Ca^{2+} signaling.[53]

TCR induces tyrosine phosphorylation of the PLC-γ1 and ζ-chain, which inhibits Ca^{2+} mobilization, interleukin-2 (IL-2) secretion, and IL-2 receptor expression.[55] This results in Lck transmitting signals from the TCR to the nucleus by phosphorylating the ζ-chain of the TCR complex and membrane proximal signal transducers such as ZAP-70 and induced interleukin 2 (IL-2) inducible T-cell kinase (Itk).[55,56]

Recently, the inhibition of TCR-mediated signaling was identified as one of the working mechanisms of many anti-inflammatory drugs.[57–59] In addition, genetic evidence has demonstrated that the Tec family tyrosine kinase, Itk, is involved in signaling through the TCR. Furthermore, mature T-cells from Itk-deficient mice were found to have reduced proliferative responses to allogeneic MHC stimulation and to anti-TCR cross-linking. Additionally, Itk is involved in T-cell development and plays an important role in proximal events in TCR-mediated signaling pathways.[60] Tec kinases are critical regulators of TCR signaling that are required for PLC-γ activation. The results of previously conducted studies indicate that T-cells from Itk-deficient mice show markedly reduced tyrosine phosphorylation of PLC-γ1 and Ca^{2+} mobilization in response to TCR stimulus.[61,62] Moreover, combined deletion of two Tec kinases in mice, Rlk and Itk, has been shown to cause marked defects in TCR responses including proliferation, cytokine production, and apoptosis *in vitro*.[61] Furthermore, T-cells from Itk-deficient mice have a defect in TCR-induced proliferation that can be overcome by bypassing the TCR with phorbol ester and calcium ionophore treatment. In addition, Itk-deficient T-cells produce virtually no IL-2 and fail to generate a calcium flux in response to TCR stimulation. Finally, Itk-deficient mice have a defect in T-cell development that is consistent with decreased TCR signaling in the thymus during positive selection.[63]

Lck has been shown to directly phosphorylate and activate Itk.[64] In addition, Kang *et al*.[65] reported that RosA inhibits Ca^{2+}-dependent pathways of TCR-mediated signaling by inhibiting PLC-γ1 and Itk activity. Furthermore, RosA has been shown to inhibit Itk activity in a ZAP-70-independent manner and to inhibit TCR-induced tyrosine phosphorylation and subsequent activation of Itk, which has been implicated as a direct

kinase of PLC-γ1. Taken together, these findings show that RosA inhibits TCR signaling by blocking upstream TCR-signaling events, specifically the tyrosine phosphorylation of Itk and PLC-γ1. The findings also demonstrate that RosA inhibits the Ca^{2+}-dependent pathways involved in TCR signaling, which shows that these effects occur in a ZAP-70-independent manner. However, the exact mechanism by which RosA downregulates Itk activity has not been elucidated. In the Kang *et al*. study, RosA was not found to inhibit Lck kinase activity, which is primarily responsible for Itk phosphorylation. In addition, it is not known if RosA inhibits the recruitment of Lck to Itk or another unknown PTK responsible for Itk phosphorylation.[65]

IN VITRO STUDIES

Rosmarinic Acid Inhibits CCL11 Expression Via the Suppression of IKK

The chemokines are a large family of small proteins involved in the activation and recruitment of specific cell populations during the course of various diseases.[66] CCL11, a CC chemokine, is a potent chemoattractant and an activator of eosinophils,[67] basophils,[68,69] and Th2 lymphocytes.[70,71] CCL11 expression has been found to be restricted to a few cell types, including eosinophils, bronchial epithelial cells, and dermal fibroblast cells.[72,73] In asthmatics, the expression of CCL11 has been found to be enhanced in these types of cells, and increased expression is associated with disease severity.[44,74] Additionally, CCL11 expression was found to be increased in epithelial cells in cases of atopic dermatitis[43] as well as in other inflammatory conditions.[75] The expression of CCL11 is induced by two potent activators: interleukin-4, which is produced by Th2 cells, mast cells, and basophils; and TNF-α, which is produced by monocytes and macrophages.[76] Although most eosinophil chemoattractants of the CC-chemokine family generally act on several receptors, CCL11 only signals through the G protein-coupled receptor CCR3.[77] CCR3 is prominently expressed on eosinophils, basophils, Th2-type lymphocytes, and fibroblasts.[44,73] However, very little is known about the mechanism by which CCR3 is regulated at the transcriptional

level, although it was recently demonstrated that CCR3 gene expression is induced by TNF-α.[78]

Recently, the inhibition of TCR-mediated signaling has been identified as one of the many effects of anti-inflammatory drugs. Even though their action points are quite different, nonsteroidal anti-inflammatory drugs (NSAIDs) such as tacrolimus, pimecrolimus,[58] and hydroxychloroquine[57] have been found to inhibit TCR-induced signaling events, including Ca^{2+} mobilization, the activation of p38 mitogen-activated protein kinase (MAPK), and expression of the CD40 ligand. In addition, RosA was recently found to inhibit the Ca^{2+}-dependent pathways of TCR-mediated signaling by inhibiting PLC-γ1 and Itk activities.[65] Furthermore, many studies conducted to evaluate the anti-inflammatory and immunomodulatory effects of RosA have indicated that RosA may exert its inhibitory effect on the expression of CCL11 and CCR3. Therefore, in this study, human dermal fibroblast cells were used to evaluate the effects and mechanisms by which RosA exerts its effects on the TNF-α-induced expression of CCL11 and CCR3.

To examine the role that RosA plays in the downregulation of CCL11 and CCR3 expression, an EIA assay for CCL11 and a Western blot assay for CCR3 were performed. The TNF-α-induced protein expression of the CCL11 and CCR3 genes was attenuated by RosA in a concentration-dependent manner. In addition, the IC_{50} of RosA against TNF-α-induced CCL11 protein expression was found to be 9.05 ± 1.47 μM. Furthermore, although RosA completely inhibited expression of the CCR3 gene at a concentration of 40 μM, CCL11 protein expression was not blocked by the same RosA concentration. Taken together, these results suggest that expression of the CCR3 gene is more dependent on the NF-κB promoter than the CCL11 gene.

To determine if the inhibitory effect of RosA on the TNF-α-induced expression of the CCL11 and CCR3 genes is mediated by suppression of NF-κB activation, the NF-κB luciferase reporter was employed in both NIH3T3 mouse fibroblast cells and human dermal fibroblast cells. TNF-α-induced NF-κB activation was inhibited in a concentration-dependent manner in both NIH3T3 cells and human dermal fibroblast cells, which suggests that RosA inhibits expression of the CCR3 and CCL11 genes via the NF-κB pathway. In addition, the IC_{50} of RosA against TNF-α-induced

NF-κB activation in NIH3T3 cells and human dermal fibroblast cells was found to be 10.89 ± 1.56 μM and 9.47 ± 1.51 μM, respectively.

To examine the effect of RosA on TNF-α-induced nuclear translocation of the NF-κB heterodimer, a Western blot assay was conducted using nuclear and cytosolic extracts. The results of this analysis revealed that nuclear translocation of NF-κB p65 from the cytosol occurred upon treatment with TNF-α. However, in cases in which cells were incubated in the presence of TNF-α along with RosA, nuclear translocation of NF-κB p65 was found to be reduced. These findings indicate that RosA inhibited translocation of NF-κB p65 from the cytosol.

Two pathways for the induction of NF-κB activity were recently discovered.[79] One is a canonical pathway in which TRAF2, MEKK and IKK-β are involved while the other is a non-canonical pathway in which NIK (NF-κB-inducing kinase) and IKK-α have been implicated. It is known that TNF-α-induced NF-κB activation is mediated by the canonical pathway. Therefore, we conducted a NF-κB luciferase reporter assay using TRAF 2, MEKK 3 and IKK-β in the absence of TNF-α to characterize the mechanism by which RosA regulates TNF-α-induced NF-κB activation. When each molecule was overexpressed by transient transfection into human dermal fibroblast cells, NF-κB luciferase reporter activity was significantly reduced by RosA. This inhibitory effect of RosA was confirmed by the results of a Western blot assay, which revealed that RosA inhibited TRAF 2-, MEKK 3- and IKK-β-induced I-κBα phosphorylation. Taken together, these findings suggest that RosA operates downstream of IKK-β in TNF-α-induced NF-κB activation.

To elucidate the action step of RosA, an *in vitro* IKK-β kinase assay was performed using immunoprecipitated IKK-β that was isolated from human dermal fibroblast cells incubated in the presence or absence of TNF-α (40 ng ml^{-1}) and RosA for 30 mins and with GST-I-κBα as a substrate. The results of this analysis revealed that IKK-β activity is reduced by RosA, which indicates that RosA directly inhibits IKK-β kinase activity.

It is well known that ICAM-1 (intracellular adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1), which are both encoded by NF-κB target genes, are involved in the transendothelial migration of leukocytes.[80] For this reason, the effects of RosA on the expression of

ICAM-1 and VCAM-1 were evaluated. The expression of both ICAM-1 and VCAM-1 was reduced by RosA. Specifically, the IC_{50} of RosA on the TNF-α-induced expression of sVCAM-1 and sICAM-1 was 14.56 ± 2.39 μM and 15.78 ± 1.56 μM, respectively. These results indicate that the anti-inflammatory properties of RosA occur via inhibition of the NF-κB pathway.

Collectively, the results of this study indicate that RosA inhibits expression of the CCL11 and CCR3 genes by suppressing NF-κB promoter activity, and that it operates downstream of IKK-β in the TNF-α-induced upregulation of CCL11 and CCR3.

Inhibition of Itk and PLC-γ1 Activity by Rosmarinic Acid

RosA has been shown to inhibit TCR-induced IL-2 expression and subsequent T-cell proliferation *in vitro*. In this study, we investigated the inhibitory mechanism of RosA on TCR signaling in detail and found that RosA inhibits TCR signaling leading to Ca^{2+} mobilization and NF-AT activation by blocking membrane-proximal events, specifically, the tyrosine phosphorylation of Itk and PLC-γ1.[65]

IL-2 is required for the development of T-cell immunologic memory, which is one of the unique characteristics of the immune system that enables expansion of the number and function of antigen-selected T-cell clones. The IL-2 promoter contains several regulatory elements that can bind different transcription factors, such as NF-AT, AP-1, Oct-1 and NF-κB.[81] In this study, RosA strongly repressed TCR-induced NF-AT promoter activity, but not AP-1. These results indicate that RosA inhibits TCR-induced IL-2 promoter activation by suppressing NF-AT activation, and that RosA acts in the membrane-proximal point(s) of TCR signaling upstream of the PMA/ionomycin working sites.

PLC-γ1 is a ubiquitous gatekeeper of calcium mobilization and diacylglycerol-mediated events induced by activation of the immunoreceptors and receptor tyrosine kinases (RTKs) such as Lck, ZAP-70 and Itk. PLC-γ is a cytoplasmic enzyme that requires membrane translocation and tyrosine phosphorylation for activation. The activated enzyme hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) to yield inositol 1,4,5-triphosphate (IP_3). The binding of IP_3 to the IP_3 receptor on the

endoplasmic reticulum leads to opening of the Ca^{2+} channel and a subsequent increase in cytosolic Ca^{2+} levels.[82] This increase then activates calcineurin, which facilitates the translocation of NF-AT into the nucleus by dephosphorylating NF-AT. In this study, incubation of Jurkat T-cells with RosA reduced the TCR-induced elevation of intracellular Ca^{2+} and inhibited TCR-induced IP_3 production. RosA also inhibited the TCR-induced tyrosine phosphorylation of PLC-γ1 in Jurkat T-cells. Taken together, these findings indicate that RosA suppressed the TCR-induced tyrosine phosphorylation of PLC-γ1 and its associated downstream signaling events, including IP_3 production and Ca^{2+} mobilization.

