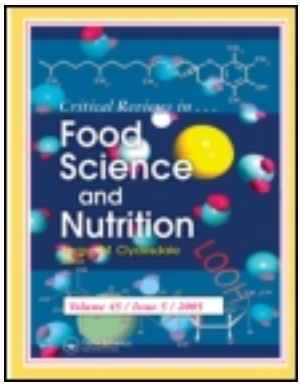

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Antioxidant Potential of Spices and Their Active Constituents

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Antioxidant Potential of Spices and Their Active Constituents

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Excessive free radical generation overbalancing the rate of their removal leads to oxidative stress. Oxidative stress has been implicated in the etiology of cardiovascular disease, inflammatory diseases, cancer, and other chronic diseases. Antioxidants are compounds that hinder the oxidative processes and thereby delay or suppress oxidative stress. There is a growing interest in natural antioxidants found in plants. Herbs and spices are most important targets to search for natural antioxidants from the point of view of safety. A wide variety of phenolic compounds present in spices that are extensively used as food adjuncts possess potent antioxidant, anti-inflammatory, antimutagenic, and cancer preventive activities. This paper reviews a host of spice compounds as exogenous antioxidants that are experimentally evidenced to control cellular oxidative stress, both in vitro and in vivo, and their beneficial role in preventing or ameliorating oxidative-stress-mediated diseases, from atherosclerosis to diabetes to cataract to cancer. The antioxidative effects of turmeric/curcumin, clove/eugenol, red pepper/capsaicin, black pepper/piperine, ginger/gingerol, garlic, onion, and fenugreek, which have been extensively studied and evidenced as potential antioxidants, are specifically reviewed in this treatise.

Keywords Spices, spice compounds, antioxidant potential, anti-inflammatory property, cancer preventive effect, hepatoprotective effect, cardioprotective effect

INTRODUCTION

Generation of reactive oxygen species (ROS) and other free radicals during metabolism is a normal process that is ideally compensated for by an elaborate endogenous antioxidant defense system. Excessive generation of free radicals overbalancing the rate of their removal leads to oxidative stress. Oxidative damage at the cellular or subcellular level is now considered to be a major event in disease processes such as cardiovascular diseases, inflammatory diseases, cataract, and cancer. Reactive oxygen radicals are detrimental to cells since they induce lipid peroxidation in cellular membranes, generating lipid peroxides that cause extensive damage to membranes and membrane-mediated chromosomal damage. Oxygen free radicals such as hydrogen peroxide, superoxide anion, and hydroxyl radical have been implicated in mediating various pathological conditions such as ischemia, atherosclerosis, inflammatory diseases, and diabetes. Endothelial cell injury is often the first stage of these disorders. The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), either present intracellular or released into the extracellular milieu, can directly scavenge these oxidants or prevent their conversion to toxic species. ROS and reactive metabolic intermediates generated from various chemical carcinogens are known to play an important role in cell damage and in the initiation and progression of carcinogenesis.

The relationship between diet and chronic diseases, particularly cardiovascular diseases and cancer has been increasingly understood in recent decades. Oxidative stress is now believed to be the primary cause for not only many degenerative diseases, such as cardiovascular diseases, inflammatory diseases, cancer, cataract, type-2 diabetes, neurodegenerative diseases, etc., but also the natural ageing process. Consequently, much scientific curiosity has now been focused on the possible role of natural antioxidants in delaying or suppressing oxidative stress. The need for exogenous antioxidants, dietary or supplementary, to augment the endogenous antioxidant machinery has been well appreciated. Both nutrient and nonnutrient components of the diet have been recognized for their antioxidant properties and consequent potential benefits.

There has been a growing interest in natural antioxidants found abundantly in plants. Apart from green tea, numerous herbs and spices are the most important targets in the search for natural antioxidants from the point of view of safety. A wide variety of phenolic compounds and flavonoids present in spices are now experimentally documented to possess potent antioxidant,

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anti-inflammatory, antimutagenic, and anticarcinogenic activities. The antioxidative and anti-inflammatory properties of these bioactive compounds (Fig. 1) from spices appear to contribute to their chemopreventive or chemoprotective activity. Cyclooxygenase-2 (COX-2) has been recognized as a molecular target of many chemopreventive as well as anti-inflammatory agents. Recent studies have shown that COX-2 is regulated by the eukaryotic transcription factor NF-kappaB. Surh (2002) recently reviewed the molecular mechanisms underlying chemopreventive effects of spice ingredients in terms of their effects on intracellular signaling cascades, particularly those involving NF-kappaB and mitogen-activated protein kinases.

In the context of oxidative stress theory of aging and age-related degenerative diseases, dietary phenolic or thiolic antioxidants have been shown to increase the life span of laboratory animals and protect against senescent immune decline. In view of the limitations of the 'lipid theory' of atherosclerosis and the current suggestion that free-radical-mediated oxidation of cholesterol is a key step in atherogenesis (Duthrie and Brown, 1994), the antioxidant compounds present in spices have gained more importance for their possible role in the prevention of atherogenesis. Although spices have been recognized for medicinal effects and have been in use in traditional systems of medicine for long, their health beneficial attributes have been experimentally verified only in the last three decades. Among the several nutraceutical attributes of common spices, their antioxidant potential has far-reaching health implication. In fact, spices were recognized for their antioxidant potency in food systems about 60 years ago (Ramaswamy and Banerjee, 1948). Nearly four decades lapsed before their role as antioxidants in protecting biological systems against oxidative damage came to be appreciated. This review considers all experimental evidences that document the potential antioxidant property of various spices or their bioactive compounds (Fig. 1) in in-vitro systems and in preventing the oxidative stress in laboratory animals and human subjects. The antioxidative effects of turmeric/curcumin, clove/eugenol, onion, garlic, ginger/gingerol, red pepper/capsaicin, black pepper/piperine, and fenugreek, which have been studied by many research groups and evidenced as potential antioxidants, are specifically dealt with in this treatise.

*TURMERIC (***Curcuma longa***) AND CURCUMIN*

The yellow compound curcumin (Fig. 1) from the rhizome of *Curcuma longa* has been claimed to be a potential antioxidant and anti-inflammatory agent with bioprotective and chemopreventive properties. The antioxidant activity of curcumin and related compounds was first reported by Sharma (1976). Curcumin has been shown to be a good inhibitor of lipid peroxidation by several investigators (Table 1). Inhibition of lipid peroxidation in human erythrocyte membranes in vitro (Salimath et al., 1986), inhibition of ascorbate/Fe^{2+}-induced lipid peroxidation in rat liver microsomes in vitro in a dose-dependent manner (Reddy and Lokesh, 1992), inhibition of superoxide anion generation in xanthine–xanthine oxidase system by 40% at a concentration of 75 μM, inhibition of the generation of hydroxyl radicals by 76% at a concentration of 50 μM, and prevention of the oxidation of Fe^{2+} in the Fenton reaction, which generates hydroxyl radicals (Reddy and Lokesh, 1994a) have been documented.

The other two curcuminoids present in *C. longa*, viz., demethoxycurcumin and bis-demethoxycurcumin (Fig. 1), are also equally effective as curcumin in their ability to inhibit iron-induced lipid peroxidation in rat brain homogenate and rat liver microsomes (Sreejayan and Rao, 1994). This suggests that the methoxy and phenolic groups contribute little to the antioxidant activity. Spectral studies showed that all three curcuminoids could interact with iron. Thus, the inhibition of iron-induced lipid peroxidation by curcuminoids may involve chelation of iron. The natural curcuminoids, curcumin, demethoxycurcumin, and bis-demethoxycurcumin, when compared for their ability to scavenge superoxide radicals and to interact with 1,1-diphenyl-2-picryl-hydrazyl (DPPH)-stable free radicals showed that curcumin is the most potent scavenger of superoxide radicals, followed by demethoxycurcumin and bis-demethoxycurcumin. This indicates that the phenolic group is essential for the free radical scavenging activity of curcumin, and that the presence of a methoxy group further increases the activity (Sreejayan and Rao, 1996).

Curcumin and its derivatives have significant abilities to protect plasmid pBR322 DNA against single-strand breaks induced by singlet oxygen ($^1O_2$), an ROS with potential genotoxic/

Table 1 Antioxidant effects of curcumin in in-vitro systems

In-vitro system	Effect demonstrated	Investigators
Human erythrocyte membranes	Lipid peroxidation was inhibited by curcumin.	Salimath et al. (1986)
Rat liver microsomes	Ascorbate-Fe^{++}-induced lipid peroxidation was inhibited by curcumin.	Reddy and Lokesh (1992)
Xanthine–xanthine oxidase system	Curcumin inhibited superoxide anion and OH radical generation.	Reddy and Lokesh (1994a)
Rat macrophages	Incubation with curcumin inhibited SO anions, hydrogen peroxide, and nitrite radical production.	Joe and Lokesh (1994)
Plasmid DNA	Curcumin diminished singlet oxygen-induced DNA strand damage.	Subramanian et al. (1994)
Rat liver microsomes and brain homogenate	Fe^{++}-induced lipid peroxidation was inhibited by curcumin and this involved chelation with iron.	Sreejayan and Rao (1994)
In vitro	Curcumin (demethoxy curcumin, bis-demethoxy curcumin) showed potent ability to scavenge superoxides and interact with DPPH radical.	Sreejayan and Rao (1996)
In vitro	Curcumin is a scavenger of nitric oxide.	Sreejayan and Rao (1997)
Human LDL	Inhibition of Cu^{2+}-induced lipid peroxidation by curcumin.	Naidu and Thippeswamy (2002)
Human PMNL cells	Aqueous extract of turmeric and curcumin inhibited 5-LO activity.	Prasad et al. (2004)

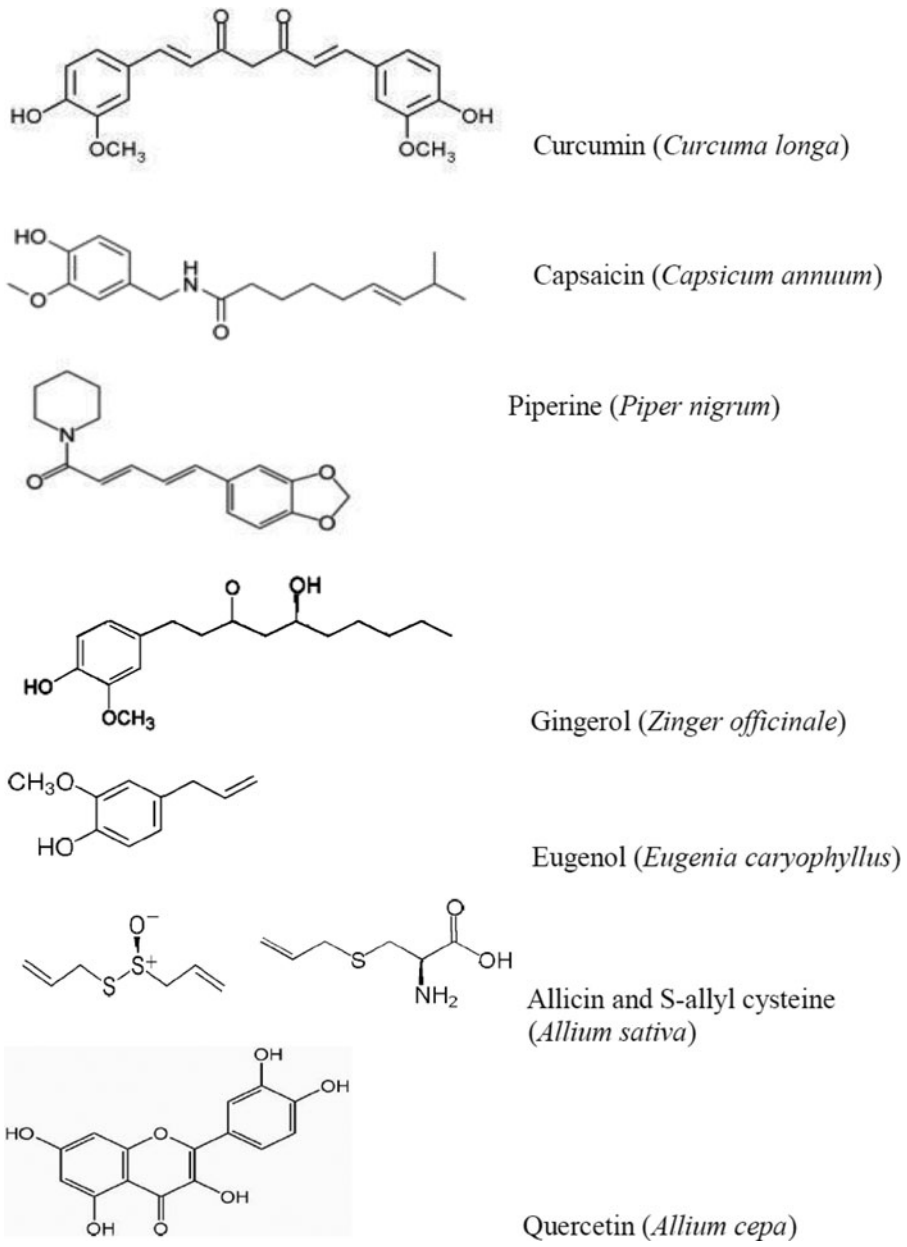

Figure 1 Structure of spice bioactive compounds.

mutagenic properties (Subramanian et al., 1994). Among the curcuminoids, curcumin was the most effective inhibitor of DNA damage, followed by demethoxycurcumin and bis-demethoxy-curcumin. The observed antioxidant activity, which was both time- and concentration-dependent, was higher than that of the well-known antioxidants lipoate, α-tocopherol, and β-carotene. The ability of curcumin to protect DNA against oxygen free radical seems to be related to its structure and may at least partly explain the therapeutic and other beneficial effects of curcumin, including its antimutagenic and anticarcinogenic properties.

The antioxidant properties of turmeric and curcumin have been evidenced in animal studies (Reddy and Lokesh, 1994b, 1994c, 1994d) (Table 2). Iron-induced lipid peroxidation (30 mg Fe^{2+}/kg, i.p.) was significantly lower in the liver of turmeric-fed (1% in the diet for 10 week) rats (Reddy and Lokesh, 1994b). The activities of SOD, CAT, and GPX were higher in the liver of turmeric-fed rats, suggesting that dietary turmeric lowers lipid peroxidation by enhancing the activities of antioxidant enzymes. Dietary curcumin (1%) reduced lipid peroxidation in serum and liver of rats fed on unsaturated lipids: peanut oil or cod-liver oil (Reddy and Lokesh, 1994c). Dietary curcumin (1%) significantly enhanced the activities of antioxidant enzymes, SOD, CAT, GPX, and glutathione-S-transferase (GST), and lowered lipid peroxides in liver of rats fed on coconut oil, peanut oil, or cod-liver oil (Reddy and Lokesh, 1994d). Thus, curcumin

Table 2 Antioxidant effects of turmeric and curcumin in in-vivo situation

Animal model	Effect demonstrated	Investigators
Mice	Orally given curcumin lowered the increased peroxidation of lipids in tissues produced by CCl_4, paraquat, and cyclophosphamide.	Soudamini et al. (1992)
Rats	Curcumin suppressed CCl_4-induced and ^{60}Co radiation-induced lipid peroxidation.	Nishigaki et al. (1992)
Rats/Fe^{2+}-induced lipid peroxidation	Dietary turmeric decreased liver lipid peroxides and enhanced activities of liver AO enzymes.	Reddy and Lokesh (1994b)
Rats fed unsaturated fat	Dietary curcumin (1.0% for 8 weeks) decreased serum and liver lipid peroxides.	Reddy and Lokesh (1994c)
Rats fed unsaturated fat	Dietary curcumin enhanced activities of antioxidant enzymes and lowered lipid peroxides.	Reddy and Lokesh (1994d)
High-fat fed rats	Beneficial influence of dietary curcumin on antioxidant status of red blood cells.	Kempaiah and Srinivasan (2004a)
High-fat fed rats	Beneficial influence of dietary curcumin on antioxidant status of blood and liver.	Manjunatha and Srinivasan (2007a)
Hypercholesterolemic rats	Beneficial influence of dietary curcumin on antioxidant status of red blood cells	Kempaiah and Srinivasan (2004b)
Hypercholesterolemic rats	Beneficial influence of dietary curcumin on antioxidant status of blood and liver	Manjunatha and Srinivasan (2007b)
Rats given irradiation	Orally given curcumin reduced lipid peroxidation in serum and liver that was increased by whole-body irradiation.	Thresiamma et al. (1996)
Hepatoprotective effect		
Rats/Fe^{2+}-induced hepatotoxicity	Oral administration of curcumin (30 mg/kg for 10 days) lowered lipid peroxides in blood and liver.	Reddy and Lokesh (1996)
Rats/Fe^{2+}-induced hepatotoxicity	Dietary curcumin (0.2% for 8 weeks) lowered liver lipid peroxides and serum enzymes.	Manjunatha and Srinivasan (2006)
Mice	Preadministration of curcumin suppressed trichloroethylene-induced oxidative stress.	Watanabe and Fukui (2000)
Rats	Curcumin treatment reduced oxidative stress induced by alcohol.	Rukkumani et al. (2004)
Chloroquine-treated rat	Daily administration of curcumin reduced lipid peroxides in induced hepatotoxicity.	Pari and Amali (2005)
Antiatherogenic and cardioprotective effect		
Human volunteers	Daily administration of curcuminoids (0.5 g) reduced serum lipid peroxides.	Soni and Kuttan (1992)
Isoproterenol-treated rat	Oral curcumin countered lipid peroxidation accompanying myocardial infarction.	Nirmala and Puvanakrishnan (1996)
Atherosclerotic rabbits	Oral *C. longa* extract decreased susceptibility LDL to lipid peroxidation.	Ramirez-Tortosa et al. (1999)
Human volunteers	Daily intake of *C. longa* extract decreased blood lipid peroxides and LDL lipid peroxidation.	Miquel et al. (2002)
High-cholesterol-fed rabbits	*C. longa* reduces oxidative stress during the development of atherosclerosis.	Quiles et al. (2002)
Isoprenaline-treated rat	Oral curcumin reduced oxidative stress during myocardial ischemia.	Manikandan et al. (2004)
Oxidative stress in diabetes		
Streptozotocin-induced diabetic rats	Dietary curcumin lowered lipid peroxides in plasma and urine.	Babu and Srinivasan (1995); Majithiya and Balaraman (2005)
Streptozotocin-induced diabetic rats	Dietary photoirradiated curcumin countered decreased enzymic/nonenzymic antioxidants.	Mahesh et al. (2004); Mahesh et al. (2005)
Antioxidant effect in experimentally induced cataract		
Rats	Naphthalene-induced opacification of eye lens lowered by curcumin treatment due to attenuation of apoptosis.	Pandya et al. (2000)
Rats	Curcumin treatment with selenium prevented oxidative damage in eye lens and delayed development of cataract.	Padmaja and Raju (2004)
Streptozotocin-induced diabetic rats	Dietary curcumin/turmeric countered oxidative stress in eye lens and progression of cataract.	Suryanarayana et al. (2005)
Rats	Dietary curcumin countered oxidative stress in eye lens and progression of cataract.	Suryanarayana et al. (2003)

(Continued on next page)

Table 2 Antioxidant effects of turmeric and curcumin in in-vivo situation *(Continued)*

Animal model	Effect demonstrated	Investigators
Antioxidant effect in nicotine-induced lung toxicity		
Nicotine administered	Curcumin lowered lipid peroxidation and enhanced antioxidant status	Kalpana and Menon (2004a), (2004b), (2004c)
Renal protective effect		
Rats	Curcumin given s.c. reduced oxidative stress during cyclosporine-induced renal toxicity.	Tirkey et al. (2005)
Neuroprotective effect		
Rats	Protective effect of curcumin against lead-induced neurotoxicity	Shukla et al. (2003)
Anticancer potential through antioxidant property		
Mice	Dietary curcumin increased activities of antioxidant enzymes and phase II drug-metabolizing enzymes.	Iqbal et al. (2003)
Mice	Curcumin showed antitumor-initiating effect in skin tumorigenesis promoted by peroxynitrite.	Nishino et al. (2004)

inhibits lipid peroxidation possibly by quenching oxygen free radicals, as inferred by in-vitro studies, and by enhancing the activity of endogenous antioxidant enzymes (Reddy and Lokesh, 1994d).

Oral administration of curcumin significantly lowered the lipid peroxidation in liver, lungs, kidneys, and brain tissues, induced by carbon tetrachloride (CCl_4), paraquat, and cyclophosphamide in mice (Soudamini et al., 1992). Administration of curcumin was also found to lower the serum and tissue cholesterol levels in these animals, indicating that curcumin helps in conditions associated with peroxide-induced injury, such as liver damage and arterial diseases. Curcumin has also been found to suppress lipid peroxidation induced in rats by CCl_4 or ^{60}Co radiation (Nishigaki et al., 1992). Oral administration of curcumin (200 μmol/kg) significantly reduced lipid peroxidation in serum and liver of rats that was increased by whole-body irradiation (Thresiamma et al., 1996).

ROS generated by activated macrophages play an important role in the initiation of inflammation. Curcumin, with known anti-inflammatory properties, has been tested for its effect on the generation of superoxide anions, hydrogen peroxide, and nitrite radical by activated rat peritoneal macrophages. Preincubation of macrophages with 10 μM curcumin completely inhibited the superoxide anions, hydrogen peroxide, and nitrite radical production in vitro by macrophages (Joe and Lokesh, 1994). The peritoneal macrophages isolated from animals fed on curcumin (by gavage for 2 weeks) produced lesser ROS compared with the macrophages from the untreated group. Curcumin, a compound with anti-inflammatory and anticancer activity, inhibited induction of nitric oxide synthase in activated macrophages (Brouet and Ohshima, 1995) and has been shown to be a potent scavenger of free radicals. Curcumin is evidenced to be a scavenger of nitric oxide too, wherein it reduced the amount of nitrite formed by the reaction between oxygen and nitric oxide generated from sodium nitroprusside (Sreejayan and Rao, 1997). Demethoxycurcumin and bis-demethoxycurcumin were as active as curcumin, indicating that the methoxy and phenolic groups are not essential for the free-radical-scavenging activity. The therapeutic properties of curcumin implicated in inflammation and cancer might be at least partly explained by its free-radical-scavenging property, including those toward nitric oxide.

Polymorphonuclear leukocytes (PMNL) play an important role in the modulation of inflammatory conditions in humans. PMNL cells recruited at the site of inflammation release inflammatory mediators such as leukotrienes, proteolytic enzymes, and ROS. Among these, leukotrienes are implicated in the pathophysiology of allergic and inflammatory disorders such as asthma, allergic rhinitis, arthritis, inflammatory bowel disease, and psoriasis. 5-lipoxygenase (5-LO) is the key enzyme in the biosynthetic pathway of leukotrienes. Prasad et al. (2004) examined 5-LO, the key enzyme involved in biosynthesis of leukotrienes, as a possible target for antioxidant spice compounds. Curcumin inhibited the formation of 5-LO product, 5-hydroperoxy eicosatetraenoic acid, in human PMNL in a concentration-dependent manner. The inhibitory effect of curcumin was similar to that of synthetic 5-LO inhibitor, phenidone, while the inhibitory potency of aqueous extracts of turmeric correlated with that of curcumin.

Dietary curcumin (0.2%), which produced the hypotriglyceridemic effect in rats fed on a high-fat diet, was also effective in reducing the oxidative stress, which was indicated by a significant countering of the depleted intracellular antioxidants, total thiols and GSH, and elevated lipid peroxides in erythrocytes (Kempaiah and Srinivasan, 2004a). The elevated lipid peroxide in blood plasma and the severely depleted hepatic GSH in high-fat treatment were also effectively reversed by dietary curcumin. The beneficial influence of dietary curcumin on the antioxidant status of red blood cells and liver in induced hypercholesterolemic rats has also been evidenced (Kempaiah and Srinivasan, 2004b). Depletion in intracellular thiols and GSH in red blood cells under hypercholesterolemic situation was effectively countered by dietary (0.2%) curcumin. Decreased hepatic total thiols and lowered activities of hepatic antioxidant enzymes, GR, GST, CAT, and SOD, in hypercholesterolemic rats were effectively countered by dietary curcumin.

Antiatherogenic and Cardioprotective Effect

Daily administration of curcuminoids (0.5 g) to healthy human volunteers produced 33% reduction in blood lipid peroxide levels (Soni and Kuttan, 1992). This was accompanied by an increase in HDL cholesterol and a decrease in total serum cholesterol as a result of curcumin administration (500 mg/day for 7 days) (Quiles et al., 2002). The reduction in serum lipid peroxides and cholesterol suggests the potential of curcumin as a chemopreventive substance against arterial diseases. The effect of a *C. longa* extract was evaluated on the development of experimental atherosclerosis (fatty streak) in rabbits and its interaction with other plasmatic antioxidants (Quiles et al., 2002). Supplementation with *C. longa* reduced oxidative stress (reduced plasma lipid peroxides and restored α-tocopherol and coenzyme Q levels at 20 and 30 days, respectively) and attenuated the development of fatty streaks in rabbits fed on a high-cholesterol diet.

Oxidation of LDL plays an important role in the development of atherosclerosis. The effect of ethanol aqueous extract of *C. longa* on the susceptibility of LDL to oxidation and on plasma lipids was evaluated in atherosclerotic rabbits (Ramirez-Tortosa et al., 1999). *C. longa* extract decreased the susceptibility of LDL to lipid peroxidation, thus suggesting its value in the management of cardiovascular diseases. The effect of phenolic and nonphenolic compounds of common spices, including curcumin, on copper-induced lipid peroxidation of human LDL was evidenced by measuring the formation of TBARS and relative electrophoretic mobility of LDL on agarose gel (Naidu and Thippeswamy, 2002). Curcumin effectively inhibited the formation of TBARS throughout the 12-hour incubation period and decreased the relative electrophoretic mobility of LDL. Curcumin at 10 μM produced 40–85% inhibition of LDL oxidation, and the inhibitory effect of curcumin was comparable to that of BHA, but relatively more potent than ascorbic acid. These data suggest that curcumin offers protection against oxidation of human LDL. In healthy humans, the daily intake of 200 mg of *C. longa* extract resulted in a decrease in total blood lipid peroxides, as well as in HDL and LDL lipid peroxidation (Miquel et al., 2002). This antiatherogenic effect was accompanied by a curcuma antioxidant-induced normalization of the plasma levels of fibrinogen and of the apo-B/apo-A ratio, thus decreasing the cardiovascular risk. The beneficial influence of dietary curcumin on the susceptibility of LDL to oxidation was examined in an animal study. Dietary curcumin significantly inhibited the in-vivo iron-induced LDL oxidation as well as copper-induced oxidation of LDL in vitro (Manjunatha and Srinivasan, 2006).

Orally administered curcumin effectively countered the biochemical changes accompanying myocardial infarction induced by *iso*-proterenol in rats (Nirmala and Puvanakrishnan, 1996). Myocardial infarction was accompanied by the disintegration of membrane PUFA expressed by increase in lipid peroxides and decreased levels of SOD, CAT, GPX, ceruloplasmin, α-tocopherol, GSH, and ascorbic acid. Oral pretreatment with curcumin two days before and during isoproterenol administration decreased the effect of lipid peroxidation. The protective effect of curcumin (15 mg/kg, administered 30 minutes before and/or after onset of ischemia) against isoprenaline-induced myocardial ischaemia was investigated by assessing oxidative-stress-related biochemical parameters in rat myocardium (Manikandan et al., 2004). Curcumin pre- and post-treatment decreased the levels of xanthine oxidase, superoxide anion, lipid peroxides, and myeloperoxidase, while the levels of SOD, CAT, GPX, and GST activities were significantly increased after curcumin post-treatment. Thus, curcumin was found to protect rat myocardium against ischemic insult and the protective effect could be attributed to its antioxidant properties as well as to its inhibitory effects on xanthine dehydrogenase/xanthine oxidase conversion and the resultant superoxide anion production.