Lck is a protein tyrosine kinase (PTK) that is primarily found in T-cells and NK cells. Lck is a key molecule involved in TCR-mediated signaling. In this study, cross-linking of purified wild-type naive CD4 cells and anti-CD3 activated Lck and initiated the signaling cascade downstream of Lck. This, in turn, led to the phosphorylation of ZAP-70, LAT and PLC-γ1, as well as Ca^{2+} mobilization and the dephosphorylation and nuclear translocation of the NF-AT. However, treatment with RosA did not suppress Lck-mediated phosphorylation of enolase, which is an exogenous substrate of Lck. This indicates that RosA does not inhibit Lck kinase activity. Based on these results, additional studies were conducted to determine the phosphorylation status of several *in vivo* Lck substrates. It is well known that Lck phosphorylates the TCR ζ-chain and ZAP-70 in response to TCR stimulus.[47–49] The results of this study demonstrated that RosA does not inhibit TCR-induced tyrosine phosphorylation of the TCR ζ-chain or of ζ-chain-associated ZAP-70, which suggests that RosA does not inhibit Lck kinase activity.

ZAP-70 plays an important role in the coupling of T-cell receptors to the PLC-γ1 Ca^{2+} and Ras signaling pathways, as well as to the activation of transcription factors including NF-AT. Activation of ZAP-70 results in the phosphorylation and activation of several downstream targets, including SLP-76 and the transmembrane adapter protein LAT. Phosphorylated LAT recruits several important proteins to the membrane, including SLP-76, Grb2, PLC-γ1, Grap, Sos and c-Cbl.[83,84] SLP-76 is recruited to membrane-bound LAT through its constitutive interaction with GADS (GRB2-related adaptor protein). Together, SLP-76 and LAT nucleate a multimolecular signaling complex, which induces a host of downstream

responses, including calcium mobilization and MAPK activation. In this study, RosA did not exert a noticeable effect on the kinetics of SLP-76 phosphorylation or the association of SLP-76 with LAT. Similarly, RosA had no effect on the TCR-induced association of LAT with SLP-76 and Grb2. Moreover, RosA did not suppress the TCR-induced tyrosine/threonine phosphorylation of Erk. The failure of RosA to inhibit the TCR-induced phosphorylation of Erk1/2 supports the previous finding that RosA does not inhibit AP-1 activation. Taken together, these results confirm the finding that RosA suppresses TCR signaling in a ZAP-70-independent manner.

Itk is a member of the Btk/Tec/Itk family of nonreceptor PTKs that has been implicated in TCR signal transduction. Mice deficient in Itk expression have a reduced number of mature T-cells, which also show reduced proliferation following TCR stimulation.[63] Itk is involved in the generation of critical second messengers (Ca^{2+}, PKC) via tyrosine phosphorylation of PLC-γ1. In this study, RosA was found to inhibit TCR-induced tyrosine phosphorylation of Itk and subsequent Itk activation. Taken together, these results indicate that RosA inhibits PLC-γ1 activity by downregulating Itk activity.

ANIMAL STUDIES

Recent studies have shown that RosA exhibits potent antioxidant activity that primarily occurs via its constituent phenolic diterpenes.[85,86] RosA has diverse immunoregulatory functions that include anticarcinogenic properties such as reduced skin tumorigenicity,[1] anti-inflammatory activity,[87] antimicrobial activity,[88] and other positive health benefits.[85,89] In studies evaluating direct dermatologic applications, RosA was shown to suppress tumorigenesis in a two-stage skin cancer model in mice,[90] as well as to exhibit photoprotective potential.[91] Additionally, the results of a study of human surface lipids in which skin was treated with rosemary extract revealed that it protected against free radical damage caused by t-butyl hydroperoxide, which supports the results of previous studies that found that RosA had antioxidant properties.[87]

RosA is also known to possess several anti-inflammatory properties[92,93] that are primarily attributed to its inhibition of the activity of

cyclooxygenase (COX) and lipoxygenase (LOX) and the activation of their complements.[8,9] In a virus animal study, RosA was found to reduce the mortality of mice infected with Japanese encephalitis virus (JEV). Clinically, infection with JEV results in increased serum and cerebrospinal fluid levels of inflammatory mediators that are correlated with the mortality rate of JE, such as TNF-α, IL-6, IL-8 and RANTES. In the present study, significant decreases in viral loads and proinflammatory cytokine levels were observed in JEV-infected animals that were treated with RosA when compared with untreated infected mice.[93] In an animal study of rheumatoid arthritis (RA), repeated administration of RosA dramatically reduced the arthritic index and number of affected paws. RA is a chronic inflammatory autoimmune disease that is characterized by the development of pathogenic T-cells and subsequent inflammatory responses in multiple joints. A murine model of collagen induced arthritis (CIA) resembles human RA in that both cellular and humoral responses are involved in the pathogenic process. In this study, RosA suppressed synovitis in a murine CIA. In addition, histopathologic observations closely paralleled clinical data, which demonstrates that RosA treated mice retained nearly normal architecture of synovial tissues, whereas control mice exhibited severe synovitis.[15]

In an allergy animal study, RosA treatment was found to inhibit increases in the numbers of eosinophils in the bronchoalveolar lavage fluids as well as in those around the airways of sensitized mice that were challenged with mite allergen. RosA has also been shown to inhibit enhanced protein expression of IL-4 and IL-5, eotaxin and allergen-specific IgG1 in the lungs of sensitized mice. Taken together, these results indicate that oral administration of perilla-derived RosA is effective in the treatment of allergic asthma, and that its effects may occur through inhibition of increases in Th2 cytokines, chemokines, and allergen-specific Ig production that occurs in response to exposure to dust mites.[94] In this study, the antiallergic effect of *Perilla frutescens* was evaluated and its active constituents were identified using the mice ear-passive cutaneous anaphylaxis (PCA) reaction, which is a common animal model of type I allergy. It was found that perilla decoction significantly suppressed the PCA reaction. In addition, when constituents of perilla decoction were orally administered to mice, RosA significantly suppressed the PCA reaction.

The decrease induced by RosA was almost equal to the decrease induced by perilla decoction, which indicates that the antiallergic effect of perilla decoction depends primarily on RosA.[95]

The antioxidative property of RosA has also been demonstrated through its ability to reduce liver injury induced by D-galactosamine[10] and lipopolysaccharides[96] through the scavenging of superoxide molecules[97] and the inhibition of COX-2. Currently, rosemary is a relatively common ingredient in cosmetics[1] as well as in cosmeceutical products. In addition, it is also considered to be an effective conditioner for greasy hair, a general tonic imparting body and sheen to hair, and an effective antidandruff ingredient when combined with sage.[86]

CLINICAL TRIALS

Atopic Dermatitis

Atopic dermatitis (AD) is a chronic inflammatory skin disease that primarily occurs during early infancy and childhood and is characterized by a chronically relapsing course. Although the pathogenesis of AD is not fully understood, it is reportedly associated with multiple immunologic abnormalities.[98,99] Symptoms of AD induced by various signal transduction pathways include inflamed skin damage and skin dryness. Accordingly, it is treated by moisturizers and topical steroids that maintain the moisture level in the skin and suppress inflammatory reactions, respectively. However, steroid hormones have been known to induce adverse side effects when used topically for prolonged periods.[100] In addition, cyclosporin A and FK506, which are used as nonsteroidal therapeutic agents, have been reported to induce cutaneous T-cell lymphoma,[101] fever,[102] extreme increases in serum alkaline phosphatase in children,[103] enhanced irritation,[104] and relapsing Kaposi's varicelliform eruption.[105] Thus, many studies are currently being conducted in an attempt to develop therapeutic agents that have high anti-inflammatory effects with few side effects.

RosA is a naturally occurring hydroxylated compound that has a broad range of applications from food preservatives to cosmetics. It is also used as an antimicrobial and an antioxidant.[1,106,107] Furthermore, RosA has

the ability to block complement fixation, inhibit lipoxygenase and cyclooxygenase activity[8,108,109] and suppress IKK-β downstream signaling during the TNF-α-induced upregulation of CCL11, which is a potent chemoattractant and an activator of eosinophils, basophils, and Th2 lymphocytes.[11] Based on these reports, we evaluated the effect of RosA on atopic dermatitis.

To accomplish this, the severity of atopic dermatitis prior to and after treatment with RosA was scored in 21 patients. The mean SCORAD index decreased from 7.37 ± 0.32 before treatment to 3.27 ± 0.21 after treatment with cream that contained RosA for 8 wks ($P < 0.05$). However, no significant change in the SCORAD score was observed in the control group (cream only) (before treatment: 6.49 ± 0.81; after treatment for 8 wks: 5.63 ± 0.97). These findings indicate that RosA improved the symptoms of AD. Specifically, following treatment with RosA for 4 wks, erythema and oozing/crust were significantly reduced ($P < 0.05$), and edema on the antecubital fossa was significantly reduced after 8 wks ($P < 0.05$). However, RosA did not have a significant effect on excoriation. In addition, although lichenification and local pruritus were significantly reduced after 8 wks of treatment with a cream containing 0.3% RosA, treatment with the cream that was used as a negative control alone also significantly reduced these symptoms. In a transepidermal water loss (TEWL) study, the TEWL of the antecubital fossa was significantly reduced after 8 wks of treatment ($P < 0.05$). Furthermore, the results of self-questionnaires evaluating the efficacy of RosA indicated that it improved dryness, pruritus, and general AD symptoms. Finally, there were no reactions observed in any patients after 4 and 8 wks of treatment when safety tests were conducted, which indicates that the RosA cream was safe for use in AD patients.

A recent study found that the transcriptional and translational expression levels of CCL11 and CCR3 were significantly higher in lesional skin from atopic dermatitis patients, but not in skin obtained from nonatopic controls. These findings indicate that CCL11 is involved in the pathogenesis of AD.[43] In addition, the results of several studies evaluating conditional IKK-β loss-of-function mutations indicate that IKK-β activity is required for inactivation of a severe inflammatory reaction that leads to multi-organ failure in response to ischemia-reperfusion.[32] IKK-β activity

is also required to prevent a considerable number of different cell types from undergoing apoptosis.[110] Finally, it has been suggested that RosA inhibits TNF-α-induced production of CCL11 and CCR3, and that this action is mediated via inhibition of IKK-β.[11] Taken together, these results indicate that RosA may ameliorate the symptoms of AD through suppression of IKK.

Seasonal Allergic Rhinoconjunctivitis

RosA is found in many medicinal species of plants belonging to the family Lamiaceae, including *Perilla frutescens*. RosA appears to be a strong anti-inflammatory agent and has been shown to have antioxidant activities in several animal models.[14,111] In addition, it was recently reported that orally-administered RosA was highly absorbed and then distributed in blood in several conjugated forms in rats[112] and humans.[113] However, few placebo-controlled clinical trials have examined the efficacy and safety of polyphenolic phytochemicals for the treatment of allergic inflammatory diseases in humans. Therefore, this study was conducted to determine if oral supplementation with RosA derived from *Perilla flutescens* is an effective method of treating patients with seasonal allergic SAR.