Hepatoprotective Effect

Oral administration of curcumin (30 mg/kg) to Wistar rats for 10 days lowered the liver and serum lipid peroxide levels and activities of serum ALAT, ASAT, and LDH, which were enhanced by i.p. injection of iron (30 mg Fe^{2+}/kg) (Reddy and Lokesh, 1996). This study indicated that curcumin reduces the iron-induced hepatic damage by lowering lipid peroxidation. Oral administration of tetrahydrocurcumin (THC), a metabolite of curcumin showed hepatoprotective effect in Wistar rats against erythromycin estolate (EME)-induced toxicity (Pari and Murugan, 2004), as indicated by countering of the increased level of serum enzymes (ASAT, ALAT, and alkaline phosphatase), bilirubin, cholesterol, triglycerides, phospholipids, free fatty acids and plasma hydroperoxides and TBARS. The antioxidant effect of THC against EME-induced (800 mg/kg) lipid peroxidation has also been evidenced in rats (Murugan and Pari, 2005), where oral administration of THC (80 mg/kg for 15 days) significantly decreased lipid peroxidation and enhanced cellular antioxidant defenses when compared with the group treated with EME alone. The effect of THC and curcumin against chloroquine (CQ)-induced hepatotoxicity has been studied in Wistar rats (Pari and Amali, 2005). Administration of THC or curcumin (80 mg/kg) for 8 days before and 7 days after single administration of CQ (970 mg/kg) significantly decreased the activities of serum enzymes (ASAT, ASAT, and alkaline phosphatase) and lipids. TBARS and hydroperoxides were also significantly decreased with concomitant increase in nonenzymic (vitamin C, vitamin E, and GSH) and enzymic antioxidants (SOD, CAT, and GPX) on treatment with THC and curcumin. THC showed more pronounced protective effect than curcumin against CQ-induced hepatotoxicity. Dietary curcumin reduced the activities of serum enzymes ASAT, ALAT, and alkaline phosphatase, and lowered liver lipid peroxide level in iron-injected rats, indicating amelioration of the severity of iron-induced hepatotoxicity (Manjunatha and Srinivasan, 2006).

In-vivo antioxidative effects of curcumin were investigated using a trichloroethylene (TCE)-induced oxidative stress in mouse liver (Watanabe and Fukui, 2000). Increase in the contents of peroxisomes and TBARS and decreases in GSH content

in the liver by TCE administration were suppressed by the preadministration of curcumin. TCE-induced changes in the activities of antioxidative enzymes: SOD, CAT, and glutathione reductase (GR). GPX and glucose-6-phosphate dehydrogenase (G6PD) were also diminished by curcumin. Thus, curcumin suppresses TCE-induced oxidative stress by scavenging free radicals, and its antioxidative activity seems to be derived from its suppressive effects on the increase in peroxisome content and decrease in GPX and G6PD activities.

Alcoholic liver disease is a major medical complication of alcohol abuse. Increasing evidence demonstrates that oxidative stress plays an important etiologic role in the development of alcoholic liver disease. Alcohol alone or in combination with a high-fat diet is known to cause oxidative injury. The protective role of curcumin on alcohol and thermally oxidized sunflower-oil (δPUFA)-induced oxidative stress was evaluated in Wistar rats (Rukkumani et al., 2004). Administration of curcumin abrogated the increased liver marker enzymes (γ-glutamyl transferase and alkaline phosphatase) and lipid peroxidative indices (TBARS and hydroperoxides and antioxidants—vitamin C, vitamin E, GSH, SOD, CAT, and GPX) in alcohol and δPUFA groups. The antioxidant status, which was decreased in alcohol and δPUFA groups, was effectively modulated by curcumin treatment. Thus, curcumin exerts its protective effect by decreasing lipid peroxidation and improving the antioxidant status.

Amelioration of Oxidative Stress in Diabetes

Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of diabetes mellitus. There is evidence for increased levels of circulating ROS in diabetic patients, as inferred by the increased lipid peroxidation. Direct measurements of intracellular generation of ROS also demonstrate an association of oxidative stress with diabetes. Streptozotocin-induced diabetic rats maintained on a 0.5% curcumin diet for 8 weeks showed lowered lipid peroxidation in plasma and urine when compared with a control diabetic group (Babu and Srinivasan, 1995). The study also revealed that curcumin feeding improved the metabolic status in diabetic conditions, despite no effect on the hyperglycemic status. The hypocholesterolemic influence, the antioxidant and free- radical-scavenging property of curcumin, was suggested to be the mechanism by which curcumin improves health in this situation. The effect of chronic curcumin treatment (200 mg/kg) on the oxidative stress in streptozotocin-induced diabetic rats was studied at 4-week intervals up to 24 weeks (Majithiya and Balaraman, 2005). Curcumin treatment significantly reduced lipid peroxidation but had no significant effect on the reduced levels of SOD, CAT, and GSH. The inability of curcumin to prevent oxidative stress during the late stage may be due to the excessive production of free radicals during chronic diabetes.

Photo-irradiated curcumin (10, 30, and 80 mg/kg) has been evaluated for the antioxidant and antihyperglycemic effect in streptozotocin-induced diabetic rats (Mahesh et al., 2004). Oral administration of photo-irradiated curcumin for 45 days resulted in a significant decrease in the elevated levels of blood glucose, together with near-normalization of the decreased enzymic and nonenzymic antioxidants and the markers of lipid peroxidation in the liver, kidneys, and brain. In another similar animal study, the antioxidant status, as revealed by circulatory lipid peroxidation, vitamin C, vitamin E, and enzymic antioxidants such as SOD and CAT, decreased in streptozotocin-diabetic animals (Mahesh et al., 2005). Oral administration of photo-irradiated curcumin (10 or 30 mg/kg) for 45 days ameliorated hyperglycemia, along with near-normalization of the antioxidant enzyme activities and the levels of lipid peroxidative markers. The antioxidant effect of curcumin as a function of changes in cellular ROS generation, tested in cells from diabetic subjects, clearly demonstrated that curcumin prevented both phorbol-12 myristate-13 acetate- and thapsigargin-induced ROS generation (Balasubramanyam et al., 2003). The dose-dependent ROS inhibitory effect of curcumin suggests that curcumin interferes with protein kinase C and calcium regulation. Simultaneous measurements of ROS and Ca^{2+} influx suggest that a rise in cytosolic Ca^{2+} may be a trigger for increased ROS generation. It is suggested that the antioxidant and antiangiogenic actions of curcumin, as a mechanism of inhibition of Ca^{2+} entry and protein kinase C activity, may be further exploited for the treatment of diabetic retinopathy and other diabetic complications.

Antioxidant Effect in Experimentally Induced Cataract

Oxidative stress has been implicated in the mechanism of naphthalene-induced cataract, a model for senile cataract. A study that examined the preventive role of dietary curcumin on naphthalene-induced opacification of rat lens demonstrated that the naphthalene-treated rats kept on a diet supplemented with 0.005% curcumin had significantly less opacification of lenses (Pandya et al., 2000). This study demonstrated that naphthalene-initiated cataract in lens is accompanied by apoptosis of lens epithelial cells and that curcumin attenuates this apoptotic effect of naphthalene. Wistar rat pups treated with curcumin administered along with selenium showed no opacities in the lens (Padmaja and Raju, 2004). Lipid peroxidation and xanthine oxidase levels in the lenses of curcumin and selenium co-treated animals were significantly less than in selenium-treated animals. Enhanced activities of SOD and CAT were seen in the eye lens of curcumin and selenium co-treated animals. Curcumin co-treatment seemed to prevent oxidative damage and delay the development of cataract.

Dietary curcumin (0.002% and 0.01%) and turmeric (0.5%) were found to be effective against development of diabetic cataract in streptozotocin-induced diabetic rats (Suryanarayana et al., 2005). Progression of cataract due to hyperglycemia at the end of 8 weeks and the biochemical parameters involved in the pathogenesis of cataract, such as oxidative stress, polyol pathway, alterations in protein content, and crystallin profile in the lens, were investigated. Curcumin and turmeric supplements delayed the progression and maturation of cataract by countering the hyperglycemia-induced oxidative stress, as indicated by

reversal of changes with respect to lipid peroxidation, GSH, protein carbonyl content, and activities of antioxidant enzymes. Turmeric or curcumin minimized osmotic stress, as assessed by polyol pathway enzymes. Aggregation and insolubilization of lens proteins due to hyperglycemia was prevented by turmeric and curcumin. Dietary curcumin (0.002%) was effective against the onset and maturation of galactose-induced cataract in rats (Suryanarayana et al., 2003). Cataract progression due to galactose feeding (30% in the diet) and biochemical parameters, lipid peroxidation, polyol pathway enzymes, GSH, protein carbonyls, advanced glycation endproducts, protein oxidation, and crystallin profile, were measured in the lens. Curcumin at 0.002% (for 4 weeks) delayed the onset and maturation of cataract, by exerting antioxidant and antiglycating effects, as it inhibited lipid peroxidation and protein aggregation.

Antioxidant Effect in Nicotine-Induced Lung Toxicity

Nicotine, the major toxic component of cigarette smoke has been identified as the main risk factor for lung diseases. The effect of curcumin on blood antioxidant status during nicotine-induced toxicity was evaluated in Wistar rats (Kalpana and Menon, 2004a). Lung toxicity was induced by nicotine injection (2.5 mg/kg, s.c.; 5 days a week, for 22 weeks). The enhanced circulatory lipid peroxides in nicotine-treated rats was accompanied by a significant decrease in the levels of ascorbic acid, vitamin E, GSH, GPX, SOD, and CAT. There was a reduction in the levels of zinc, with an elevation in copper and ferritin levels in nicotine-treated rats. Administration of curcumin significantly lowered lipid peroxidation and enhanced the antioxidant status by modulating the levels of zinc, copper, and ferritin. In another study, the protective effect of curcumin on tissue lipid peroxidation and antioxidants was evaluated in nicotine-treated Wistar rats (Kalpana and Menon, 2004b). Curcumin (80 mg/kg) was orally administered simultaneously for 22 weeks. Curcumin lowered the level of nicotine-induced lipid peroxidation and enhanced the antioxidant status by countering the decrease in the levels of ascorbic acid, vitamin E, GSH, GPX, SOD, and CAT. This suggested that curcumin exerts its protective effect against nicotine-induced lung toxicity by modulating the extent of lipid peroxidation and augmenting the antioxidant defense system. The protective effects of curcumin on lipid peroxidation and the antioxidant status were evaluated in bronchoalveolar lavage fluid and bronchoalveolar lavage of rats with nicotine-induced (2.5 mg/kg for 22 weeks) lung toxicity (Kalpana and Menon, 2004c). Curcumin was given orally (80 mg/kg) simultaneously for 22 weeks. Curcumin significantly lowered the marker enzymes (alkaline phosphatase and LDH), decreased lipid peroxidation, and enhanced the antioxidant status (GSH, GPX, SOD, and CAT), suggesting that it exerts its protective effect against nicotine-induced lung toxicity by modulating the biochemical marker enzymes and lipid peroxidation, and by augmenting the antioxidant defense system.

Renal Protective Effect

The beneficial effect of curcumin in preventing acute renal failure and related oxidative stress caused by chronic administration of cyclosporine (CsA) was examined in rats (Tirkey et al., 2005). Curcumin administered concurrently with CsA (20 mg/kg/day s.c.) for 21 days effectively attenuated CsA-induced nephrotoxicity through its antioxidant activity, as revealed by markedly countered elevated levels of TBARS, attenuated renal dysfunction, increased the levels of antioxidant enzymes in CsA-treated rats, and normalized the altered renal morphology.

Neuroprotective Potential

Free-radical-induced neuronal damage is implicated in cerebral ischemia reperfusion (IR) injury and antioxidants are reported to have neuroprotective activity. The neuroprotective potential of curcumin investigated in middle-cerebral-artery-occlusion-induced focal cerebral IR injury suggested the neuroprotective potential of curcumin in cerebral ischemia, which is mediated through its antioxidant activity (Thiyagarajan and Sharma, 2004). Curcumin treatment (300 mg/kg, i.p.) prevented IR-injury-mediated fall in GPX activity and elevation in lipid peroxidation. The protective effect of curcumin against lead-induced neurotoxicity has been evidenced in rats (Shukla et al., 2003). Co-treatment with curcumin (100 mg/kg, p.o.) and lead (50 mg/kg, p.o) for 45 days caused a significant decrease in the elevated LPO with concomitant decrease in lead levels in all the brain regions. It also significantly reversed the decrease in GSH levels, and SOD and CAT activities in all the four brain regions in rats.

Effectiveness in Wound Healing

Turmeric/curcumin are potential agents for wound healing. The therapeutic mechanism of wound healing was studied by examining the antioxidant effects of curcumin on hydrogen peroxide and hypoxanthine–xanthine-oxidase-induced damage to cultured human keratinocytes and fibroblasts (Phan et al., 2001). Exposure of human keratinocytes and human dermal fibroblasts to curcumin at 10 and 2.5 μg/mL respectively, showed significant protective effect against hydrogen peroxide. No protective effects of curcumin on either fibroblasts or keratinocytes against hypoxanthine–xanthine-oxidase-induced damage were found.

Anticancer Potential Through Antioxidant Property

Dietary antioxidants protect laboratory animals against the induction of tumors by a variety of chemical carcinogens. One of the possible mechanisms of. antitumor properties could be through protection against chemical carcinogenesis via antioxidant-dependent induction of detoxifying enzymes. The

effect of dietary curcumin on the activities of antioxidant and phase II drug-metabolizing enzymes involved in detoxification and production of ROS was evaluated in mice (Iqbal et al., 2003). Dietary curcumin (2% for 30 days) significantly increased the activities of GPX, GR, G6PD, and CAT in liver and kidneys. Curcumin feeding also enhanced the activity of phase II drug-metabolizing enzymes, viz., GST and quinone reductase, in liver and kidneys. Induction of detoxifying enzymes by curcumin suggests the potential of this compound as a protective agent against chemical carcinogenesis and other forms of electrophilic toxicity.

Incidence of cancer at different sites may be related to the oxidative damage to host genome by genotoxicants. The nonnutritive dietary constituents that possess antimutagenic property appear to be promising chemopreventive agents. The role of nitric oxide (NO) in carcinogenesis has been pointed out, since large quantity of NO has been detected in cancer tissues, and the expression of inducible NO synthase (iNOS) was found to correlate with tumor growth and metastasis. NO possesses tumor-initiating activity in mouse skin carcinogenesis. It has been suggested that pathological effects induced by NO may partly depend on peroxynitrite, an active metabolite of NO. It was found that while treatment with peroxynitrite (initiator) plus TPA (promoter) resulted in the formation of skin tumors, curcumin showed antitumor-initiating effect (Nishino et al., 2004). Curcumin, endowed with antioxidant and other cytoprotective properties, has been reported to reduce nitrite formation during NO oxidation in solution. This decrease in nitrite production was attributed to the direct sequestration of NO by curcumin (Johnston and DeMaster, 2003).

Bis-demethoxycurcumin was found to be the most active among curcuminoids of turmeric in the inhibition of Ehrlich ascites tumors in mice (Ruby et al., 1995). These compounds were also checked for their antioxidant activity, which possibly indicates their potential use as antipromoters. The amount of curcuminoids (curcumin, demethoxycurcumin, and bis-demethoxy-curcumin) needed for 50% inhibition of lipid peroxidation was 20, 14, and 11 μM; for 50% inhibition of superoxides was 6.25, 4.25, and 1.9 μM; and for 50% inhibition of hydroxyl radical was 2.3, 1.8, and 1.8 μM, respectively. The ability of these compounds to suppress the superoxide production by macrophages activated with phorbol-12-myristate-13 acetate indicated that all the three curcuminoids inhibited superoxide production, and bis-demethoxycurcumin produced the maximum effect.

Elevated level of methylglyoxal (MG), a reactive dicarbonyl compound endogenously produced in diabetic patients, is believed to contribute to diabetic complications as it is cytotoxic through induction of apoptosis. The effect of curcumin on MG-induced apoptotic events was investigated in human hepatoma G2 cells (Chan et al., 2005). It was observed that curcumin prevented MG-induced cell death and apoptotic biochemical changes, such as mitochondrial release of cytochrome C, caspase-3 activation, and cleavage of poly [ADP-ribose] polymerase. Curcumin prevented MG-stimulated intracellular oxidative stress, as indicated by attenuation of ROS generation, ROS being responsible for triggering cytochrome C release, caspase activation, and subsequent apoptotic biochemical changes. Curcumin inhibited the MG-induced DNA fragmentation, along with the inhibition of the MG-stimulated increase in ROS in ESC-B5 cells in vitro (Hsuuw et al., 2005). In addition, curcumin prevented the MG-induced apoptosis of mouse blastocysts isolated from pregnant mice. The results support the hypothesis that curcumin inhibits MG-induced apoptosis in mouse ESC-B5 cells and blastocysts by blocking ROS formation and subsequent apoptotic biochemical events.

Thus, the antioxidant properties curcumin explains the diverse pharmacological potential of this phytochemical or the parent spice—turmeric (Fig. 2). The antioxidant property of curcumin involves its potential to scavenge free radicals, to inhibit the iNOS activity, and enhance the activities of endogenous antioxidant enzymes. As a result of all these, lipid peroxidation is lowered. The antioxidant property is also implicated in cancer chemopreventive effects of curcumin against the induction of tumors in various target organs.

*CLOVE (***Eugenia caryophyllus***) AND EUGENOL*

The antioxidant potency of eugenol, the principal flavor compound of clove (Fig. 1), was first recognized by the inhibition of copper-induced lipid peroxidation in human erythrocyte membranes (Nagashima, 1989) (Table 3). An in-vitro study has shown that eugenol inhibits liver microsomal monooxygenase activities and CCl_4-induced lipid peroxidation (Nagababu and Lakshmaiah, 1994). Eugenol effectively inhibited the generation of ROS in model systems (Reddy and Lokesh, 1994a). Eugenol inhibited superoxide anion generation in xanthine–xanthine oxidase system, to an extent of 50% at a concentration of 250 μM, and the generation of hydroxyl radicals, to an extent of 70%, as measured by deoxyribose degradation. The hydroxyl radical formation measured by the hydroxylation of salicylate to 2,3-dihydroxy benzoate was inhibited to an extent of 46% at 250 μM. Eugenol also prevented the oxidation of Fe^{2+} in the Fenton reaction, which generates hydroxyl radicals. Eugenol (25–150 μM) inhibited ascorbate/Fe^{2+}-induced lipid peroxidation in rat liver microsomes in vitro in a dose-dependent manner (Reddy and Lokesh, 1992). While investigating the effect of spice principles on scavenging of superoxide anions, as measured by nitrobluetetrazolium reduction in xanthine–xanthine oxidase system, superoxide anions were observed to be inhibited by eugenol in a dose-dependent manner, with a K_i value of 64 μM (Krishnakantha and Lokesh, 1993). Spice principles with known anti-inflammatory properties have been tested for their effect on the generation of superoxide anions, H_2O_2, and nitrite radical by activated rat peritoneal macrophages (Joe and Lokesh, 1994). Preincubation of macrophages with 500 μM eugenol completely inhibited the superoxide anions and hydrogen peroxide release by macrophages.

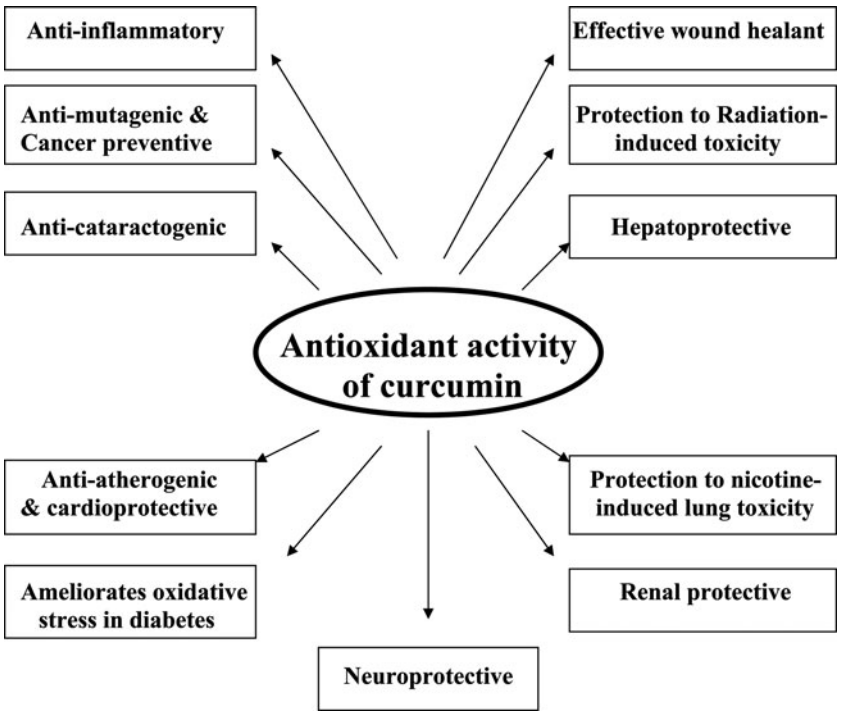

Figure 2 Diverse pharmacological activities of curcumin mediated by antioxidant potential.

Evaluation of inhibitory effects of eugenol on tobacco-induced mutagenesis using Ames *Salmonella*/microsome assay revealed inhibition at concentrations of 0.5 and 1 mg/plate (Sukumaran and Kuttan, 1995). Eugenol also inhibited the nitrosation of methylurea and enzymatic lipid peroxidation catalyzed by soybean lipoxygenase in an in-vitro system in a dose-dependent manner (Naidu, 1995). Enzyme kinetic studies showed that eugenol noncompetitively inhibited lipid peroxidation. The inhibitory mechanism implies that eugenol does not inactivate the enzyme directly but may interfere with fatty acid radical intermediates due to its hydroxyl-radical-scavenging ability and thus play a role in inhibiting the propagation of lipid peroxidation. In in-vitro models of peroxynitrite-induced nitration and lipid peroxidation, eugenol decreased 3-nitrotyrosine formation, with IC_{50} value of 46.7 μM, and also inhibited the peroxynitrite-induced lipid peroxidation, with IC_{50} value of 13.1 μM (Chericoni et al., 2005). It is believed that eugenol might inhibit lipid peroxidation at the stage of

Table 3 Antioxidant influence of eugenol in in-vitro systems

Animal model/In-vitro system	Effect demonstrated	Investigators
Human erythrocyte membrane	Inhibition of Cu^{++}-induced lipid peroxidation.	Nagashima (1989)
Ames *Salmonella*/microsome assay	Inhibitory effects of eugenol on tobacco-induced mutagenesis.	Sukumaran and Kuttan (1995)
Rat liver microsomes	Ascorbate-Fe^{++}-induced lipid peroxidation was inhibited by eugenol.	Reddy and Lokesh (1992)
ROS generation in xanthine–xanthine oxidase system	Inhibition of generation of reactive oxygen species by 250 μM eugenol; prevention of oxidation of Fe^{2+} in the Fenton reaction, which generates OH radicals.	Reddy and Lokesh (1994a)
In-vitro study	Eugenol inhibited liver microsomal monooxygenase-activities CCl_4-induced lipid peroxidation.	Nagababu and Lakshmaiah (1994)
Soybean LO-catalyzed lipid peroxidation	Noncompetitive inhibition of enzymatic lipid peroxidation by eugenol.	Naidu (1995)
Peroxynitrite-induced nitration/lipid peroxidation	Decreased nitrotyrosine formation and lipid peroxidation by eugenol.	Chericoni et al. (2005)
Hydroxyl radical from 3,4-dihydroxyphenylalanine	Direct trapping of hydroxyl radicals by eugenol.	Ogata et al. (2005)
Lipopolysaccharide-activated macrophages	Methanolic extract of cloves found to inhibit inhibit prostaglandin E(2) production.	Kim et al. (2003)
Human PMNL cells	Eugenol inhibited 5-lipoxygenase and formation of leukotrienes.	Raghavendra et al. (2006)
Human promyelocytic leukemia cells	Eugenol treatment displayed apoptosis, including DNA fragmentation through ROS scavenging.	Yoo et al. (2005)
In-vitro study	Copper-dependent oxidation of LDL was potently inhibited by eugenol.	Ito et al. (2005)

Table 4 Antioxidant influence of eugenol in animal models

Animal model/In-vitro system	Effect demonstrated	Investigators
Rats fed unsaturated fat	Dietary eugenol (0.05% for 8 weeks) decreased serum and liver lipid peroxides.	Reddy and Lokesh (1994c)
Rats fed unsaturated fat	Dietary eugenol (0.17% for 10 weeks) enhansed activities of AO enzymes and lowered lipid peroxides in liver.	Reddy and Lokesh (1994d)
7,12-dimethyl benz (α) anthracene applied to mice	Eugenol pretreatment inhibited number of tumors due to radical scavenging activity.	Sukumaran et al. (1994)
CCl_4-administered rat	Eugenol pretreatment (25 mg/kg body wt. for 3 days) countered hepatotoxicity.	Nagababu et al. (1995)
CCl_4-administered rat	Simultaneous administration of eugenol inhibited accumulation of TBARS and the decrease in drug metabolizing enzymes.	Kumaravelu et al. (1995)
Rats injected i.p. Fe^{2+}	Eugenol pretreatment (100 mg/kg daily for 10 days) lowered lipid peroxides in liver; lowered plasma specific enzymes.	Reddy and Lokesh (1996)
Rats	Oral eugenol (1 g/kg daily for 15 days/90 days) resulted in higher level of glutathione and GSH transferase in intestine.	Vidhya and Devaraj (1999)
Swiss mice	Eugenol exerted protection against oxidative stress during exposure to gamma radiation.	Tiku et al. (2004)

initiation, propagation, or both, and many attempts have been made to elucidate the mechanism of its antioxidant activity. Ogata et al. (2005) investigated the trapping effect of eugenol on hydroxyl radical generated from L-3,4-dihydroxyphenylalanine (DOPA) in an in-vitro system and found that eugenol trapped hydroxyl radicals directly, because it had no iron-chelating action, and did not trap DOPA semiquinone radical and inhibited hydroxyl radicals with or without iron ion.

Inducible COX-2 has been implicated in the processes of inflammation and carcinogenesis. Thus, the potential COX-2 inhibitors have been considered as anti-inflammatory or cancer chemopreventive agents. The methanolic extract of the cortex of *Eugenia caryo-phyllata*, which contains eugenol, was found to potently inhibit the prostaglandin-E2 production in lipopolysaccharide-activated mouse macrophage RAW264.7 cells (Kim et al., 2003). This suggests that eugenol might be a plausible COX-2 inhibitor, and thus act as an anti-inflammatory or cancer chemopreventive agent. PMNL cells play an important role in the modulation of inflammatory conditions in humans. PMNL cells recruited at the site of inflammation release inflammatory mediators, such as leukotrienes, proteolytic enzymes, and ROS. Among these, leukotrienes are implicated in pathophysiology of allergic and inflammatory disorders such as asthma, allergic rhinitis, arthritis, inflammatory bowel disease, and psoriasis. 5-Lipoxygenase (5-LO) is the key enzyme in biosynthetic pathway of leukotrienes. Eugenol has been shown to significantly inhibit 5-LO in human PMNL (Raghavenra et al., 2006). It is further evidenced that eugenol inhibits 5-LO noncompetitively and also inhibits formation of leukotriene C in human PMNL cells and thus may have a beneficial role in modulating the 5-LO pathway in human PMNL cells.

Eugenol has been investigated for its effects on cytotoxicity, induction of apoptosis, and the putative pathways of its actions in human promyelocytic leukemia cells (HL-60) (Yoo et al., 2005). Eugenol-treated HL-60 cells displayed features of apoptosis, including DNA fragmentation, and demonstrated that ROS plays a critical role in eugenol-induced apoptosis in HL-60, and this is a possible mechanism of the anticancer effect of eugenol. The antioxidant action of eugenol has been evaluated in relation to the role of transition metals (Ito et al., 2005). While iron-mediated lipid peroxidation and autooxidation of Fe^{2+} ion were inhibited less effectively by eugenol, copper-induced oxidation of LDL was potently inhibited. Antioxidant properties of eugenol are the result of complex formation with reduced metals. Inhibition of LDL oxidation by eugenol is due to the suppression of a free radical cascade of lipid peroxidation in LDL by reducing the copper ion.

The antioxidant properties of eugenol have been documented in animal studies (Reddy and Lokesh, 1994c, 1994d) (Table 4). Dietary eugenol (0.05%) significantly lowered lipid peroxidation in serum and liver of rats fed on peanut oil or cod-liver oil, which are essentially unsaturated lipids (Durak et al., 2004a). Dietary eugenol (0.17%) enhanced the activities of antioxidant enzymes SOD, CAT, GPX, and GST and lowered lipid peroxides in liver of rats fed on coconut oil, peanut oil, or cod-liver oil (Reddy and Lokesh, 1994c). This study indicated that dietary lipids and eugenol modulate lipid peroxidation in rat liver by influencing the antioxidant defense system. Eugenol pretreatment was found to considerably inhibit the number of tumors produced by the application of 7,12-dimethyl benz (α-) anthracene as initiator and croton oil as promoter in mice (Sukumaran et al., 1994). There was considerable decrease in the number of tumor-bearing animals and their onset. Eugenol inhibited superoxide formation and lipid peroxidation, suggesting that the radical scavenging activity may be responsible for its chemopreventive action.