In this 21-day, randomized, double-blind, age-matched, placebo-controlled parallel group study, patients with SAR were treated daily with RosA [200 mg ($n = 10$) or 50 mg ($n = 9$)] or placebo ($n = 10$). Patients then recorded their symptoms daily in a diary card. In addition, profiles of infiltrating cells and concentrations of eotaxin, IL-1β, IL-8, and histamine were measured in nasal lavage fluid. Serum IgE concentrations and routine blood tests were also conducted. When compared to placebo supplementation, supplementation with RosA resulted in a significant increase in responder rates for itchy nose, watery eyes, itchy eyes, and total symptoms ($p < 0.05$). RosA also significantly decreased the numbers of neutrophils and eosinophils in nasal lavage fluid ($p < 0.05$). Additionally, patients reported no adverse events and no significant abnormalities were detected during routine blood tests.

The results of this study provide solid evidence that RosA derived from *Perilla flutescens* is an effective intervention for SAR. In addition,

the results of this study indicate that its effects may be mediated, at least in part, through inhibition of PMNL infiltration into the nostrils.

CONCLUSIONS

RosA has a number of interesting biological effects, including antiviral, antibacterial, antioxidant, anti-inflammatory and immunomodulatory activities. As a result of its antiviral activity, RosA-containing extracts of *Melissa officinalis* are used to treat Herpes simplex infections. It is believed that the anti-inflammatory properties of RosA occur via inhibition of lipoxygenase and cyclooxygenases, interference with the complement cascade, and the inhibition of CCL11. Therefore, it has been proposed that RosA has the potential for use as a therapeutic agent for the treatment of atopic dermatitis. The results of the human clinical study described here confirmed that RosA can be useful for the treatment of atopic dermatitis. In addition, RosA was found to inhibit TCR-induced T-cell activation and proliferation, which suggests that RosA may be useful for the treatment of T-cell-mediated immunological disorders.

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Mint and Its Constituents

Ajaikumar B. Kunnumakkara*, Jing-Gung Chung, Cemile Koca and Sanjit Dey

Besides its culinary uses, mint is also used in traditional systems of medicine for the treatment of biliary disorders, dyspepsia, enteritis, flatulence, gastritis, intestinal colic and for spasms of the bile duct, gallbladder and gastrointestinal tract. The major active ingredient of this plant, menthol and carvone, has been found to possess antioxidant, antimicrobial, anti-inflammatory and antitumor activities. This chapter describes the biological activities of mint and its constituents.

INTRODUCTION

Mentha (mint) is a genus with almost 25 species (and many hundreds of varieties) of flowering plants in the family Lamiaceae (Mint Family).[1] The word "mint" descends from the Latin word "menthe," which is rooted in the Greek word "minthe," mentioned in Greek mythology as Minthe, a nymph who was transformed into a mint plant.[2] There are different types of mint including *Mentha aquatica* — water mint, or marsh mint; *Mentha arvensis* — corn mint, wild mint, Japanese peppermint, field mint, pudina; *Mentha asiatica* — asian mint; *Mentha australis* — Australian mint; *Mentha citrata* — bergamot mint; *Mentha crispata* — wrinkled-leaf mint; *Mentha diemenica* — slender mint; *Mentha laxiflora* — forest mint; *Mentha longifolia* or *Mentha sylvestris* — horse mint; *Mentha*

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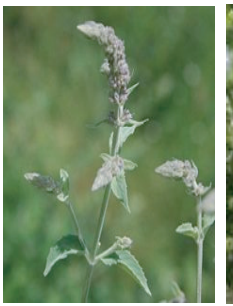
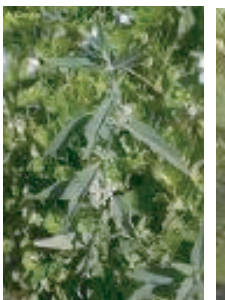
Horse mint
(Mentha longifolia)

Water mint
(Mentha aquatica)

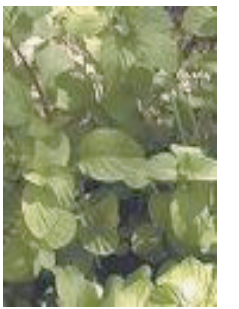
Japaneese mint
(Mentha arvensis)



Asian mint
(Mentha asiatica)



Astralian mint
(Mentha astralis)

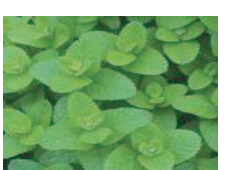


Bergamot mint
(Mentha citrata)

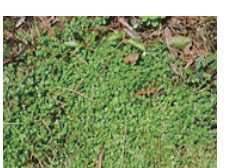
Slender mint
(Mentha diemenica)

Garden mint
(Mentha sachalinensis)

Spear Mint
(Mentha spicata)



Apple mint
(Mentha suaveolens)



Corsican mint
(Mentha requienii)

Fig. 1. Different mint species and their common names.

piperita — peppermint; *Mentha requienii* — corsican mint; *Mentha sachalinensis* — garden mint; *Mentha spicata — M. cordifolia*, spearmint, curly mint; *Mentha suaveolens* — apple mint, pineapple mint; and *Mentha vagans* — gray mint (Fig. 1). These plants are used in traditional systems of medicine against a wide variety of diseases. The leaves have a pleasant, warm, fresh, aromatic, sweet flavor with a cool aftertaste. Mint leaves are used in teas, beverages, jellies, syrups, candies, and ice cream. In Middle Eastern cuisine mint is used in lamb dishes. In British cuisine, mint sauce is popular with lamb. Mint is a necessary ingredient in Touareg tea, a popular tea in northern African and Arab countries. Mint leaves, without a qualifier like peppermint or apple mint, generally refers to spearmint leaves. Research over the past few years has revealed that mint possesses antibacterial, antinoceceptive, anti-androgenic and anti-inflammatory activities.

TRADITIONAL USES OF MINT

Mentha spicata Labiatae, commonly known as spearmint, is used for various kinds of illnesses in the herbal medicine and food industries. The plant is typically used in the treatment of loss of appetite, common cold, bronchitis, sinusitis, fever, nausea and vomiting, and is ingested as a herbal agent.[3] Peppermint plants have been used as a herbal medicine for many conditions, including loss of appetite, common cold, bronchitis, sinusitis, fever, nausea, vomiting and indigestion.[4] *Mentha arvensis*, is known to possess abortifacient property in folklore medicine.[5]

CHEMICAL CONSTITUENTS OF MINT

During the past years, the researchers have identified several biologically active compounds from mint plants. Haudenschild *et al*. identified carvone and menthol from *Mentha spicata*[6] (Fig. 2). These two chemicals are the major constituents of mint. The oil of this plant contains 40.12% carvone.[7] In 2003, Díaz-Marota *et al*.[8] identified 28 compounds from this plant including carvone, limonene, and 1,8-cineole. The researchers also identified several other compounds from this plant such as monoterpenoid

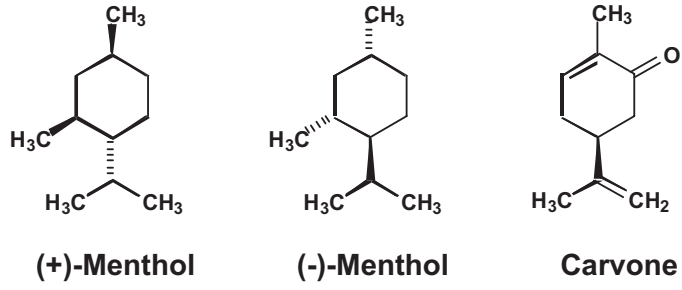


Fig. 2. Structure of carvone and menthol.

glycosides, spicatoside A ((+)-5-[1-(beta-D-glucopyranosyloxymethyl) ethenyl]-2-methyl-2-cyclohexen-1-one) and spicatoside B ((−)-5-[[2-beta-D-glucopyranosyloxy)-1-hydroxy-l-methyl]ethyl]-2-methyl-2-cyclohe xen-1-one)[9]; ursane, 3-methoxy-4-methylbenzaldehyde, veratric acid, 5-hydroxy-3′,4′,6,7-tetramethoxyflavone, diosmetin, thymonin, daucosterol[10]; protocatechuic aldehyde, protocatechuic acid, chrysoeriol, 5,6-dihydroxy-7,8,3′,4′-tetramethoxyflavone, nodifloretin[11]; and lignans named spicatolignan A and spicatolignan B.[12] Oinonen *et al*. identified linarin, a selective acetyl cholinesterase inhibitor from *M. arvensis*.[13] Recent studies identified two new ceramides from the methanolic extract of *M. longifolia*, longifoamide A {6′-tetracosenamide, (6′-Z)-N-[2, 3-dihydroxy-1-(hydroxymethyl)octadecyl]} and B{6′-tetracosenamide, (6′-Z)-N-[2,3,4-trihydroxy-1-(hydroxymethyl)octadecyl]}.[14]

BIOLOGICAL ACTIVITIES OF MINT

The research over the past several years has shown that mint and its constituents possess different biological activities including antimicrobial, antioxidant, anti-inflammatory and anticancer properties.

Mint as an Acetyl Cholinesterase and Butyrylcholinesterase Inhibitor

Acetylcholinesterase, also known as AChE, is an enzyme that degrades (through its hydrolytic activity) the neurotransmitter acetylcholine,

producing choline and an acetate group. It is mainly found at neuromuscular junctions and cholinergic synapses in the central nervous system, where its activity serves to terminate synaptic transmission. Adsersen *et al*. showed that spearmint inhibited AChE by more than 15% at 0.1 mg/ml.[15] The studies also showed that the essential oils and its main constituent (−)-carvone from spearmint inhibited acetylcholinesterase activity by a spectrophotometric method of Ellman at 1 mg/ml concentration.[16] Linarin (acacetin-7-O-β-d-rutinoside), a compound isolated from the flower extract of *Mentha arvensis*, also showed dose-dependent inhibition of acetylcholinesterase.[13]

Butyrylcholinesterase (BCHE), also called serum cholinesterase, is very similar to the neuronal acetylcholinesterase. It is believed to play a role in the body's ability to metabolize the drug cocaine. Mutant alleles at the BCHE locus are responsible for suxamethonium sensitivity. The studies showed that the essential oils and its main constituent (−)-carvone from spearmint inhibited BCHE activity by a spectrophotometric method of Ellman at 1 mg/ml concentration.[16]

Insecticidal Activity of Mint

Mint is also known to exhibit insecticidal activity against a wide variety of insects. Studies have shown that essential oils of spearmint were highly effective against *Lycoriella ingenua* (Diptera: Sciaridae) at 20×10^{-3} mg/ml.[17] *Anopheles stephensi* (Liston) is a well-known vector of the malarial parasite in tropical countries. The developing trend of resistance in mosquitoes toward synthetic mosquitocidal agents makes their management extremely difficult. Tripathi *et al*.[18] evaluated the effects of piperitenone oxide isolated from the essential oil of *Mentha spicata* L. variety viridis against various stages of *A. stephensi*. The results indicated that piperitenone oxide is more potent than the crude essential oil of *M. spicata* variety viridis. Piperitenone oxide and the oil showed mortality of the fourth instar Liston larvae (LD50; 61.64 and 82.95 µg/ml, respectively). *A. stephensi* female adults exposed to the oil at a dose of 60.0 µg/ml inhibited oviposition approximately 42 times less than the control, whereas exposure of piperitenone oxide at the same dose completely inhibited the oviposition. Piperitenone oxide also completely inhibited egg

hatching at the dose of 75.0 μg/ml in ovicidal assay. Developmental toxicity studies showed the significant developmental inhibition potential of the compound and oil. Moreover, piperitenone oxide was found to be highly toxic and repellent toward *A. stephensi* adults as compared with oil.[18]