The protective effect of eugenol against CCl_4 toxicity has been studied (Nagababu et al., 1995). Eugenol (5 or 25 mg/kg) administered orally to rat 48 hours, 24 hours, and 30 minutes before a single oral dose of CCl_4 (2.5 ml/kg) prevented the rise in serum ASAT level without appreciable improvement in morphological changes in liver. The protective effect of eugenol against CCl_4-induced hepatotoxicity was more evident when it was given concurrently or soon after rather than much before CCl_4 treatment. Oral administration of eugenol (100 mg/kg) for 10 days lowered the liver and serum lipid peroxide levels, and serum ALAT, ASAT, and LDH, enhanced by i.p. injection of iron, indicating that eugenol reduces the iron-induced hepatic damage by lowering lipid peroxidation (Reddy and Lokesh, 1996). Chemoprotection extended by eugenol against CCl_4 toxication was established by studies on drug-metabolizing phase I and phase II enzymes (Kumaravelu et al., 1995). An overall

decrease in drug-metabolizing enzymes was observed with CCl_4 toxication, with a subsequent decrease in cytochromes P_{450} and b_5. CCl_4 increased TBARS, while simultaneous administration of eugenol with CCl_4 inhibited the accumulation of TBARS. Further, the inactivation of the drug-metabolizing system by CCl_4 was countered by eugenol. Eugenol appeared to act as an antioxidant and inducer of phase II and phase I enzymes, respectively.

The effect of eugenol on the antioxidant status of rat intestine after short- and long-term oral administration (1 g/kg for 15 days and 90 days) has been studied (Vidhya and Devaraj, 1999). The level of lipid peroxidation products (TBARS) and the activities of GPX, GR, SOD, and CAT were found to be near normal on eugenol treatment. GSH was increased significantly on a 90-day eugenol treatment. The activity of GST was increased in eugenol-treated groups. The results suggest that eugenol is protective and induces GST, and thereby, it may facilitate the removal of toxic substances from the intestine. Tiku et al. (2004) have evidenced that eugenol exerts significant protection against oxidative stress in mice during exposure to γ-radiation.

The influence of an in-vivo treatment with eugenol on established mutagens was studied to determine whether eugenol has antigenotoxic potential. In-vivo treatment of rats with eugenol resulted in a reduction of the mutagenicity of benzo[α]pyrene (B[α]P) in the *Salmonella typhimurium* mutagenicity assay (Rompelberg et al., 1996). On the other hand, in-vitro treatment of cultured cells with eugenol resulted in an increase in the genotoxicity of B[α]P. These findings indicate that there is only limited support for the antigenotoxic potential of eugenol in vivo.

*GARLIC (*Allium sativum*) AND ONION (*Allium cepa*)*

Diallyl sulfides and diallyl disulfides, which are active components of garlic, have known anti-inflammatory, antimutagenic activities. Lipid peroxidation has been implicated as a major cause in cancer development. While investigating the effect of S-allylcysteine (SAC) of garlic on 7,12-dimethylbenz[α]anthracene (DMBA)-induced hamster buccal pouch carcinogenesis in Syrian hamsters, it was observed that SAC modulated DMBA-induced decreased susceptibility of the buccal pouch to lipid peroxidation (Table 5). While simultaneously enhancing SOD and CAT activities in the liver and circulation system, SAC decreased the extent of lipid peroxidation (Balasenthil et al., 2001). This suggests that SAC exerts its chemopreventive effects by modulating lipid peroxidation and enhancing antioxidant enzyme activities in the target organ as well as in the liver and circulation system. The anticarcinogenic effect of dietary garlic (2%) on azoxymethane-induced colonic precancerous lesion studied in Sprague–Dawley rats (Sengupta et al., 2003) revealed that after 12 weeks of first azoxymethane injection (15 mg/kg), along with reduction in aberrant crypt foci by 32%, GST activity was found to be induced in both liver

Table 5 Antioxidant influence of garlic (*Allium sativum*) and its constituents

System	Effect observed	Investigators
Hamster: DMBA-induced buccal pouch carcinogenesis	S-allylcysteine suppressed incidence of tumors accompanied by decreased lipid peroxidation and enhanced antioxidant enzyme activities.	Balasenthil et al. (2001)
Rats: Azoxymethane-induced colon tumor	Garlic intake (2% for 3 months) reduced incidence of crypt foci, accompanied by reduction in lipid peroxidation and higher GST activity.	Sengupta et al. (2003)
Rats: NDA-induced liver cancer	S-allylcysteine administration inhibited tumor incidence and lipid peroxidation, with simultaneous increase in antioxidants.	Sundaresan and Subramanian (2003)
Streptozotocin-induced diabetic rats	Garlic oil administration for 15 days increased plasma total thiols and decreased lipid peroxides; higher GST activity in erythrocytes and SOD in liver and kidney.	Anwar and Meki (2003)
Alloxan-induced diabetic rats	Garlic juice (for 4 weeks) countered changes in lipid peroxides and activities of GST in plasma and tissues.	El-Demerdash et al. (2005)
Rats	Garlic oil increased hepatic GSH transferase, GSH reductase, and superoxide dismutase activities.	Chen et al. (2003)
Atherosclerotic patients	Garlic extract ingestion (1ml/kg/day for 6 months) lowered plasma and erythrocyte malondialdehyde levels.	Durak et al. (2004a)
Hypercholesterolemic volunteers	Garlic extract consumption increased blood antioxidant oxidant potential, oxidation resistance, and superoxide radical scavenger activity, and decreased MDA level.	Durak et al. (2004b)
Bovine PAE cells	AGE suppressed generation of hydrogen peroxide and superoxide anion and increased activity of AO enzymes.	Wei and Lau (1998)
Human volunteers	Garlic supplementation increased resistance of LDL to oxidation.	Lau (2001)
Rat erythrocytes	AGE prevented decrease of RBC deformability induced by lipid peroxidation and reduced TBARS increase.	Moriguchi et al. (2001)
Human LDL	Garlic compounds inhibited superoxide production; suppressed copper-induced LDL oxidation.	Ou et al. (2003)

and colon, whereas considerable reduction in lipid peroxidation level was observed in liver as well as in colon. This suggests that garlic has a protective effect on colon carcinogenesis, which is mediated by modulation of the oxidative stress during carcinogenesis.

Effects of SAC of garlic on circulatory lipid peroxidation and antioxidant levels were evaluated in N-nitrosodiethylamine (NDA)-induced hepatocarcinogenesis in Wistar rats (Sundaresan and Subramanian, 2003). Significantly elevated TBARS in the circulation system of rats bearing carcinoma indicated the higher levels of lipid peroxidation, which was accompanied by significantly decreased levels of antioxidants. SAC-administered rats showed the inhibition of tumor incidence and lipid peroxidation with simultaneous elevation in antioxidants (GSH, β-carotene, ascorbic acid, α-tocopherol, GPX, SOD, and CAT). Thus, SAC exerts its chemopreventive effects by decreasing lipid peroxidation and enhancing the levels of antioxidants in NDA carcinogenesis by reducing the formation of free radicals.

The effect of garlic oil on the oxidative stress in streptozotocin-induced diabetic rats has also been examined (Anwar and Meki, 2003). The results suggested that garlic oil (10 mg/kg i.p. for 15 days) may effectively normalize the impaired antioxidants status (circulatory total thiols and ceruloplasmin, and activities of GST and SOD in liver and kidneys) in streptozotocin-induced diabetes. The effects of this antioxidant may be useful in delaying the complicated effects of diabetes such as retinopathy, nephropathy, and neuropathy due to imbalance between free radicals and the antioxidant system. Garlic oil (0–200 mg/kg thrice a week for 6 weeks) increased hepatic GST, GR, and SOD activities in rats in a dose-dependent manner (Chen et al., 2003). The study indicated that garlic oil modulates the antioxidant capacity of animals. Treatment of the alloxan-induced diabetic rats with repeated doses of either garlic or onion juice (equivalent to 0.4 g/100 g for 4 weeks) could restore the increase in TBARS and the activity of GST in plasma, liver, testes, brain, and kidneys (El-Demerdash et al., 2005). The results suggested that garlic and onion juices exert an antioxidant effect and consequently may alleviate liver and renal damage caused by alloxan-induced diabetes.

The ingestion of garlic extract (1 ml/kg daily for 6 months) by atherosclerotic patients leads to significantly lowered plasma and erythrocyte malondialdehyde levels without any changes in SOD and GPX activities (Durak et al., 2004a). The results also demonstrated amelioration of the oxidative stress by the ingestion of garlic extract. Thus, it is possible that reduced peroxidation processes may play a part in some of the beneficial effects of garlic in atherosclerotic diseases. Effects of garlic extract supplementation on the blood lipid profile and antioxidant status were investigated in volunteers with high blood cholesterol (Durak et al., 2004b). The use of garlic extract for 4 weeks increased the blood antioxidant potential, oxidation resistance, and nonenzymatic superoxide radical scavenger activity values and decreased the MDA level, suggesting that garlic extract supplementation strengthens the blood antioxidant potential. It also leads to a decrease in the level of MDA in the blood samples, which demonstrates reduced oxidation reactions in the body. Aged garlic extract (AGE) has been shown to prevent oxidant-induced injury of endothelial cells. AGE exhibited both concentration- and time-dependent suppression of the generation of hydrogen peroxide and superoxide anion, and also increased the activity of three antioxidant enzymes (SOD, CAT, and GPX) in bovine pulmonary artery endothelial cells (Wei and Lau, 1998). This suggested that AGE may be an effective antioxidant in preventing or treating disorders related to endothelial cell injury associated with free radicals.

While hypercholesterolemia is considered a major risk factor for atherosclerosis and that lowering of cholesterol can significantly reduce risk for cardiovascular diseases, oxidation of LDL has been recognized as playing an important role in the initiation and progression of atherosclerosis. Oxidized LDL promotes vascular dysfunction by exerting direct cytotoxicity toward endothelial cells, by increasing chemotactic properties for monocytes, by transforming macrophages to foam cells via scavenger receptors, and by enhancing the proliferation of various cell types, e.g., endothelial cells, monocytes, and smooth muscle cells; all of these events are recognized as contributing to atherogenesis. Several garlic compounds can effectively suppress LDL oxidation in vitro (Lau, 2001). Short-term supplementation of garlic in human subjects has demonstrated an increased resistance of LDL to oxidation. These data suggest that suppressed LDL oxidation may be one of the powerful mechanisms accounting for the antiatherosclerotic properties of garlic. The effects of AGE on lipid peroxidative damage and the deformability of erythrocytes have been evaluated in rats (Moriguchi et al., 2001). AGE significantly prevented the decrease in erythrocyte deformability induced by lipid peroxidation in a dose-dependent manner. Addition of AGE significantly inhibited an increase in TBARS and the hemolysis rate. It has been shown that several extracts and compounds derived from garlic are able to inhibit Cu^{2+}-induced LDL oxidation. Heat processing of aqueous extracts of raw garlic or garlic powder or garlic cloves does not affect garlic's ability to inhibit Cu^{2+}-induced lipoprotein oxidation in human serum (Pedraza-Chaverri et al., 2004). A study of the protective action of four organosulfur compounds (diallyl sulfide, DAS; diallyl disulfide, DADS; S-ethylcysteine, SEC; N-acetylcysteine, NAC) derived from garlic against oxidation and glycation in human LDL showed significant inhibition of superoxide production by xanthine–xanthine oxidase and showed marked copper-chelating capability. (Ou et al., 2003). DAS and DADS had greater antioxidant activity against copper- and amphotericin B-induced LDL oxidation than SEC and NAC, while the latter two were more effective in sparing LDL-associated α-tocopherol. These results suggest that the four organosulfur compounds derived from garlic are potent agents for protecting LDL against oxidation and glycation.

Onion is a major source of flavonoids, especially the two quercetin glycosides, quercetin 4′-*o*-β-glucoside and quercetin 3,4′-*o*-β-diglucosides, which are recognized as bioactive substances. The extract from onion and various flavonoids induce

Table 6 Antioxidant influence of onion (*Allium cepa*) and its constituents

System	Effect observed	Investigators
Nicotine-injected rats	Onion oil (administered for 3 weeks) showed increased resistance to lipid peroxidation and increased concentrations of tissue antioxidants.	Helen et al. (2000)
Nicotine-injected rats	Garlic oil or onion oil supplementation increased activities of antioxidant enzymes and increased concentrations of glutathione.	Helen et al. (1999)
Alloxan-induced diabetic rats	S-methyl cysteine sulfoxide (treated for 2 months) lowered levels of malondialdehyde, hydroperoxide, and conjugated dienes in tissues.	Kumari and Augusti (2002)
Alloxan-induced diabetic rats	Onion juice (for 4 weeks) countered changes in lipid peroxides and activities of GST in plasma and tissues.	El-Demerdash et al. (2005)
Rats fed on the high-fat/high-sucrose diet	Green-leafy Welsh onion (dietary 5%) reduces superoxide generation and increases the NO availability in the aorta.	Yamamoto et al. (2005)
Human volunteers	Ingestion of fried onions resulted in increased total antioxidant capacity	McAnlis et al. (1999)
Epidermal cells incubated in the presence/absence of phorbol ester	Garlic oil/onion oil/dipropenyl sulfide increased GSH peroxidase activity.	Perchellet et al. (1986)
Rat tissues: In vitro	Garlic- and onion-derived compounds increased in vitro the activity of phase II enzymes—quinone reductase and glutathione transferase	Munday and Munday (2001)
Human PMNL cells In vitro	Aqueous extract of onion or quercetin inhibited 5-lipoxygenase	Prasad et al., 2004

the cellular antioxidant system (Table 6). Onion extract and quercetin were able to increase the intracellular concentration of GSH by approximately 50% in a reporter construct (Myhrstad et al., 2002). Onion oil, garlic oil, and dipropenyl sulfide of onion increased GPX activity in isolated epidermal cells incubated in the presence or absence of the potent tumor promoter 12-*o*-tetradecanoylphorbol-13-acetate (TPA) (Perchellet et al., 1986). The stimulatory effects of these on epidermal GPX activity are concentration-dependent and long-lasting, and thus prevent the inhibitory effect of TPA on this enzyme. Garlic oil also increased remarkably the GPX activity in the presence of various nonphorbol ester tumor promoters. Since garlic or onion oil treatments inhibit the sharp decline in the intracellular ratio between reduced glutathione and oxidized glutathione caused by TPA, it is suggested that some of the inhibitory effects of garlic and onion oils on skin tumor promotion may result from their enhancement of the natural GSH-dependent antioxidant protective system of the epidermal cells.