Antibacterial and Antifungal Activities of Mint

Studies have shown that mint possesses antibacterial activity against a variety of harmful pathogens. The essential oils of peppermint, spearmint and Japanese mint showed the inhibition of the proliferation of *Helicobacter pylori*, *Salmonella enteritidis*, *Escherichia coli* O157:H7, methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococccus aureus* (MSSA) *in vitro* assays.[19] The effects of essential oils and their components on the bactericidal activity of nitrofurantoin against *Enterobacter cloacae* were also studied. Using disk-diffusion and agar-dilution methods, Rafii and Shahverdi showed that the oil, carvone and piperitone equally increased the bactericidal activity of nitrofurantoin.[7] Coutinho *et al*.[20] showed that *M. arvensis* extract inhibited the growth of multiresistant *Escherichia coli*. Studies have also shown that *M. suaveolens* showed antibacterial (both Gram+ and Gram−) and antifungal activities.[21] Peppermint extract and its chemical constituents also exhibited high antibacterial activity against human and plant pathogens. These extracts and compounds inhibited human pathogens such as *Staphylococcus aures*, *S. epidermis*, *Listeria monocytogenes*, *Salmonella enteritidis*, *Enterococcus faecium*, *Shigella sonei*, *Helicobacter pylori*, *Escherichia coli* and *Micrococcus flavus*[22–27] as well as plant pathogens such as *Pseudomonas* and *Xanthomonas* strains.[28]

Antiviral Activity of Mint

The studies showed that *M. longifolia* methanolic as well as ethyl acetate extracts inhibited the growth of HIV-1BaL infection by about 40% and 55%, respectively. But, only ethyl acetate extract shows significant inhibitory activity (50% inhibition) against HIV-1 reverse transcriptase.[29] The different extracts and the essential oils of peppermint showed significant antiviral activities. The aqueous extracts of the leaves of

peppermint showed antiviral activities against influenza A, Newcastle disease virus, herpes simplex virus (HSV) and Vaccinia virus.[30] Aqueous extracts also inhibited human immunodeficiency virus-1 (HIV-1). The combination of alcoholic extracts of peppermint and *Thymus serpyllum*, *Viscum album*, *Salvia officinalis* and *Glycyrrhiza glabra* inhibited influenza virus A, Gabrovo (H1N1), A/Hong Kong (H3N2) and A/PR/8 (H1N1). The essential oils from this plant also suppressed the replicative ability of HSV-1 and HSV-2.[31,32]

Antioxidant Activities of Mint

Kiselova *et al*.[33] studied the antioxidant activities of spearmint by ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) cation radical decolorization assay. They showed that spearmint possess significant antioxidant activities. In another study, Choudhury *et al*.[34] showed that diethyl ether extract of mint possess 100% free radical scavenging activity at approximately 40 µg/L.[34] Arumugam *et al*.[35] studied the different fractions (hexane, chloroform, ethyl acetate and water) of the ethanolic extract of dried leaves of spearmint for total antioxidant activity (TAA) and relative antioxidant activity (RAA) and compared with standard antioxidants such as quercetin, beta-carotene, L-ascorbic acid and glutathione using ABTS*+ decolorization assay (ABTS/potassium persulphate). They showed that total phenolics are found to be highest in the ethyl acetate fraction (54 mg/g) and least in the hexane fraction (13 mg/g) and more or less similar in the water and chloroform fractions (30–32 mg/g). TAA was found to be less in hexane and chloroform fractions (< 53% at 50 µg/ml) and highest in ethyl acetate (95% at 20 µg/ml) and water (84% at 30 µg/ml) fractions. The RAA of the ethyl acetate fraction is 1.1 compared to quercetin (at 5 µM/ml), but greater when compared to beta-carotene (15 µM/ml), L-ascorbic acid (15 µM/ml) and glutathione (15 µM/ml). The essential oils from peppermint exhibited higher antioxidant effects against sunflower oil peroxidation than butylated hydroxytoluene (BHT).[36]

Benzoyl peroxide (BPO) is an effective cutaneous tumor promoter acting through the generation of oxidative stress, induction of ornithine decarboxylase activity and by enhancing DNA synthesis. Saleem *et al*.[37]

showed that BPO treatment increased cutaneous microsomal lipid peroxidation and hydrogen peroxide generation and decreased activity of cutaneous antioxidant enzymes, catalase, glutathione peroxidase, glutathione reductase and glutathione S-transferase, and the level of cutaneous glutathione was depleted. The prophylactic treatment of mice with spearmint extract (10, 15 and 20 mg/kg) 1 hr before BPO treatment resulted in the diminution of BPO-mediated damage. Moreover, depleted levels of glutathione, inhibited activity of glutathione-dependent and antioxidant enzymes were recovered to a significant level in the spearmint treatment group. The studies also showed that treatment with spearmint extract inhibited ornithine decarboxylase activity and the 3H-thymidine uptake in DNA synthesis in murine skin. However, some other studies have shown that the administration of the extract decreased activities of enzymes like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in the hypothalamus of rats.[38]

Antimutagenic Activity of Mint

Hot water extracts of spearmint have shown antimutagenic activity against the direct-acting mutagens 4-nitro-1,2-phenylenediamine (NPD) and 2-hydroxyamino-3-methyl-3H-imidazo[4,5-f]quinoline (N-OH-IQ) using *Salmonella typhimurium* strain TA98. Nontoxic concentrations of spearmint extract inhibited the mutagenic activity of N-OH-IQ in a concentration-dependent fashion but had no effect against NPD. In rats the administration of spearmint water extract in drinking fluid before, during, and after 2-wk treatment with IQ inhibited colonic aberrant crypt foci significantly at 8 wks.[39]

Antinoceceptive Activity of Mint

Nociception (synonyms: nociperception, physiological pain) is the afferent activity produced in the peripheral and central nervous system by stimuli that have the potential to damage tissue.[40,41] This activity is initiated by nociceptors (also called pain receptors), which can detect mechanical, thermal or chemical changes above a set threshold. Once stimulated, a nociceptor transmits a signal along the spinal cord to

the brain. Nociception triggers a variety of autonomic responses and may also result in the experience of pain in sentient beings. Studies have shown that the intraperitoneal administration of (−)-carvone (100 mg/kg and 200 mg/kg) in mice inhibited acetic acid-induced writhing in mice. Moreover, the administration of (−)-carvone inhibited the licking response of the injected paw in mice in the first and second phases of the formalin test. Studies also showed that naloxone (5 mg/kg, s.c.), an opioid antagonist, had no influence on the antinociceptive action of (−)-carvone (100 mg/kg) and suggested nonparticipation of the opioid system in the modulation of pain induced by (−)-carvone. Moreover, the sucrose gap technique showed that (−)-carvone (10 mM) was able to reduce the excitability of the isolated sciatic nerve through a diminution of the compound action potential amplitude by about 50% from control recordings. These results suggested antinociceptive activity of (−)-carvone associated with decreased peripheral nerve excitability.[42]

Suppression of Neutrophil Recruitment in Mice by Mint Essential Oil

Mint also possesses anti-inflammatory activities. To assess their anti-inflammatory potential, the effects of essential oils on neutrophil recruitment in mice were studied. The results showed that the administration of spearmint oil inhibited casein induced leukocyte and neutrophil recruitment in mice.[43] Moreno *et al*.[44] showed that *M. suaveolens* possesses anti-inflammatory activity against rat paw edema induced by carrageenin. Moreover, studies showed that this extract possesses significant diminution in the contractile effects induced by histamine, serotonin and acetylcholine *in vitro*.

Effect of Mint on Iron Absorption in Rats

Studies have shown that tea containing spearmint and peppermint decreased iron absorption in rats. The rats were divided into four groups of 12 animals: group I received no herbal tea (control group); group II received 20 g/L peppermint tea; group III received 20 g/L *M. spicata* tea; group IV received 40 g/L *M. spicata* tea. Herbal teas were prepared

daily and provided at all times to the rats over 30 days as drinking water. The results showed that peppermint tea caused a decrease in serum iron and ferritin levels and an increase in unsaturated iron-binding capacity (UIBC). Spearmint tea caused no significant change in serum iron, ferritin levels and UIBC.[45]

Mint Possess Anti-Androgenic Activity *In Vivo*

One of the prominent functions of this plant extract is its anti-androgenic activity. Studies have shown that the aqueous extract of this plant induced oxidative stress in the hypothalamic region and anti-androgenic activity in rats. The administration of the extract decreased activities of enzymes like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in the hypothalamus of rats. RT-PCR and immunoblot analysis showed the decreased expression of the steroidogenic enzymes, cytochrome P450scc, cytochrome P450C17, 3beta-hydroxysteroid dehydrogenase (3beta-HSD), 17beta-hydroxysteroid dehydrogenase (17beta-HSD) and related proteins like steroidogenic acute regulatory protein, androgen receptor and scavenger receptor class B-1 and testicular 3beta-HSD and 17beta-HSD enzymes. Histopathological analysis showed a decreased sperm density in cauda epididymis and degeneration of ductus deference.[38]

In another study Akdogan *et al.*[46] showed the effect of peppermint and spearmint teas on plasma total testosterone, luteinizing hormone, and follicle-stimulating hormone levels and testicular histologic features in rats. The animals were randomized into four groups of 12 rats each. The control group was given commercial drinking water, and the experimental groups were given 20 g/L peppermint tea, 20 g/L spearmint tea, or 40 g/L spearmint tea. The follicle-stimulating hormone and luteinizing hormone levels increased and total testosterone levels decreased in the experimental groups compared with the control group. The administration of mint tea increased the mean seminiferous tubular diameter compared with the control group. The only effects of *M. piperita* on testicular tissue was segmental maturation arrest in the seminiferous tubules; however, the effects of *M. spicata* extended from maturation arrest to diffuse germ cell aplasia in relation to the dose.[46]

Contraceptive Activity of Mint and Its Reversibility

Sharma and Jacob[47] studied the contraceptive activity of Japaneese mint in mice and its reversibility. They showed that the oral administration of the methanolic extract of mint caused inhibition of fertility in mice, while maintaining their normal sexual behavior. With the increase in treatment duration, there occurred a corresponding decrease in the mean weight of testis and accessory organs of reproduction. Sperm concentration, motility and viability in the cauda epididymis were also decreased. Spermatozoa with coiled tails also appeared in the epididymal smear. However, all the induced effects returned to normalcy within 30 days following withdrawal of 60-day treatment. The methanolic extract of mint did not affect the body weight of the mice and their blood cell count, packed cell volume, hemoglobin and blood/serum biochemistry.