The antioxidant effect of onion oil and garlic oil (100 mg/kg for 21 days) on nicotine-induced (0.6 mg nicotine/kg) lipid peroxidation was studied in rat tissues (Helen et al., 1999). TBARS, conjugated dienes, and hydroperoxides were significantly increased in the liver, lungs, heart, and kidney tissues of nicotine-treated rats. Both the garlic oil and onion oil supplementation to nicotine-treated rats increased resistance to lipid peroxidation. The activities of catalase, SOD, and GPX that decreased in nicotine-treated rats had increased with garlic oil or onion oil supplementation, indicating that oils of garlic and onion are effective antioxidants against the oxidative damage caused by nicotine. Lipid peroxidation products and the antioxidant defense system were studied in tissues of rats injected with nicotine (0.6 mg/kg) and simultaneously given onion oil (100 mg/kg) or vitamin E (100 mg/kg) for 21 days (Helen et al., 2000). Onion oil supplement to nicotine-treated rats showed increased resistance to lipid peroxidation, as indicated by the concentrations of FFA, TBARS, conjugated dienes, and hydroperoxides, and the effect was comparable to that of vitamin E. On onion oil or vitamin E supplementation, the concentration of antioxidants, GSH, ascorbic acid, and retinol, were significantly raised in all the tissues studied. The results indicated that onion oil is an effective antioxidant against the oxidative damage caused by nicotine as compared with vitamin E.

The aqueous extract of onion as well as quercetin inhibited 5-LO in human PMNL cells, the key enzyme involved in the biosynthesis of leukotrienes in a concentration-dependent manner, with IC_{50} value of 1.0 mg for onion extract and 25 μM for quercetin, respectively (Prasad et al., 2004). The inhibitory effect of quercetin was similar to that of synthetic 5-LO inhibitor, phenidone. Treatment of alloxan-induced diabetic rats (for 2 months) with S-methyl cysteine sulfoxide (SMCS) isolated from onion not only ameliorated the diabetic condition significantly, but also had an antioxidant effect (Kumari and Augusti, 2002). Similar to insulin or glibenclamide, SMCS lowered the levels of MDA, hydroperoxide, and conjugated dienes in tissues exhibiting an antioxidant effect on lipid peroxidation in experimental diabetes. This is achieved by their stimulating effects on glucose utilization and the antioxidant enzymes, SOD and CAT. SMCS proved to be a more effective antioxidant compared with the drugs tested. It has been recently reported that green-leafy Welsh onion reduces superoxide generation by suppressing angiotensin II production, increasing the NO availability in the aorta, and consequently, lowering the blood pressure in rats fed on a high-fat/high-sucrose diet when included at 5% level (Yamamoto et al., 2005). The radical-scavenging and -reducing antioxidative activities of green Welsh onion may also be effective in decreasing superoxides.

Total antioxidant capacity and susceptibility of LDL to oxidation were measured following the ingestion of fried onions (225 g) by healthy volunteers (McAnlis et al., 1999). Blood quercetin levels reached peak after 2 hours, decreasing to baseline after 24 hours. This was accompanied by an increase in the total antioxidant activity of the plasma after 2 hours. There was no significant change in the susceptibility of plasma LDL to oxidation over the 48-hour period after consumption of fried

onions. Quercetin was not detected in either LDL or VLDL, but was present in the HDL fraction. The results suggested that quercetin can be absorbed in humans from dietary sources to high enough concentrations to increase the overall antioxidant activity of the plasma, and it provides no direct protective effect during LDL oxidation.

*RED PEPPER (***Capsicum annuum***) AND CAPSAICIN*

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide; Fig. 1) is the major pungent and irritating ingredient of red pepper. The antioxidant property of capsaicin in terms of inhibiting lipid peroxidation in human erythrocyte membranes (Salimath et al., 1986) and in rat liver microsomes has been documented (Reddy and Lokesh, 1992) (Table 7). The antioxidant activity of capsaicin in the biomembrane has been evidenced by measuring the oxidation of methyl linoleate micelles in soybean phosphatidylcholine liposomal membrane, and it has been observed that capsaicin inhibited the oxidation almost as effectively as α-tocopherol (Okada and Okajima, 2001).

Capsaicin is observed to inhibit copper ion-induced lipid peroxidation of human LDL, suggesting that it is an effective antioxidant offering protection against oxidation of human LDL (Naidu and Thippeswamy, 2002). The beneficial influence of dietary capsaicin on the susceptibility of LDL to oxidation was examined in an animal study. Dietary capsaicin significantly inhibited the in-vivo iron-induced LDL oxidation as well as copper-induced oxidation of LDL in vitro (Manjunatha and Srinivasan, 2006). Capsaicin significantly inhibited lipid peroxidation in rat liver mitochondria induced by ADP/Fe^{2+}, and was more effective than α-tocopherol (Kogure et al., 2002). Capsaicin was also found to scavenge DPPH radicals in membranes, both at/near the membrane surface and in the interior of the membrane. Vanillin and 8-methyl-6-noneamide were major reaction products of capsaicin with DPPH radicals, thus suggesting that the radical-scavenging site of capsaicin is the C7-benzyl carbon. Phenolic compounds of various spices, including capsaicin, modulate 5-LO activity in human PMNL cells, the key enzyme involved in the biosynthesis of leukotrienes (Prasad et al., 2004).

Wistar rats administered capsaicin (i.p. 3 mg/kg) for 3 consecutive days showed a reduction in the oxidative stress, measured as MDA in the liver, lungs, kidneys, and muscles (Lee et al., 2003). It is hypothesized that capsaicin could be a potent antioxidant even when consumed for a short period. The influence of hypolipidemic spice principle capsaicin on the antioxidant status of red blood cells and liver in hyperlipidemic rats has been reported (Kempaiah and Srinivasan, 2004a). Capsaicin (0.015%) in the diet, which produced the hypotriglyceridemic effect, was also effective in reducing the oxidative stress, which was indicated by countering of the depleted antioxidant molecules and antioxidant enzymes in erythrocytes and liver, and by decreasing the elevated lipid peroxide content. The beneficial influence of capsaicin on the antioxidant status of red blood cells and liver in induced hypercholesterolemic rats has also also evidenced (Kempaiah and Srinivasan, 2004b). The depletion in intracellular thiols and GSH in erythrocytes under hypercholesterolemic situation was effectively countered by dietary (0.015%) capsaicin. The activity of GR that was lowered in hypercholesterolemic conditions was completely countered by dietary capsaicin. Decreased hepatic antioxidant enzymes GR, GST, CAT, and SOD in hypercholesterolemic rats were effectively countered by dietary capsaicin.

*BLACK PEPPER (***Piper nigrum***) AND PIPERINE*

Black pepper (*Piper nigrum*) is one of the most widely used among spices. It is valued for its distinct biting quality attributed to piperine (Fig. 1). Piperine has been demonstrated in in-vitro experiments to protect against oxidative damage by inhibiting or

Table 7 Antioxidant influence of red pepper and capsaicin

Animal model	Effect demonstrated	Investigators
Human erythrocyte membranes	Lipid peroxidation was inhibited by capsaicin.	Salimath et al. (1986)
Rat liver microsomes	Ascorbate-Fe^{++}-induced lipid peroxidation was inhibited by capsaicin.	Reddy and Lokesh (1992)
Soybean phospholipid liposomal membrane	Inhibition of oxidation of methyl linoleate micelles by capsaicin.	Okada and Okajima (2001)
Rat liver mitochondria	Inhibition of lipid peroxidation induced by ADP/Fe^{2+} and scavenging of DPPH radicals by capsaicin.	Kogure et al. (2002)
Human LDL	Inhibition of Cu^{2+} induced lipid peroxidation by capsaicin.	Naidu and Thippeswamy (2002)
Human PMNL cells	Inhibition of 5-lipoxygenase.	Prasad et al. (2004)
Rats	Capsaicin administration for 3 days reduced oxidative stress in the liver, lungs, kidneys, and muscles.	Lee et al. (2003)
High-fat-fed rats	Beneficial influence of dietary capsaicin on antioxidant status of red blood cells.	Kempaiah and Srinivasan (2004a)
High-fat-fed rats	Beneficial influence of dietary capsaicin on antioxidant status of blood and liver.	Manjunatha and Srinivasan (2007a)
Hypercholesterolemic rats	Beneficial influence of dietary capsaicin on antioxidant status of red blood cells.	Kempaiah and Srinivasan (2004b)
Hypercholesterolemic rats	Beneficial influence of dietary capsaicin on antioxidant status of blood and liver.	Manjunatha and Srinivasan (2007b)

Table 8 Antioxidant influence of black pepper (*Piper nigrum*) and piperine

Animal model	Effect demonstrated	Investigators
Rat liver microsomes	Marginal inhibitory effect of piperine on ascorbate-Fe^{++}-induced lipid peroxidation.	Reddy and Lokesh (1992)
Rats	Piperine treatment protected against oxidative stress induced in intestinal lumen by carcinogens.	Khajuria et al. (1998)
Streptozotocin-induced diabetic rats	Intraperitoneal administration of piperine for 2 weeks partially protected against diabetes-induced oxidative stress.	Rauscher et al. (2000)
In vitro	Inhibition/quenching of superoxides and hydroxyl radicals by piperine; inhibition of lipid peroxidation.	Mittal and Gupta (2000)
Human LDL	Piperine protects Cu^{++}-induced lipid peroxidation of human LDL.	Naidu and Thippeswamy (2002)
Mice	Piperine treatment decreased mitochondrial lipid peroxidation and augmented antioxidant defense system during benzo(α)pyrene- induced lung carcinogenesis.	Selvendiran et al. (2004)
Rats fed high-fat diet	Dietary black pepper/piperine reduces high-fat-diet-induced oxidative stress by lowering lipid peroxidation and restoring activities of antioxidant enzymes and GSH.	Vijayakumar et al. (2004)
In vitro	Black pepper aqueous extract and piperine inhibit human PMNL 5-lipoxygenase.	Prasad et al. (2004)

quenching free radicals and ROS and by inhibiting lipid peroxidation (Mittal and Gupta, 2000) (Table 8). Piperine was found to act as a hydroxyl radical scavenger at low concentrations, but at higher concentrations, it activated the Fenton reaction, resulting in increased generation of hydroxyl radicals. Whereas piperine acts as a powerful superoxide scavenger, with IC_{50} value of 1.82 mM, a 52% inhibition of lipid peroxidation was observed at a dose of 1.4 mM, with IC_{50} value of 1.23 mM. Piperine marginally inhibited ascorbate/Fe^{2+}-induced lipid peroxidation in rat liver microsomes at a concentration of 600 μM (Reddy and Lokesh, 1992). Piperine has been shown to be an effective antioxidant and offers protection against oxidation of human LDL, as evaluated by copper ion-induced lipid peroxidation of human LDL (Naidu and Thippeswamy, 2002). An aqueous extract of black pepper as well as piperine has been examined for its effect on human PMNL 5-LO (Prasad et al., 2004). The formation of 5-LO product 5-HETE was significantly inhibited in a concentration-dependent manner, with IC_{50} value of 0.13 mg for aqueous extracts of pepper and 60 μM for piperine. Thus, piperine might exert an antioxidant physiological role by modulating the 5-LO pathway.

The efficacy of piperine treatment (10 mg/kg/day, i.p. for 14 days) in diabetes-induced oxidative stress in rats has been reported (Rauscher et al., 2000). All tissues from streptozotocin-induced diabetic animals exhibited disturbances in antioxidant defense when compared with normal rats. Treatment with piperine reversed the disturbances in antioxidant defense in nonhepatic tissues (GSH in brain, renal GPX and SOD activities, and cardiac GR activity and lipid peroxidation). Thus, subacute treatment with piperine for 14 days was partially effective as an antioxidant in diabetes. Khajuria et al. (1998) investigated the ability of piperine to inhibit or reduce the oxidative changes induced by chemical carcinogens in a rat intestinal model. Carcinogenesis was initiated in the intestinal lumen of rats with 7,12-dimethyl benzanthracene, dimethyl aminomethyl azobenzene and 3-methyl cholanthrene. Oxidative alterations were assessed by determining TBARS as a measure of lipid peroxidation, the thiol status, and the expression of γ-glutamyl transpeptidase (γ-GT) and Na^+,K^+-ATPase activity in the intestinal mucosa. Data indicated that carcinogen induced glutathione depletion, with substantial increase in TBARS and enzyme activities. A protective role of piperine against the oxidative alterations by the carcinogen was indicated by the observed inhibition of TBARS, a significant increase in the GSH levels, and restoration of γ-GT and Na^+, K^+-ATPase activity.

Oral supplementation of piperine (50 mg/kg) effectively suppressed experimental lung carcinogenesis by benzo(α)pyrene in mice, as revealed by a decrease in the extent of mitochondrial lipid peroxidation and concomitant increase in the activities of enzymatic antioxidants (SOD, CAT, and GPX) and nonenzymatic antioxidant (GSH, vitamin E, and vitamin C) levels (Selvendiran et al., 2004). This suggests that piperine may extend its chemopreventive effect by modulating lipid peroxidation and augmenting the antioxidant defense system. Supplementation of black pepper or piperine has been observed to reduce high-fat-diet-induced oxidative stress in terms of tissue lipid peroxidation, and enzymic and nonenzymic antioxidants in rats (Vijayakumar et al., 2004). Groups of Wistar rats were fed on high-fat diet (20% coconut oil, 2% cholesterol, and 0.125% bile salts), high-fat diet plus black pepper (0.25 g or 0.5 g/kg), and high-fat diet plus piperine (0.02 g/kg) for a period of 10 weeks. Significantly elevated levels of TBARS, conjugated dienes, and lowered activities of SOD, CAT, GPX, and GST, and GSH levels in the liver, heart, kidneys, intestine, and aorta were observed in high-fat-diet-fed rats. Simultaneous supplementation with black pepper or piperine lowered TBARS and conjugated diene levels, and maintained activities of SOD, CAT, GPX, and GST, and GSH levels near to those of control rats.

*FENUGREEK (***Trigonella foenum-graecum***)*

Fenugreek seed is reported to counter the increased lipid peroxidation and alterations in the content of circulating antioxidant molecules, GSH, β-carotene, and α-tocopherol, in

Table 9 Antioxidant effects of fenugreek (*Trigonella foenum-graecum*) and ginger (*Zingiber officinale*)

Animal model	Effect demonstrated	Investigators
Fenugreek		
Alloxan-induced diabetic rats	Decreased lipid peroxidation and countering of alteration in circulatory antioxidant molecules.	Ravikumar and Anuradha (1999)
Alloxan-induced diabetic rats	Restoration of tissue antioxidant molecules by dietary fenugreek.	Anuradha and Ravikumar (2001)
Alloxan-induced diabetic rats	Reversal of alterations in tissue antioxidant enzymes and peroxidative damage by fenugreek administration.	Genet et al. (2002)
Ginger and gingerol		
Phospholipid liposomes	6-Gingerol decreased peroxidation by Fe^{3+}/Ascorbate.	Aeschbach et al. (1994)
Lipopolysaccharide-activated macrophages	6-Gingerol protected against peroxynitrite-mediated oxidation and nitration reactions.	Ippoushi et al. (2003)
In vitro	Inhibitory effect of alcohol extract of ginger (DPPH scavenging and conjugated diene production).	Stoilova et al. (2007)

alloxan-induced diabetic rats (Ravikumar and Anuradha, 1999) (Table 9). The enhanced lipid peroxidation and increased susceptibility to oxidative stress associated with the depletion of antioxidants in liver, kidneys, and pancreas observed in alloxan-induced diabetic rats were normalized with fenugreek seed powder treatment (2 g/kg for 30 days) (Ravikumar and Anuradha, 1999). The protective effect of the aqueous extract of fenugreek seeds on the activity of Ca^{2+}-ATPase activity in liver homogenate in the presence of Fe^{2+}/ascorbate in vitro has been reported (Anuradha and Ravikumar, 2001). The findings suggest that the soluble portion of the seeds could be responsible for the antioxidant property. Oxygen free radicals are presumably responsible for the severity and complications of diabetes. The activities of antioxidant enzymes CAT, SOD, and GPX, as well as the oxidative damage, were examined in the tissues of diabetic rats treated with fenugreek (Genet et al., 2002). Fenugreek administration to diabetic animals reversed the disturbed antioxidant levels and peroxidative damage, thus suggesting that fenugreek seeds have a beneficial antioxidant property that can be exploited for the treatment/reversal of the complications of diabetes.

*GINGER (*Zingiber officinale*) AND GINGEROL*

The antioxidant effect of total phenols of ginger extract has been recently studied in vitro (Stoilova et al., 2007) (Table 9). The total phenols of the alcohol extract were found to be 870 mg/g dry extract. DPPH radical scavenging reached 90% and exceeded that of BHT; the IC_{50} concentration for inhibition of DPPH was 0.64 μg/ml. The ginger extract inhibited the hydroxyl radicals by 80% at 37°C and by 75% at 80°C, which showed a higher antioxidant activity than quercetin. Ginger contains pungent ingredients, such as 6-gingerol and 6-paradol, that possess antioxidative and anti-inflammatory properties (Surh, 1999). These phenolic compounds also have antitumor promotional and antiproliferative effects. The chemopreventive and chemoprotective effects exerted by 6-gingerol are often associated with their antioxidative and anti-inflammatory activities. Aeschbach et al. (1994) have evidenced useful antioxidant properties of gingerol, which decreased peroxidation of phospholipid liposomes in the presence of Fe^{3+} and ascorbate. The compound was a good scavenger of peroxyl radicals generated by pulse radiolysis. Reactive nitrogen species, such as NO and its derivative peroxynitrite, have been thought to influence signal transduction and cause DNA damage, contributing to carcinogenic processes. Gingerol exhibited dose-dependent inhibition of NO production and significant reduction of iNOS in lipopolysaccharide-stimulated macrophages, thus evidencing the protective ability of this compound against peroxynitrite-mediated oxidation and nitration reactions (Ippoushi et al., 2003). Moreover, gingerol effectively suppressed peroxynitrite-induced oxidation of dichlorodihydrofluorescein, oxidative single-strand breaks in plasmid DNA, and formation of 3-nitrotyrosine in bovine serum albumin and macrophage cells. The data indicated that gingerol is a potent inhibitor of NO synthesis and also an effective protector against peroxynitrite-mediated damage.

CONCLUSIONS

Oxidative stress is clearly implicated in a wide range of diseases, including cardiovascular diseases, cancer, inflammatory diseases, neurodegenerative diseases, cataract, etc. Epidemiological data generally suggest a benefit of consuming diets rich in antioxidant nutrients and phytochemicals. Spices that are normal ingredients of our diet are now known to exert health beneficial antioxidant effects in the mammalian system and in several model systems through their bioactive compounds. In many instances, the observed antioxidant effect of the spice compound in the studied biological system compared well with that of known antioxidant nutrient such as α-tocopherol or the commercial antioxidant chemical such as BHT or BHA. Antioxidant properties of spices are of particular interest in view of the impact of oxidative modification of LDL cholesterol in the development of atherosclerosis, and supression of oxidative stress and inflammation by spices is important in their cancer preventive role, since both oxidative stress and inflammation are risk factors for cancer initiation and promotion.

Among the several nutraceutical attributes of common spices (Srinivasan, 2005), their antioxidant potential has a far-reaching health implication. The studies to this effect are exhaustive and experimental evidences are plenty, particularly in the case of

curcumin of turmeric and eugenol of clove. The antioxidant activity of the spice compounds in the mammalian system involve one or more of the following: (1) free radical scavenging, (2) suppressing lipid peroxidation, (3) enhancing the antioxidant molecules in tissues, (4) stimulating the activities of endogenous antioxidant enzymes, (5) inhibiting the activity of iNOS, (6) inhibiting LDL oxidation, and (6) inhibiting 5-LO and COX-2 enzymes. All the available information that are reviewed here essentially endorses that using antioxidant spices at high levels is beneficial to the markers of health, although it is less clearly evident in many instances that these are actually beneficial in preventing or protecting oxidative-stress-mediated diseases. It is invariably extrapolated by these independent investigators that by virtue of their antioxidant activity, these spice bioactive compounds can be anti-inflammatory, antimutagenic and cancer preventive, antiatherogenic and cardioprotective, hepatoprotective, neuroprotective, anticataractogenic, etc. The challenge lies in integrating this knowledge to ascertain whether any effects can be observed in humans.

It may also be argued that oxidative stress is not a consequence of excessive free radicals overcoming the system, and that oxidative stress is a natural consequence that plays a required role in regulating cell physiology. Thus, it may just be considered as a transient pro-oxidant state. There is also a possibility that antioxidants can act as pro-oxidants in certain circumstances. In addition, there is frequent mention of the fact that certain antioxidant enzymes are induced by treatment with antioxidants, while exactly the opposite response would be predicted; this is because the production shuts down when exogenous antioxidant sources are plentiful. An alternative interpretation is that minor oxidative stress caused by the antioxidants may provoke a compensatory upregulation of antioxidant enzymes, as has been frequently observed. Thus, in some instances, the tested antioxidant spice compounds may have shown differential influences on different antioxidant parameters in the same system.

All the available information on the health-benefiting antioxidant potency of spices and their bioactive components recommends these phytochemical agents as part of the food-based strategy to derive the health benefits. Most of the animal studies that documented the beneficial antioxidant influence of spices have employed spice concentrations roughly 5–10 times the dietary levels found normally in Indian diets (Thimmayamma et al., 1983. Although the effective dose of these dietary spices evidenced to produce the desired antioxidant influence far exceeds the normal levels encountered in our daily diet (Table 10), their dietary consumption can certainly be considerably enhanced without any deleterious effects to derive the health benefit. The effectiveness of lower doses of these spices cannot be ruled out, although it is not experimentally documented. There is also the possibility of deriving the beneficial effect by chronic consumption of these spices in our daily diet. More research is required, particularly examining the effects of chronic consumption patterns.

The liberal consumption of spices to derive beneficial effects on the antioxidant status has been proved to be safe. Extensive animal studies carried out to evaluate the safety aspect of spices have indicated that even at much higher dietary levels (up to 100 times the normal intake), red pepper (Srinivasan et al., 1980), black pepper (Srinivasan and Satyanarayana, 1981; Bhat and Chandrasekhara, 1986), turmeric (Sambaiah et al., 1982), and fenugreek (Udayasekhararao, 1996) have no adverse effects on growth, organ weights, the feed efficiency ratio, nitrogen balance, and blood constituents.

Table 10 Intake of a few common spices by Indian population

Spice/active principle	Intake/day	
	g/adult*	mg/kg body wt.
Turmeric	0.2–4.8	3.3–80
Curcumin[a]	0.004–0.1	0.06–1.6
Red pepper	2.4–4.1	40–70
Capsaicin[b]	0.007–0.012	0.12–0.21
Fenugreek	0.3–0.6	5–10
Garlic	0–20	0–350
Onion	0–500	0–85

*Adult body weight: 60 kg.
[a]2% in turmeric.
[b]300 mg% in red pepper.
Source: Thimmayamma et al. (1983).

In view of the antioxidant potential of a number of spices with a far-reaching health implication, these food adjuncts deserve to be considered as the natural and necessary component of our daily nutrition, beyond their role in imparting taste and flavor to our food. As several metabolic diseases and age-related degenerative disorders are closely associated with oxidative processes in the body, the use of spices as a source of antioxidants to combat oxidation warrants further attention, in terms of validating the antioxidant capacity of spices as well as testing their effects on markers of oxidation, more so in clinical trials that are aiming to establish antioxidants as mediators of disease prevention. With time, we can expect a greater body of scientific evidence supporting the benefits of spices in the overall maintenance of health and protection from diseases.

ABBREVIATIONS

ALAT = Alanine aminotransferase
ASAT = Aspartate aminotransferase
BHA = *t*-butylhydroxy anisole
BHT = *t*-butylhydroxy toluene
GSH = Reduced glutathione
LDH = Lactate dehydrogenase
LDL = Low-density lipoprotein
HDL = High-density lipoprotein;
VLDL = Very-low-density lipoprotein
TBARS = Thiobarbituric acid reactive substances

REFERENCES

Aeschbach, R., Loliger, J., Scott, B. C., Murcia, A., Butler, J., Halliwell, B. and Aruoma, O. I. (1994). Anti-oxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxy-tyrosol. *Food Chem. Toxicol.* **32**:31–36.

Anuradha, C. V. and Ravikumar, P. (2001). Restoration of tissue antioxidants by fenugreek seeds (*Trigonella foenum-graecum*) in alloxan-diabetic rats. *Indian J. Physiol. Pharmacol.* **45**:408–420.

Anwar, M. M. and Meki, A. R. (2003). Oxidative stress in streptozotocin-induced diabetic rats: Effects of garlic oil and melatonin. *Comp. Biochem. Physiol. Mol. Integr. Physiol.* **135**:539–547.

Babu, P. S. and Srinivasan, K (1995). Influence of dietary curcumin and cholesterol on the progression of experimentally induced diabetes in albino rat. *Mol. Cell. Biochem.* **152**:13–21.

Balasenthil, S., Ramachandran, C. R. and Nagini, S. (2001). S-allylcysteine, a garlic constituent, inhibits 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis. *Nutr. Cancer.* **40**:165–172.

Balasubramanyam, M., Koteswari, A. A., Kumar, R. S., Monickaraj, S. F., Maheswari, J. U. and Mohan, V. (2003). Curcumin-induced inhibition of cellular reactive oxygen species generation: Novel therapeutic implications. *J. Biosci.* **28**:715–721.

Bhat, B. G. and Chandrasekhara, N. (1986). Lack of adverse influence of black pepper, its oleoresin and piperine in the weanling rat. *J. Food Safety.* **7**:215–223.

Brouet, I., and Ohshima, H. (1995). Curcumin, an anti-tumour promoter and anti-inflammatory agent, inhibits induction of nitric oxide synthase in activated macrophages. *Biochem. Biophys. Res. Commun.* **206**:533–540.

Chan, W. H., Wu, H. J. and Hsuuw, Y. D. (2005). Curcumin inhibits ROS formation and apoptosis in methylglyoxal-treated human hepatoma G_2 cells. *Ann. NY Acad. Sci.* **1042**:372–378.

Chen, H. W., Tsai, C. W., Yang, J. J., Liu, C. T., Kuo, W. W. and Lii, C. K. (2003). The combined effects of garlic oil and fish oil on the hepatic antioxidant and drug-metabolizing enzymes of rats. *Br. J. Nutr.* **89**:189–200.

Chericoni, S., Prieto, J. M., Iacopini, P., Cioni, P. and Morelli, I. (2005). In vitro activity of the essential oil of *Cinnamomum zeylanicum* and eugenol in peroxynitrite-induced oxidative processes. *J. Agric. Food Chem.* **53**:4762–4765.

Durak, I., Aytac, B., Atmaca, Y., Devrim, E., Avci, A., Erol, C. and Oral, D. (2004a). Effects of garlic extract consumption on plasma and erythrocyte antioxidant parameters in atherosclerotic patients. *Life Sci.* **75**: 1959–1966.

Durak, I., Kavutcu, M., Aytac, B., Avci, A., Devrim, E., Ozbek, H. and Ozturk, H. S. (2004b). Effects of garlic extract consumption on blood lipid and oxidant/antioxidant parameters in humans with high blood cholesterol. *J. Nutr. Biochem.* **15**:373–377.

Duthrie, G. G. and Brown, K. M. (1994). Reducing the risk of cardiovascular disease. **In:** *Functional Foods*, pp. 19–38. Goldberg, I. Ed., Chapman & Hall, London.

El-Demerdash, F. M., Yousef, M. I. and El-Naga, N. I. (2005). Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. *Food Chem. Toxicol.* **43**:57–63.

Genet, S., Kale, R. K. and Baquer, N. Z. (2002). Alterations in antioxidant enzymes and oxidative damage in experimental diabetic rat tissues: Effect of vanadate and fenugreek (*Trigonella foenum-graecum*). *Mol. Cell. Biochem.* **236**:7–12.

Helen, A., Krishnakumar, K., Vijayammal, P. L. and Augusti, K. T. (2000). Antioxidant effect of onion oil (*Allium cepa*. Linn.) on the damages induced by nicotine in rats as compared to α-tocopherol. *Toxicol. Lett.* **116**:61–68.

Helen, A., Rajasree, C. R., Krishnakumar, K., Augusti, K. T. and Vijayammal, P. L. (1999). Antioxidant role of oils isolated from garlic (*Allium sativum* Linn.) and onion (*Allium cepa* Linn.) on nicotine-induced lipid peroxidation. *Vet. Hum. Toxicol.* **41**:316–319.

Hsuuw, Y. D., Chang, C. K., Chan, W. H. and Yu, J. S. (2005). Curcumin prevents methylglyoxal-induced oxidative stress and apoptosis in mouse embryonic stem cells and blastocysts. *J. Cell. Physiol.* **205**:379–386.

Ippoushi, K., Azuma, K., Ito, H., Horie, H. and Higashio, H. (2003). [6]-Gingerol inhibits nitric oxide synthesis in activated J774.1 mouse macrophages & prevents peroxynitrite-induced oxidation & nitration reactions. *Life Sci.* **73**:3427–3437.

Iqbal, M., Sharma, S. D., Okazaki, Y., Fujisawa, M. and Okada, S. (2003). Dietary supple-mentation of curcumin enhances antioxidant and phase II metabolizing enzymes in ddY male mice: Possible role in protection against chemical carcinogenesis and toxicity. *Pharmacol. Toxicol.* **92**:33–38.