Radioprotective Activity of Mint

Studies have shown that Japaneese mint (*M. arvensis*) possesses radioprotective activity against gamma radiation in mice. The administration of mint extract protected mice against gastrointestinal death as well as bone marrow death. The results also showed that the drug was nontoxic up to doses as high as 1,000 mg/kg body weight.[48]

Immunomodulatory Activity of Mint

Studies have also shown that Japaneese mint possesses immunomodulatory activity in rats. Immunologic anaphylactic reaction was generated by sensitizing the skin with anti-dinitrophenyl (DNP) IgE followed 48 hrs later with an injection of antigen. The oral and intravenous administration of the mint extract inhibited histamine release from rat peritoneal mast cells and it inhibited anti-DNP IgE-mediated tumor necrosis factor-alpha (TNF-alpha) production.[49] In another study Raphael and Kuttan[50] showed that the administration of carvone increased total antibody production, antibody producing cells in spleen, bone marrow cellularity and alpha-esterase positive cells significantly compared to normal mice, indicating its potentiating effect on the immune system.

Gastroprotective Activity of Mint

The gastroprotective effect of peppermint has been examined in many different animal models. Aqueous extracts of peppermint showed significant relaxation effect on isolated rabbit duodenum.[51] Menthol and peppermint oil inhibited the binding of the labeled calcium channel blockers 3H-nitrendipine and 3H-PN 200-110 in isolated guinea-pig ileal smooth muscle.[52] Peppermint oil also suppressed calcium influx in rabbit jejunum and guinea-pig colon.[53] Beesley *et al*.[57] showed that in Wistar rat jejunum, mucosal side application of peppermint oil (1 and 5 mg/mL) significantly inhibited active sodium dependent glucose absorption and active transport of the amino acid glycine, while serosal application (1 mg/mL) inhibited acetylcholine-induced secretion. Other studies showed that peppermint oil and menthol effectively stimulate choleretic activity (bile flow) in rats.[54–56] The plant also inhibited the intestinal bacteria-induced production of hydrogen sulfide, methanethiol (MeSH) and ammonia in the large intestine of pigs.[58] Akdogan *et al*.[46] found that administration of 20 g/L of *M. piperita* tea for 30 days inhibited iron absorption, significantly reduced serum iron and ferritin levels, and increased unsaturated iron-binding capacity in rats. Naseri *et al*.[59] studied the effects of *Mentha longifolia* leaf extract on rat ileal smooth muscle contractions. The last portion of the ileum from male adult Wistar rat was mounted in an organ bath containing Tyrode's solution. The tissue was contracted by carbachol (CCh, 10 μM), KCl (60 mM) and BaC12 (4 mM). The ilium was then incubated with mint extract. The results showed that *Mentha longifolia* hydroalcoholic leaf extract induces spasmolytic activity mainly through disturbance in calcium mobilization and partly by potassium channels activation.

The Effect of Mint on the Nervous System

Galeotti *et al*.[60] studied the local anesthetic effect of menthol in rabbits using a conjunctival reflex test, where they showed that an increased number of stimuli were necessary to provoke the reflex. The administration of the ethanolic extract of peppermint inhibited acetic acid induced analgesic activity in Swiss albino mice.[61] In another study,

Umezu and Ho[62] observed that intraperitoneal administration of peppermint oil significantly increases ambulatory activity in mice.

Cardiovascular Effects of Mint

Bello *et al*.[63] evaluated the effects of methanolic and dichloromethanolic extracts of the leaves and stems of *Mentha suaveolens* Ehrh. on resting arterial blood pressure, heart rate and noradrenaline induced hypertension. Intravenous administration of both extracts bolus to urethane anesthetized normotensive rats reduced the mean arterial blood pressure and heart rate, while only the dichloromethanol extract prevented the noradrenaline induced hypertension.

Antitumor Activities of Mint

Studies over the last several years have shown that peppermint and its constituents inhibit the proliferation of several cancer cell lines and induces cytotoxicity. Lu *et al*.[64] showed that menthol induced cell death in HL-60 cells is not apoptosis, but necrosis. The studies also showed that (−)-Menthol inhibited the proliferation of human gastric SNU-5 cancer cells in a dose- and time-dependent manner, by inhibiting gene expression of topoisomerase I, IIα and IIβ and promoting the gene expression of NF-κB.[65] Studies also showed that menthol displayed a dose-dependent inhibition of arylamine N-acetyltransferase (NAT) in human liver tumor cells.[66] Ohara and Matsuhisa[67] showed that peppermint inhibited okadaic acid (OA) induced tumor promotion by inhibiting phosphatase-2A.

Studies have shown that menthol suppresses carcinogenesis in animals. The administration of peppermint inhibited oral leukoplakia and frank tumors in the oral cavity of Syrian golden hamsters.[68] The studies also showed that administration of aqueous peppermint suspension inhibited 7,12-dimethylbenz (a) anthracene (DMBA) induced skin papillomagenesis in mice.[69] Samarth *et al*.[70,71] showed that peppermint extract treatment resulted in a significant reduction in the number of lung adenomas from an incidence of 67.92% in animals given only benzo[a]pyrene (BP) to 26.31%,

an inhibition of 61.26% in Swiss albino mice. The studies also showed that carvone has no effect on lung metastasis induced by B16F-10 melanoma cells in C57BL/6J mice.[72]

CLINICAL STUDIES

Mint Inhibits Androgen Levels in Women with Hirsutism

Clinical trials in women with hirsutism showed that mint tea inhibited androgen levels in these patients. Twenty-one female hirsute patients, 12 with polycystic ovary syndrome and nine with idiopathic hirsutism (the growth of excessive male-pattern hair on a woman) were included in this study. The patients took a cup of herbal tea which was steeped with spearmint for 5 days twice a day in the follicular phase of their menstrual cycles. The treatment with spearmint tea showed a significant decrease in free testosterone and increase in luteinizing hormone, follicle-stimulating hormone and estradiol. There were no significant decreases in total testosterone or dehydroepiandrostenedione sulphate levels. The study suggests that spearmint could be an alternative to antiandrogenic treatment for mild hirsutism.[73]

Mint Inhibits Irritable Bowel Syndrome

The efficacy of an herbal medicine, *Carmint*, on the relief of abdominal pain and bloating in patients with irritable bowel syndrome was also studied. Carmint contains total extracts of *Melissa officinalis*, *Mentha spicata*, and *Coriandrum sativum*, which have antispasmodic, carminative, and sedative effects. Thirty-two irritable bowel syndrome patients were randomly assigned to receive either Carmint or placebo, plus loperamide or psyllium (based on their predominant bowel function), for 8 wks. The results showed that the severity and frequency of abdominal pain/discomfort were significantly lower in the Carmint group than the placebo group at the end of the treatment. The study suggests that Carmint plus loperamide or Carmint plus psyllium (depending on the irritable bowel syndrome subtype) might be effective in patients.[74]

Mint and Respiratory Tract Disorders

Eccles *et al*.[75] studied the effects of inhalation of L-menthol, D-isomenthol and D-neomenthol upon nasal resistance and sensation to airflow in 40 subjects. The results showed that L-menthol caused a highly significant enhancement of nasal sensation of airflow but the isomers D-isomenthol and D-neomenthol had no effect on nasal sensation of airflow. The same group also showed the effects of menthol on reaction time and nasal sensation of airflow in human subjects suffering from the common cold. The results showed that menthol ingestion compared to placebo caused a significant increase in nasal sensation of airflow which persisted for up to 30 mins.[76,77] They also studied the effects of oral administration of (−)-menthol on nasal resistance to airflow and nasal sensation of airflow in subjects suffering from nasal congestion associated with the common cold, and the results showed that there was no effect on nasal resistance to airflow (NAR) as measured by posterior rhinomanometry but there was a marked change in nasal sensation of airflow with a subjective sensation of nasal decongestion.[76,77] Naito *et al*.[78] studied the effect of L-menthol stimulation of the major palatine nerve on nasal patency. They showed that menthol stimulation exerted an indirect effect on the nasal cavity in that all subjects mentioned a cold sensation in their nose and a sensation of increased nasal patency, but no influence on nasal resistance to airflow was detected. Morice *et al*.[79] studied the effect of inhaled menthol on citric acid induced cough in normal subjects. The results showed that menthol inhalation caused a reduction in evoked cough when compared with placebo. In a recent study Kenia *et al*.[80] showed that menthol has no effect on objective measures of flow but significantly increases the perception of nasal patency.

Nishino *et al*.[81] investigated the effects of nasal inhalation of L-menthol (a specific stimulant of cold receptors) on the respiratory sensation and ventilation during loaded breathing in 11 normal subjects. Their results showed that stimulation of cold receptors in the upper airway with nasal inhalation of L-menthol reduces the sensation of respiratory discomfort associated with loaded breathing. This effect is more effective during flow-resistive loading than during elastic loading.

Haidl *et al*.[82] investigated the effect of the inhalation of a 1% L-menthol solution in the premedication of fiberoptic bronchoscopy (FB)

on the frequency of cough and the irritability of the tracheobronchial mucosa during FB in a blinded, randomized and placebo-controlled study. The results showed that the inhalation of 1% L-menthol did not enhance the tolerability of FB. However, L-menthol induced a significant increase in peak respiratory flow (PEF) immediately after inhalation. Finally, sensation of dyspnea was decreased in both groups at the day post-FB.

Dresser *et al*.[83] determined the effect of peppermint oil and ascorbyl palmitate on cytochrome P4503A4 (CYP3A4) activity *in vitro* and oral bioavailability of felodipine in humans. The results showed that peppermint oil, menthol, menthyl acetate, and ascorbyl palmitate were moderately potent reversible inhibitors of CYP3A4 activity.

The Effect of Mint Against Gallbladder Stones

Ahrens *et al*.[84] investigated the effects of menthol in the adjuvant treatment of gallbladder stones fragmented by extracorporeal shock wave lithotripsy (ESWL) as an option for reducing doses of urso- and chenodeoxycholic acid (UDC/CDC). The results showed that patients become stone-free more quickly on the standard UDC plus CDC therapy. In the menthol group, there was no substantial, statistically relevant difference in the efficacies of the two treatments.

The Effect of Mint on the Skin

To determine the efficacy of menthol penetration-enhanced tetracaine gel in the management of pain, a double-blind, placebo-controlled, randomized controlled trial (RCT) design was used. The mean verbal pain scores (VPS) were significantly lower in volunteers treated with penetration-enhanced tetracaine gel than those in volunteers receiving non-penetration-enhanced tetracaine gel or placebo. Menthol improved the analgesic efficacy of the tetracaine 4% gel in part through enhanced percutaneous permeation.[85] Brain *et al*.[86] showed that menthol produces local vasodilation and reduces skin barrier function in human subjects. In another study Panahi *et al*.[87] showed that a 1% phenol and 1% menthol combination has significant therapeutic effects for mustard gas-induced pruritus in chemical warfare-injured veterans, compared with placebo.

The Effect of Peppermint on Gastrointestinal Disorders

Studies have shown that the administration of peppermint oil does not affect gastric emptying time, but causes a complete inhibition of gallbladder volume during the refilling phase, compared to placebo.[88] But in another study Dalvi *et al*.[89] showed that treatment with peppermint oil increased gastric emptying rate. Leicester and Hunt[90] showed that the administration of peppermint oil reduces painful muscle spams in patients undergoing endoscopy. In another study Asao *et al*.[91] showed that intracolonic administration of peppermint oil decreased the spasmolytic effect.

Sparks *et al*.[92] showed that a suspension of barium sulfate and peppermint oil eliminated residual spasm in 60% of patients undergoing double contrast barium enema examination. In another study Micklefield *et al*.[93] showed that the administration of peppermint oil decreased the number of duodenal contractions and the contraction amplitudes.

The leaves of peppermint or its oil have been found to decrease abdominal pain and dyspepsia.[94–97] The administration of peppermint oil was also found to be helpful in relieving symptoms of irritable bowel syndrome in both adults and children.[98]

TOXICITY STUDIES OF MINT

The Effect of Mint on Uterine Tissue in Rats

Akdogan *et al*.[4] studied the effect of mint tea on rat kidneys. They showed that the administration of mint tea increased levels of plasma urea and creatinine in rats. The administration of both peppermint and spearmint teas decreased the levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) in rat kidney homogenates. Treatment with mint tea showed hydropic degeneration of tubular epithelial cells, epithelial cells with picnotic nuclei and eosinophilic cytoplasm, tubular dilatation and enlargements in Bowman capsules. The study showed that peppermint is not nephrotoxic but spearmint presents markedly nephrotoxic changes in rats. In another study Güney *et al*.[3] showed that the consumption of mint tea increased malondialdehyde (MDA) levels in the uterine tissues of rats. Moreover, the administration of mint tea induced changes like apoptosis and diffuse eosinophil

leucocyte infiltration in surface and stromal glandular epithelium in both endometrium and endocervix.[3]

The Effect of Mint on Liver Tissues of Rats

The effect of peppermint and spearmint teas on the liver tissues of rats has also been studied. The administration of both peppermint tea and spearmint tea increased the levels of liver function enzymes such as aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT). The homogenates of the liver tissues from peppermint tea treated animals showed an increase in antioxidant enzyme levels such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), whereas the administration of spearmint tea (20 g/L) decreased CAT levels. Interestingly, treatment with spearmint tea at a 40 g/L dose decreased SOD, GSH-Px and CAT activities in the liver homogenates of rats.[99]

Toxicity of Mint in Humans

One report has suggested that mint oil was an allergen in a patient with oral lichen planus.[100]

MINT BASED RECIPES

1. Mint Fried Rice

Ingredients

1 bunch mint, rinsed; leaves and tender stems plucked
2 cups basmati rice, washed and soaked in 4 cups of water
3 green chillies and one onion, sliced thinly, lengthwise
¼ cup fresh coconut, chopped
1 inch piece ginger and 3 cloves garlic, peeled and chopped
4 each: cloves, cardamom, small cinnamon pieces, bay leaves and 1 star anise
¼ cup roasted cashews
1 tablespoon ghee
1 teaspoon salt, or to taste

Method

Put mint leaves, coconut, green chillies, ginger and garlic in a mixer. Add a pinch of salt and blend to fine consistency without adding water.

Put the rice cooker pot on the stove on a medium flame. (To make the dish as a one-pot meal, saute the masala for pulao in the rice cooking pot first, then add the soaked basmati rice along with water to the same pot and cook.)

Heat ghee on medium heat in the rice cooker pot. When it is hot, add the spices (cardamom, cloves, cinnamon, bay leaves and star anise) and saute. Add the onions and saute until they start to brown. Add the pureed mint-green chilli-coconut paste. Fry until the mint paste changes color from bright green to light-green. Take care not to burn/brown the masala paste.

Into this sauteed masala, empty the basmati rice and the water it was soaked in. Add salt and stir so that all the ingredients mix together. Remove the pot from stove, put it back in the rice cooker and switch on to cook. Once the rice cooks to tender, remove the lid, add cashews and mix once. Put the lid back on and let it stand for another 5 mins.

2. Mint Sandwich

Ingredients

4 slices of bread

For first filling:
1 potato (if large)
1 teaspoon garam masala
½ teaspoon corriander powder
1 teaspoon ginger garlic paste
3–4 green chilies, chopped
Salt to taste

For second filling:
½ cup pudina
½ cup corriander leaves

3–5 green chillies
¼ cup raw mango (or 1 tablespoon lime juice)
Salt to taste

Method

Boil potato and mash it along with the first filling ingredients except the green chillies. Add the green chillies and keep aside.

Make a paste with all the second filling ingredients. If there is no raw mango, you can substitute it with lime juice.

If the bread slices are thick, you can spread both fillings one by one.

Otherwise, spread one filling and use another slice to separate the other filling.

Place in a sandwich toaster or on pan with a small amount of ghee. Toast/fry until golden brown and enjoy with tomato sauce.

CONCLUSION

Besides the traditional uses of mint to treat various disorders, research over the past few years has identified several additional uses of mint and its components menthol and carvone. These chemicals are promising antimicrobial, antioxidant, anti-inflammatory and antitumor agents. Further studies are needed to validate these uses.

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Turmeric (Curcumin)

Jen-Kun Lin and Shoei-Yn Lin-Shiau

The essential role of spices in human health has become an important issue in modern food science and molecular nutrition. Turmeric is an orange-yellow spice often found in curry powder. The major active principle of turmeric is curcumin, and the spice also contains more than 28 phytocompounds. In recent years a lot of interest has been focused on curcumin due to its use in the treatment of a wide variety of disorders without appreciable side effects. Curcumin has been shown to inhibit carcinogensis at its initiation and promotion stages. It possesses anti-inflammatory activity and is a potent inhibitor of reactive oxygen species (ROS), generating enzymes such as cyclooxygenase-2, lipooxygenase, xanthine oxidase and nitric oxide synthase. Curcumin is also a potent inhibitor of protein kinase C, EGFR tyrosine kinase and I-κB kinase (IKK). In addition, curcumin inhibits the activation of NF-κB and the expression of c-jun, c-fos, c-myc and iNOS. It has been proposed that curcumin may suppress tumor promotion and progression by blocking signal transduction pathways in target cells. Fundamentally, although the inflammation is a protective effect, persistent inflammation is believed to be involved in multiple stages of cancer development. As a result, aberrant increased activity of NF-κB, the major factor that plays a key role in inflammation is implicated in a variety of human cancers. Curcumin is known to exert significant anti-inflammatory effects by interrupting NF-κB signaling in tumor cells at multiple levels. In addition, further studies demonstrate that NF-κB is suppressed by curcumin

through inhibiting the activity of IKK. All these observations indicate that curcumin indeed shows valuable potential in the treatment of cancer. In this chapter, the anticancer effects of curcumin and turmeric and their underlying mechanisms are discussed. We also provide a summary of the recent literature focusing on NF-κB signaling pathways and their potential involvement in the development of anticancer strategies.

INTRODUCTION

The essential role of spices in the maintenance of human health has become a hot issue in modern food science and molecular nutrition. Current biomedical efforts are concentrated on their scientific merits and molecular mechanisms in order to provide science-based evidence for traditional uses and to develop either functional foods or nutraceuticals. The Indian and Chinese traditional medical systems use turmeric for healing wounds, rheumatic disorders, gastrointestinal systems, deworming, rhinitis and as a cosmetic.[1–3] Studies in Oriental herbal medicines have explored its anti-inflammatory, cholekinetic and anti-oxidant potentials. Recent studies have focused on its preventive effect on precarcinogenic, anti-inflammatory and anti-atherosclerotic effects in biological systems both under *in vitro* and *in vivo* conditions in animals and humans.[1,3] Both turmeric and curcumin have been found to increase detoxifying enzymes, prevent DNA damage, improve DNA repair, and decrease mutations and tumor formation.

Limited clinical studies suggest that turmeric can significantly impact the excretion of mutagens in urine in smokers and regress precancerous palatal lesions. Turmeric has been shown to reduce DNA adducts and micronuclei in oral epithelial cells.[2] It prevents formation of nitroso compounds both *in vitro* and *in vivo*. It also delays induced cataract in diabetes and reduces hyperlipidemia in obese rats. Recently, several molecular targets have been identified for the therapeutic or preventive effects of turmeric and curcumin.[4]

TURMERIC AND CURRY POWDER

Turmeric is an orange-yellow spice often found in curry powder. The major active principle is curcumin. In recent years, considerable interest has been

focused on curcumin due to its effective treatment of a wide variety of disorders without any side effects. One of the major curcuminoids of turmeric is responsible for imparting its characteristic orange-yellow color. Turmeric was used in ancient times on the Indian subcontinent to treat various diseases, including rheumatism, bodyache, skin diseases, intestinal worms, diarrhea, intermittent fever, hepatic disorders, biliousness, urinary discharges, dyspepsia, inflammation, constipation, leukederma, amenorrhea and colic.[5] Based on the literature on folk medicine, turmeric and curcumin have the potential to treat a wide variety of inflammatory disorders, including cancer, diabetes, cardiovascular diseases, arthritis, Alzheimer's disease, and psoriasis[1,2] through modulation of numerous molecular targets.[4]

CURCUMA SPECIES

Curcumin (diferuloylmethane) is a major orange-yellow pigment that has been isolated from the ground rhizome of the Curcuma species, Zingiberaceae (Fig. 1). The appearance of the leaves and roots of *Curcuma longa* are shown in Figs. 1a and 1b, respectively. Nine major species of Curcuma have been cultivated and their compositions of curcuminoids are summarized in Table 1.[6] Three major curcuminoids — curcumin, demethoxycurcumin and bisdemethoxycurcumin — occur naturally in these Curcuma species. The proportions of the curcuminoids of these plants vary with site and cultivation period as illustrated in Table 1. It is notable that *Curcuma longa* L. (turmeric) has the highest concentration of curcumin as compared to the other species.

CHEMICAL CONSTITUENTS OF TURMERIC

Turmeric contains a wide variety of phytocompounds, including curcuminoids and other phenolic compounds (more than 28 compounds) as shown in Table 2. Curcumin is the phytochemical that imparts the yellow to orange-yellow color to turmeric and is now recognized as being responsible for most of the therapeutic effects. It is estimated that 2–5% of turmeric is curcumin. Curcumin was first isolated from turmeric in 1815, and the structure was described chemically in 1910 as diferuloylmethane. Most commercially available preparations of curcumin contain

Fig. 1. **(a)** Cultivated plant of *Curcuma longa* L. **(b)** Harvested roots of *Curcuma longa* L.

Table 1. Curcuminoid contents in rhizome of Curcuma species.[a]

Curcuma Species	Site of Cultivation (yr)	Curcuminoids (%)[b]			
		Total	Cur	Dcur	Bcur
Curcuma longa L.	Nang-ning (1981)	3.91	1.84	1.09	1.01
Curcuma longa L.	Cheng-du (1979)	3.83	2.03	1.12	0.82
Curcuma longa L.	Beijing (1980)	3.82	1.79	1.11	0.75
Curcuma longa L.	Nang-Chang (1965)	1.41	0.70	0.35	0.23
Curcuma longa L.	Kwang-Chou (1979)	1.28	0.53	0.40	0.32
Curcuma phaeocaulis	—	3.00	—	—	—
Curcuma xanthorrhiza Raxb	Kwang-Chou (1980)	2.10	1.43	0.86	0.12
Curcuma wenyujin	Che-chiang (1979)	0.20	0.13	0.07	0.02
Curcuma sichuanensis	Si-chuan (1980)	0.04	0.01	0.01	< 0.001
Curcuma kwangsinensis	Yun-nan (1980)	1.54	0.89	0.57	0.23
Curcuma zedoaria	—	0.10	—	—	—
Curcuma aeruginosa Roxb.	Si-chuan (1980)	0.04	0.01	0.01	< 0.001
Curcuma elata Roxb.	Kwang-see (1980)	0.01	< 0.001	< 0.001	< 0.001

[a]*Source*: Chen and Fang[6] (except *Curcuma phaeocaulis* and *Curcuma zedoaria* from Aggarwal *et al*.[1]).
[b]Curcuminoids are expressed in percent of dried root analyzed. Abbreviations are: Cur — curcumin; Dcur — demethoxycurcumin; Bcur — bisdemethoxycurcumin, and total — total curcuminoids including cur, Dcur and Bcur.

approximately 77% diferuloymethane, 18% demethoxycurcumin and 5% bisdemethoxycurcumin. Curcumin is hydrophobic in nature and soluble in dimethylsulfoxide, acetone, ethanol and oils. It has an absorption maxima around 420 nm. When exposed to acidic conditions, the color of turmeric or curcumin turns from yellow to deep red.[1]

BIOLOGICAL ACTIVITIES OF CURCUMIN *IN VITRO* AND *IN VIVO*

Curcumin has several biological activities as demonstrated in cell cultures and experimental animal model systems. We will focus on its ROS scavenging effects, inhibition of carcinogenesis, induction of apoptosis, and neuroprotective effects.

Table 2. Chemical composition of Curcuma species.

I.	Curcuminoids
	Curcumin
	Demethoxycurcumin
	Bisdemethoxycurcumin
	Cyclocurcumin
	Tetrahydrocurcumin
	Hexahydrocurcumin
	Hexahydrocurcuminol
	Curcumin sulfate
	Curcumin glucuronide
II.	Other phytocompounds
	β-elemene, β-sesquiphelandrene, β-zingiberene, γ-elemene, β-curcumene, α-curcumene, turmerin, curdione, α-tumerone, β-turmerone, curdione
	Furanodiene
	Curzerene
	Curzerenone
	Germacrone
	Eugenol
	Curcumol
	Linderazulene
	Isocurcumenol
	Curcumenol

Sources: Chen and Fang[6] and Aggarwal.[1]

Scavenging of ROS

Curcumin is a potent scavenger of ROS, including superoxide anions,[7] hydroxyl radicals, singlet oxygen,[8] nitric oxide and peroxynitrite. Curcumin has the ability to protect lipids, hemoglobin and DNA against oxidative degradation. Pure curcumin has more potent superoxide anion scavenging activity than demethoxycurcumin or bisdemethoxycurcumin.[7] Curcumin is also a potent inhibitor of ROS-generating enzyme cyclooxygenase and lipoxygenase in mouse epidermis.[9]

Chronic exposure of humans to high concentrations of arsenic in drinking water is associated with skin lesions, peripheral vascular disease, hypertension, blackfoot disease and a high risk of cancer. Arsenic causes

DNA damage through the generation of ROS and the enhancement of lipid peroxidation levels. Curcumin has been shown to counteract the damage by quenching ROS, decreasing the level of lipid peroxidation and increasing the level of phase II detoxification enzymes like catalase, superoxide dismutase, and glutathione peroxidase.[10]

Inhibition of Carcinogenesis

Studies have shown that curcumin inhibits chemical carcinogenesis at different tissue sites in several experimental animal models. Curcumin inhibited tumor initiation by benzo[α]pyrene (BaP) and 7,12-dimethylbenz[α] anthracene (DMBA) in mouse epidermis.[11] Topical application of curcumin strongly inhibited tumor production in the skin of DMBA-initiated mice.[12] Including curcumin in the diet decreased the number of N-ethyl-N-nitro-N-nitrosoguanidine (ENNG) induced duodenal tumors per mouse.[13] Administration of curcumin in the diet decreased the number of azoxymethane (AOM) induced colon tumors in mice[13] and rats.[14]

Induction of Apoptosis

We have demonstrated that curcumin induces apoptosis in several tumor cell lines.[15] The curcumin-induced apoptosis is highly dependent on the origin and malignancy of the cell lines. It appears that typical apoptosis can only be induced in immortalized mouse embryo fibroblast NIH 3T3, erbB2 oncogene-transformed NIH 3T3, mouse sarcoma 180, human colon cancer cell HT29, human kidney cancer cell 293 and human hepatocellular carcinoma HepG2 cells — but not in primary cultures of mouse embryonic fibroblast C3H10T1/2, rat embryonic fibroblast or human foreskin fibroblast cells.[15] Treatment of NIH3T3 cells with the PKC inhibitor staurosporine, the tyrosine kinase inhibitor herbimycin A or arachidonic acid metabolism inhibitor quinacrine induces typical apoptosis. These results suggest that blocking the cellular signal transduction of protein kinase cascade in immortalized or transformed cells might trigger the executive steps of apoptosis.

We have found that curcumin (3.5 μg) induces apoptosis in human promyelocytic HL-60 cells.[16] The apoptotic effect of curcumin was not

affected by cycloheximide, actinomycin D, EGTA, W7 (calmodulin inhibitor), sodium orthovanadate, or geanistein and, whereas an exonuclease inhibitor ZnSO4 and a proteinase inhibitor N-tosyl-L-lysine chloromethyl-ketone (TLCK) could markedly abrogate curcumin-induced apoptosis. The antioxidants N-acetyl-L-cysteine (NAC), L-ascorbic acid, alpha-tocopherol, catalase and superoxide dismutase all effectively prevented curcumin-induced apoptosis. Furthermore, overexpression of Bcl-2 in HL-60 cells delayed the entry of curcumin-treated cells into apoptosis, suggesting that Bcl-2 plays an important role in the early stage of curcumin-triggered apoptotic cell death.[16]

Recent studies have demonstrated that both Bax and Bak genes are essential for maximum apoptotic response by curcumin in mouse embryonic fibroblast. Curcumin treatment resulted in increase in the protein levels of Bax and Bak, and mitochondrial translocation and activation of Bax in fibroblast to trigger a drop in mitochondrial membrane potential, cytosolic release of apoptogenic molecules (cytochrome *c*, etc.), activation of caspase-9 and caspase-3, and ultimately induction of apoptosis.[17]

It is interesting to note that curcumin inhibited growth of LNCaP xenografts in nude mice by inducing apoptosis (TUNEL staining) and inhibiting proliferation (PCNA and Ki57 staining) and sensitized these tumors to undergo apoptosis by TRAIL.[18] In xenograft tumors, curcumin upregulated the expression of TRAIL-R1/DR-4, TRAIL-R/DR-5, Bax, Bak, p21(WAF-1) and p27 (kip1) and inhibited the activation of NF-κB and its gene products such as cyclin D1, VEGF, μPA, MMP-2, MMP-9, Bcl-2 and Bcl-XL. The regulation of death receptors and members of the Bcl-2 family and inactivation of NF-κB may sensitize TRAIL-resistant LNCaP xenografts.[18]

Neuroprotective Effects of Curcumin

Neurodegenerative diseases result in the loss of functional neurons and synapses. Although future therapies like stem cell approaches offer some hope, current treatments for most of these diseases are unable to prevent these devastating diseases. Progressive accumulation of molecular damage is a hallmark of cellular aging, which is amenable to intervention and prevention by hormesis through mild stress. Neuroprotective approaches work

best prior to the initiation of damage, suggesting that some safe and effective naturally occurring compounds would be highly promising. Curcumin has an outstanding safety profile and a number of pleiotropic actions with potential for neuroprotective efficacy, including anti-inflammatory, antioxidant, and anti-protein-aggregate activities.[19] In addition, these effects can be achieved at submicromolar levels.[20] In our preliminary results, curcumin's dose-response curves are strongly dose dependent and often biphasic so that *in vitro* data must be cautiously interpreted; many effects might not be achievable in target tissues *in vivo* with oral dosing. Indeed, accumulating cell culture and animal model data show that dietary curcumin is a strong candidate for use in the prevention or treatment of major disabling age-related neurodegenerative diseases like Alzheimer's, Parkinson's, and stroke.[21] Promising results have already led to ongoing pilot clinical trials. In phase I clinical studies, curcumin with doses up to 3,600–8,000 mg daily for four months did not result in discernible toxicities except mild nausea and diarrhea.[22] It is imperative that well-designed clinical trials supported by better formulations of curcumin and its derivatives be conducted in the near future.

ANTICANCER EFFECTS OF CURCUMIN

Cancers, with their diverse histological origins, have therapeutically specific targets as well as common molecular markers involved in their initiation and progression. Based on this concept, curcumin has been shown to affect several intracellular targets regulating survival or death of cancer cells. To exert its anticancer activity, curcumin is considered to counteract the altered functionality of proliferative and apoptotic pathways. Interestingly, accumulating evidence suggests that curcumin shows anticancer effects at lower doses compared to other anticancer drugs. These effects are mediated through the regulation of numerous biochemical cascades, including various transcription factors, growth factors, inflammatory cytokines, protein kinases, and other enzymes.[4] Among these molecular targets, particularly, curcumin is apparently a highly effective interacts with several inflammatory targets. This suggests that curcumin's reported beneficial effects might be due in part to its ability to modulate the immune system. In this regard, we have suggested the

molecular mechanisms underlying the cancer prevention of curcumin.[3,23] In 1991, our laboratory demonstrated that curcumin can suppress the expression of c-jun, a proliferation stimulating gene in immune cells.[24,25] The apoptotic effects of curcumin have been demonstrated by Jiang *et al*.[15] and Kuo *et al*.[16] In 1993, the inhibition of protein kinase C (PKC) and xanthine oxidase were also described.[26,27] Finally, we further demonstrated that nuclear factor-kappa B (NF-κB), the major factor playing a key role in inflammatory and immune response, was suppressed by curcumin by inhibiting the activity of I-κB kinase (IKK).[28]

In principle, curcumin has been widely demonstrated to have potent antioxidant activities. It is well-known that reactive oxygen species (ROS) play a key role in enhancing inflammation through the activation of stress kinases and redox-sensitive transcription factors such as NF-κB. Oxidative stress activates NF-κB-mediated transcription of pro-inflammatory mediators either through the activation of its activating inhibitor of I-κB kinase or the enhanced recruitment or activation of transcriptional co-activators. Although numerous different pathways are activated during the inflammatory response, NF-κB is thought to be of the most important in cancer-induced inflammation.[29]

Curcumin is an ROS scavenger, increases antioxidant glutathione levels by induction of glutamate cysteine ligase, and acts as an anti-inflammatory agent through the inhibition of NF-κB signaling.[30]

MOLECULAR TARGETS OF CURCUMIN

The molecular targets of curcumin in cancer chemoprevention have been intensively discussed.[4] Curcumin possesses an anti-inflammatory activity and is a potent inhibitor of ROS-generating enzymes such as lipoxygenase, cyclooxygenase, xanthine oxidase and inducible nitric oxide synthase (iNOS). An effective inducer of heme oxygenase-1, curcumin also possesses a cancer chemopreventive activity. Curcumin is a potent inhibitor of protein kinase C (PKC), EGFR-tyrosine and I-κB kinase. Subsequently, curcumin inhibits the activation of NF-κB and the expression of oncogenes including c-jun, c-fos, c-myc, NIK, MAPKs, ERK, ELK, PI3K, Akt, CDKs, and iNOS. It is considered that PKC, mTOR and EGFR tyrosine kinase are the major upstream molecular targets for curcumin

intervention, whereas the nuclear oncogenes such as c-jun, c-fos, c-myc, CDKs, fatty acid synthase (FAS) and iNOS might act as downstream molecular targets for curcumin actions. It has been proposed that curcumin might suppress tumor promotion by blocking signal transduction pathways in the target cells. The oxidant tumor promoter TPA activates PKC by reacting with zinc thiolate present within the regulatory domain, whereas the oxidized form of cancer chemopreventive agents such as curcumin can inactivate PKC by oxidizing the vicinal thiols present within the catalytic domain. Recent studies have indicated that proteasome-mediated degradation of cell proteins plays a pivotal role in the regulation of several basic cellular processes, including differentiation, proliferation, cell cycling and apoptosis. It has been demonstrated that curcumin-induced apoptosis is mediated through the impairment of the ubiquitin-proteasome pathways.[4]

THE PIVOTAL ROLE OF NF-κB SIGNALING IN CANCER CHEMOPREVENTION BY CURCUMIN

NF-κB was initially reported in the 1980s as a regulator of immunoglobin κ gene transcription in B lymphocytes.[31] To date, five mammalian NF-κB family members have been discovered, including p50, p52, p65 (RelA), c-rel and RelB. To activate specific downstream gene expression, NF-κB molecules form dimers, dissociate with the inhibitor of I-κB proteins, enter the nucleus upon activation, and bind DNA (Fig. 2). Aberrant increased NF-κB activity is implicated in a variety of human cancers. In principle, interruption of NF-κB signaling by curcumin in tumor cells can be achieved at multiple levels. Curcumin-inhibited inducible NF-κB activation and suppressed proliferation has been observed in breast cancer,[32] ovarian cancer,[33] pancreatic cancer,[34] leukemia and multiple myeloma,[35] oral cancer,[36] bladder cancer,[37] and prostate cancer.[38] Most downstream effects through curcumin are NF-κB-regulated gene products, including apoptosis (Bcl, TRAF), cell cycles (cyclin D1, cyclin D2), growth factors (interleukin, TNF-α, VEGF), receptors (CD40, CD44, CD86, CCR7, CXCL) and metalloproteinases (MMP-2, MMP-9). Curcumin also sensitizes human cancer cells through this pathway. In human pancreatic cancer, the curcumin

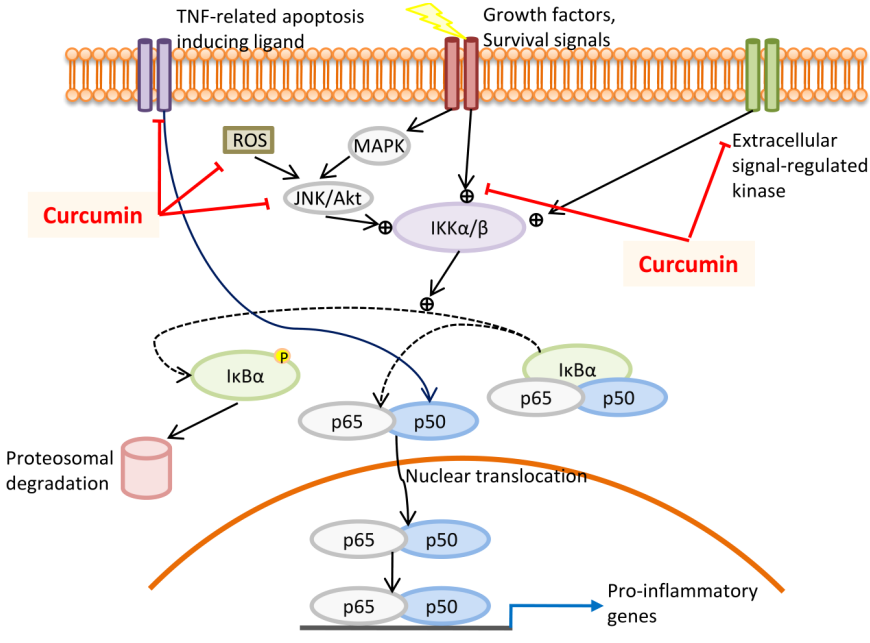

Fig. 2. Possible molecular mechanisms for the anti-inflammatory and anticarcinogenic effects of curcumin.[66] Curcumin is known to exert its anti-inflammatory and anticarcinogenic effects by interrupting NF-κB signaling at multiple sites and multiple levels. For example, ROS mediate inflammation through the activation of stress kinases and redox-sensitive transcription factors such as NF-κB, however, curcumin is an ROS scavenger and thus prevents inflammatory signaling. In addition, curcumin can interfere with the functions of Akt and MAPKs, and in turn downregulate the downstream molecule, NF-κB, and other oncogenic proteins.[66]

combination therapy with TNF-related apoptosis inducing ligand (TRAIL) suggests that the inhibition of NF-κB stimulates TRAIL-induced apoptosis.[39] Moreover, Notch-1, Hes-1, and Bcl-XL expression levels were concomitantly downregulated by curcumin treatment, and correlated with the inactivation of NF-κB activity in increasing apoptosis.[40] In human prostate cancer, curcumin induces apoptosis through Bax translocation to mitochondria[41] and caspase activation,[42] which enhances the therapeutic efficiency when combined with TRAIL. Similarly, curcumin sensitization to TRAIL by inhibiting Akt-regulated NF-κB and NF-κB-dependent antiapoptotic targets Bcl-2, Bcl-xL, and

XIAP in LNCaP and PC3 prostate cancer cells.[43] Thus, curcumin may play an adjuvant role in inducible cancer chemoresistance through the inhibition of NF-κB signaling.

Curcumin has also been shown to interfere with Akt and mitogen-activated protein kinases (MAPK) pathways, two key survival signaling systems. Because NF-κB is a downstream target of Akt, the inhibition of Akt and MAPK by curcumin is implicated in mediating the beneficial effects in anticancer therapy. For instance, curcumin decreased expression and activation of epidermal growth factor receptor (EGFR), HER-2, HER-3 and IGF-1R as well as their downstream effectors Akt and cycloxygenase-2 (COX-2) in HCT-116 and HT-29 colon cancer cells.[44] It has been observed that curcumin inhibited EGFR to block Akt, indicating that cancer cell apoptosis is induced by the inhibition of NF-κB activity treated with curcumin at low concentrations.[45] Curcumin also interrupts extracellular signal-regulated kinase (ERK) signaling, reduces NF-κB activity and results in the suppression of connective tissue growth factor (CTGF) expression in activated hepatic stellate cells.[46] Likewise, TNF-α-stimulated Akt phosphorylation mediated Src transactivation and stimulated recruitment and assembling NF-κB p65 to induce MMP-9 expression.[47] As a consequence, curcumin treatments showed that the blockage of Akt nuclear translocation inhibits MMP-9 expression. Curcumin was also reported to block histone deacetylase (HDAC) and p300/Notch 1 signaling by preventing the degradation of I-κBα in leukemia.[48] Moreover, in C6 glioma cells, curcumin reduced cell survival correlated with the inhibition NF-κB signaling pathways via prevention of constitutive JNK and Akt activation.[49]

It is also known that curcumin with its potent antioxidant property is anticipated to exert its bioactivities. In K562 leukemia cells, curcumin-induced topo I and topo II-DNA complexes were prevented by the antioxidant N-acetylcysteine; this suggests ROS may directly mediate the formation of these complexes.[50] However, the suppression of TNF-induced NF-κB activation by curcumin suggests the critical role of its structural signature rather than its ROS scavenging ability.[51] Curcumin acts through the inhibition of phosphorylation of the I-κB, which leads to their degradation by the proteosome. Work by Marin *et al*. has shown that a dose-dependent inhibition of NF-κB correlated with decreased levels

of phospho-I-κBα selectively induces apoptosis of cancer cells but not normal cells.[52] Furthermore, the inhibition of I-κB degradation leads to a downregulated expression of COX-2.[53] By inhibition of I-κB degradation, curcumin suppressed the expression of NF-κB, COX-2, and MMP-9, and indeed suppressed the incidence of breast cancer metastasis.[54] Other than inhibiting I-κB degradation, previous studies reported that curcumin also inhibits ligand-independent dimerization such as the TLR4 receptor complex in addition to the IKK pathway.[55] In MDA-MB-468 breast cancer cells and HT29 colon cancer cells, treatment with curcumin inhibited Stat3 phosphorylation, resulting in reduction of the nicotinamide N-methyltransferase (NNMT) level.[56] In human endometrial cancer cells, curcumin downregulates Ets-1 and Bcl-2 expression and induces apoptosis, suggesting a novel molecular mechanism for antitumor activity.[57] Curcumin also inhibits acid sphingomyelinase, and this might be involved in its antiproliferative effect against colon cancer cells.[58] Furthermore, studies have demonstrated curcumin-induced apoptosis selectively in human papilloma virus (HPV)-associated cervical cancer cells.[59]

CLINICAL APPLICATION OF CURCUMIN

Curcumin has long been promised to be a therapeutic or preventive agent for several major human diseases because of its antioxidative, anti-inflammatory, and anticancerous effects. The absorption, bioavailability and metabolism of curcumin have been studied in humans.[60] In 2001, a phase I clinical trial demonstrated that curcumin is not toxic to humans up to 8,000 mg/day when taken for three months, and the safety of curcumin applied in human subjects was confirmed by our laboratory.[61] The data also suggest a biologic effect of curcumin in the chemoprevention of cancer. It has been suggested that constitutive NF-κB is a crucial event for enhanced proliferation and survival of malignant cells. In 1986 Satoskar *et al*. showed curcumin's anti-inflammatory response in a preclinical trail.[62] In 2004 Sharma *et al*. designed a dose-escalation study to explore the pharmacology of curcumin in humans ($n = 50$). By measuring patient blood leukocytes, levels of inducible prostaglandin E2 (PGE_2) production were reduced after treatments.

Because curcumin is known to inhibit NF-κB-stimulated inflammatory signaling by blocking I-κB degradation, the results indicated that curcumin downregulates COX2 transcription, at least in part, by inhibition of NF-κB signaling.[63] In addition to cancer therapy, the effects of curcumin on other conditions were also studied, including rheumatoid arthritis, atherosclerosis, chronic pancreatitis, psoriasis, hyperlipidemia, and neurodegenerative diseases. For example, a short-term human study ($n = 36$) has suggested that curcumin significantly decreases serum cholesterol concentration.[64] In addition, Zhang *et al.* demonstrated that curcumin treatments stimulated immune clearance of amyloidosis in AD brain, suggesting a potential neuropreventive role for curcumin.[65] Another study found curcumin exerted its inhibition in patients with *H. pylori*-induced chronic inflammation, suggesting curcumin has therapeutic effects in patients with inflammation-related diseases.

CONCLUSION

Decades of extensive research have concluded overwhelmingly that curcumin appears to have beneficial therapeutic effects for inflammatory-related diseases including cancer. Marked by chronic inflammation modulators, NF-κB is the major molecular target of curcumin treatments (Fig. 2). Since blocking I-κB degradation and its control by IKK are essential steps in NF-κB activation, targeting this node for NF-κB specific blockade without safety concerns is worth exploring in future.[66] Another challenge is how the bioactivities of curcumin metabolites undergo physiological processing. The absorption, bioavailability and metabolism of curcumin have been studied *in vivo* in our laboratory. We found curcumin-glucuronoside, dihydrocurcumin-glucuronoside, THC-glucuronoside and THC to be the major metabolites of curcumin.[3,28] This data shows that although curcumin is poorly absorbed, it is rapidly metabolized so that the concentrations detected in free serum are very low. In the absence of detailed information on the biological effects of curcumin metabolites, efforts are necessary to establish their role as well as key elements of their structure-activity relationships. Understanding the molecular mechanisms underlying the anti-inflammatory and anticancer

actions of curcumin and its derivatives should ultimately prove to be helpful toward the designing of safer and more effective drugs in the treatment of human cancer.

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