Ito, M., Murakami, K. and Yoshino, M. (2005). Antioxidant action of eugenol compounds: Role of metal ion in the inhibition of lipid peroxidation. *Food Chem. Toxicol.* **43**:461–466.

Joe, B. and Lokesh, B. R. (1994). Role of capsaicin, curcumin and dietary n-3 fatty acids in lowering the generation of reactive oxygen species in rat peritoneal macrophages. *Biochim. Biophys. Acta.* **1224**:255–263.

Johnston, B. D. and DeMaster, E. G. (2003). Suppression of nitric oxide oxidation to nitrite by curcumin is due to the sequestration of the reaction intermediate nitrogen dioxide, not nitric oxide. *Nitric Oxide.* **8**:231–234.

Kalpana, C. and Menon, V. P. (2004a). Inhibition of nicotine-induced toxicity by curcumin and curcumin analog: A comparative study. *J. Med. Food.* **7**:467–471.

Kalpana, C. and Menon, V. P. (2004b). Modulatory effects of curcumin on lipid peroxidation and antioxidant status during nicotine-induced toxicity. *Pol. J. Pharmacol.* **56**:581–586.

Kalpana, C. and Menon, V. P. (2004c). Curcumin ameliorates oxidative stress during nicotine-induced lung toxicity in Wistar rats. *Ital. J. Biochem.* **53**:82–86.

Kempaiah, R. K. and Srinivasan, K. (2004a). Influence of dietary curcumin, capsaicin and garlic on the antioxidant status of red blood cells and the liver in high-fat-fed rats. *Ann. Nutr. Metab.* **48**:314–320.

Kempaiah, R. K. and Srinivasan, K. (2004b). Antioxidant status of red blood cells and liver in hypercholesterolemic rats fed hypolipidemic spices. *Int. J. Vitam. Nutr. Res.* **74**:199–208.

Khajuria, A., Zutshi, U. and Bedi, K. L. (1998). Permeability characteristics of piperine on oral absorption: An active alkaloid from peppers and a bioavailability enhancer. *Indian J. Exp. Biol.* **36**:46–50.

Kim, S. S., Oh, O. J., Min, H. Y., Park, E. J., Kim, Y., Park, H. J., Han, Y. and Lee, S. K. (2003). Eugenol suppresses cyclooxygenase-2 expression in lipopolysaccharide-stimulated mouse macrophage RAW264.7 cells. *Life Sci.* **73**:337–348.

Kogure, K., Goto, S., Nishimura, M., Yasumoto, M., Abe, K., Ohiwa, C., Sassa, H., Kusumi, T. and Terada, H. (2002) Mechanism of potent antiperoxidative effect of capsaicin. *Biochim. Biophys. Acta.* **1573**:84–92.

Krishnakantha, T. P. and Lokesh, B. R. (1993). Scavenging of superoxide anions by spice principles. *Indian J. Biochem. Biophys.* **30**:133–134.

Kumaravelu, P., Dakshinamoorthy, D. P., Subramaniam, S., Devaraj, H. and Devaraj, N. S. (1995). Effect of eugenol on drug-metabolizing enzymes of carbon tetrachloride-intoxicated rat liver. *Biochem. Pharmacol.* **49**:1703–1707.

Kumari, K. and Augusti, K. T. (2002). Antidiabetic and antioxidant effects of S-methyl cysteine sulfoxide isolated from onions (*Allium cepa* Linn.) as compared to standard drugs in alloxan diabetic rats. *Indian J. Exp. Biol.* **40**:1005–1009.

Lau, B. H. (2001). Suppression of LDL oxidation by garlic. *J. Nutr.* **131**:985S–988S.

Lee, C. Y., Kim, M., Yoon, S. W. and Lee, C. H. (2003). Short-term control of capsaicin on blood and oxidative stress of rats in vivo. *Phytother. Res.* **17**:454–458.

Mahesh, T., Balasubashini, M. S. and Menon, V. P. (2004). Photo-irradiated curcumin supplementation in streptozotocin-induced diabetic rats: Effect on lipid peroxidation. *Therapie.* **59**:639–644.

Mahesh, T., Balasubashini, M. S. and Menon, V. P. (2005). Effect of photo-irradiated curcumin treatment against oxidative stress in streptozotocin-induced diabetic rats. *J. Med. Food.* **8**:251–255.

Majithiya, J. B. and Balaraman, R. (2005). Time-dependent changes in antioxidant enzymes and vascular reactivity of aorta in streptozotocin-induced diabetic rats treated with curcumin. *J. Cardiovasc. Pharmacol.* **46**:697–705.

Manikandan, P., Sumitra, M., Aishwarya, S., Manohar, B. M., Lokanadam, B. and Puvanakrishnan, R. (2004). Curcumin modulates free radical quenching in myocardial ischaemia in rats. *Int. J. Biochem.Cell. Biol.* **36**:1967–1980.

Manjunatha, H. and Srinivasan, K. (2006). Protective effect of dietary curcumin and capsaicin on induced oxidation of low-density lipoprotein, iron-induced hepatotoxicity and carrageenan-induced inflammation in rats. *FEBS J.* **273**:4528–4537.

Manjunatha, H. and Srinivasan, K. (2007a). Hypolipidemic and antioxidant effect of curcumin and capsaicin in high fat fed rats. *Can. J. Physiol. Pharmacol.* **85**:588–596.

Manjunatha, H. and Srinivasan, K. (2007b). Hypocholesterolemic and antioxidant effect of curcumin and capsaicin in induced hyper-cholesterolemic rats. *Lipids.* **42**:1133–1142.

McAnlis, G. T., McEneny, J., Pearce, J. and Young, I. S. (1999). Absorption and antioxidant effects of quercetin from onions, in man. *Eur. J. Clin. Nutr.* **53**:92–96.

Miquel, J., Bernd, A., Sempere, J. M., Diaz-Alperi, J. and Ramirez, A. (2002). The curcuma antioxidants: Pharmacological effects and prospects for future clinical use (A review). *Arch.Gerontol. Geriatr.* **34**:37–46.

Mittal, R. and Gupta, R. L. (2000). In vitro antioxidant activity of piperine. *Exp. Clin. Pharmacol.* **22**:271–274.

Moriguchi, T., Takasugi, N. and Itakura, Y. (2001). The effects of aged garlic extract on lipid peroxidation and the deformability of erythrocytes. *J. Nutr.* **131**:1016S–1019S.

Munday, R. and Munday, C. M. (2001). Relative activities of organosulfur compounds derived from onions and garlic in increasing tissue activities of quinone reductase and glutathione transferase in rat. *Nutr. Cancer*, **40**:205–210.

Murugan, P. and Pari, L. (2005). Effect of tetrahydrocurcumin on erythromycin estolate-induced lipid peroxidation in rats. *J. Basic Clin. Physiol. Pharmacol.* **16**:1–15.

Myhrstad, M. C., Carlsen, H., Nordstrom, O., Blomhoff, R. and Moskaug, J. O. (2002). Flavonoids increase the intracellular glutathione level by transactivation of the gamma-glutamylcysteine synthetase catalytical subunit promoter. *Free Radic. Biol. Med.* **32**:386–393.

Nagababu, E. and Lakshmaiah, N. (1994). Inhibition of microsomal lipid peroxidation and mono-oxygenase activities by eugenol. *Free Radic. Res.* **20**:253–266.

Nagababu, E., Sesikeran, B. and Lakshmaiah, N. (1995). The protective effects of eugenol on carbon tetrachloride induced hepatotoxicity in rats. *Free Radic. Res.* **23**:617–627.

Nagashima, K. (1989). Inhibitory effect of eugenol on Cu^{++} catalyzed lipid peroxidation in human erythrocyte membrane. *Int. J. Biochem.* **21**:745–749.

Naidu, K. A. (1995). Eugenol: An inhibitor of lipoxygenase-dependent lipid peroxidation. *Prostaglandins Leukot. Essent. Fatty Acids.* **53**:381–383.

Naidu, K. A. and Thippeswamy, N. B. (2002). Inhibition of human low density lipoprotein oxidation by active principles from spices. *Mol. Cell. Biochem.* **229**:19–23.

Nirmala, C. and Puvanakrishnan, R. (1996). Protective role of curcumin against isoproterenol induced myocardial infarction in rats. *Mol. Cell. Biochem.* **159**:85–93.

Nishigaki, I., Kuttan, R., Oku, H., Ashoori, F., Abe, H. and Yagi, K. (1992). Suppressive effect of curcumin on lipid peroxidation induced in rats by CCl_4 or ^{60}Co irradiation. *J. Clin. Biochem. Nutr.* **13**:23–30.

Nishino, H., Tokuda, H., Satomi, Y., Masuda, M., Osaka, Y., Yogosawa, S., Wada, S., Mou, X. Y., Takayasu, J., Murakoshi, M., Jinnno, K. and Yano, M. (2004). Cancer prevention by antioxidants. *Biofactors.* **22**:57–61.

Ogata, M., Kaneya, D., Shin-Ya, K., Li, L., Abe, Y., Katoh, H., Seki, S., Seki, Y., Gonda, R., Urano, S. and Endo, T. (2005). Trapping effect of eugenol on hydroxyl radicals induced by L-DOPA in vitro. *Chem. Pharm. Bull.* (Tokyo). **53**:1167–1170.

Okada, Y. and Okajima, H. (2001). Antioxidant effect of capsaicin on lipid peroxidation in homogeneous solution, micelle dispersions and liposomal membranes. *Redox Rep.* **6**:117–122.

Ou, C. C., Tsao, S. M., Lin, M. C. and Yin, M. C. (2003). Protective action on human LDL against oxidation and glycation by four organosulfur compounds derived from garlic. *Lipids.* **38**:219–224.

Padmaja, S. and Raju, T. N. (2004). Antioxidant effect of curcumin in selenium induced cataract of Wistar rats. *Indian J. Exp. Biol.* **42**:601–603.

Pandya, U., Saini, M. K., Jin, G. F., Awasthi, S., Godley, B. F. and Awasthi, Y. C. (2000). Dietary curcumin prevents ocular toxicity of naphthalene in rats. *Toxicol. Lett.* **115**:195–204.

Pari, L. and Amali, D. R. (2005). Protective role of tetrahydrocurcumin (THC) an active principle of turmeric on chloroquine induced hepatotoxicity in rats. *J. Pharm. Pharm. Sci.* **8**:115–123.

Pari, L. and Murugan, P. (2004). Protective role of tetrahydrocurcumin against erythromycin estolate-induced hepatotoxicity. *Pharmacol. Res.* **49**:481–486.

Pedraza-Chaverri, J., Gil-Ortiz, M., Albarran, G., Barbachano-Esparza, L., Menjivar, M. and Medina-Campos, O. N. (2004). Garlic's ability to prevent in vitro Cu^{2+}-induced lipoprotein oxidation in human serum is preserved in heated garlic: Effect unrelated to Cu^{2+}-chelation. *Nutr. J.* **3**:10–11.

Perchellet, J. P., Perchellet, E. M., Abney, N. L., Zirnstein, J. A. and Belman, S. (1986). Effects of garlic and onion oils on glutathione peroxidase activity, the ratio of reduced/oxidized glutathione and ornithine decarboxylase induction in isolated mouse epidermal cells treated with tumor promoters. *Cancer Biochem. Biophys.* **8**:299–312.

Phan, T. T., See, P., Lee, S. T. and Chan, S. Y. (2001). Protective effects of curcumin against oxidative damage on skin cells in vitro: Its implication for wound healing. *J. Trauma.* **51**:927–931.

Prasad, N. S., Raghavendra, R., Lokesh, B. R. and Naidu, K. A. (2004). Spice phenolics inhibit human PMNL 5-lipoxygenase. *Prostaglandins Leukot. Essent. Fatty Acids.* **70**:521–528.

Quiles, J. L., Mesa, M. D., Ramirez-Tortosa, C. L., Aguilera, C. M., Battino, M., Gil, A. and Ramirez-Tortosa, M. C. (2002). *Curcuma longa* extract supplementation reduces oxidative stress and attenuates aortic fatty streak development in rabbits. *Arterioscler. Thromb. Vasc. Biol.* **22**:1225–1231.

Raghavenra, H., Diwakar, B. T., Lokesh, B. R. and Naidu, K. A. (2006). Eugenol—The active principle from cloves inhibits 5-lipoxygenase activity and leukotriene-C4 in human PMNL cells. *Prostaglandins Leukot. Essent. Fatty Acids.* **74**:23–27.

Ramaswamy, T. S. and Banerjee, B. N. (1948). Vegetable dyes as antioxidants for vegetable oils. *Ann. Biochem. Exp. Med.* **8**:55–68.

Ramirez-Tortosa, M. C., Mesa, M. D., Aguilera, M. C., Quiles, J. L., Baro, L., Ramirez-Tortosa, C. L., Martinez-Victoria, E. and Gil, A. (1999). Oral administration of a turmeric extract inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental atherosclerosis. *Atherosclerosis.* **147**:371–378.

Rauscher, F. M., Sanders, R. A. and Watkins, J. B. (2000). Effects of piperine on antioxidant pathways in tissues from normal and streptozotocin-induced diabetic rats. *J. Biochem. Mol. Toxicol.* **14**:329–334.

Ravikumar, P. and Anuradha, C. V. (1999). Effect of fenugreek seeds on blood lipid peroxidation and antioxidants in diabetic rats. *Phytother. Res.* **13**:197–201.

Reddy, A. C. P. and Lokesh, B. R. (1992). Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. *Mol. Cell. Biochem.* **111**:117–124.

Reddy, A. C. P. and Lokesh, B. R. (1994a). Studies on the inhibitory effects of curcumin and eugenol on the formation of reactive oxygen species and the oxidation of ferrous iron. *Mol. Cell. Biochem.* **137**:1–8.

Reddy, A. C. P. and Lokesh, B. R. (1994b). Effect of dietary turmeric (*Curcuma longa*) on iron-induced lipid peroxidation in the rat liver. *Food Chem. Toxicol.* **32**:279–283.

Reddy, A. C. P. and Lokesh, B. R. (1994c). Dietary unsaturated fatty acids, vitamin E, curcumin and eugenol alter serum and liver lipid peroxidation in rats. *Nutr. Res.* **14**:1423–1437.

Reddy, A. C. P. and Lokesh, B. R. (1994d). Alterations in lipid peroxides in rat liver by dietary n-3 fatty acids: Modulation of antioxidant enzymes by curcumin, eugenol, and vitamin E. *J. Nutr. Biochem.* **5**:181–188.

Reddy, A. C. P. and Lokesh, B. R. (1996). Effect of curcumin and eugenol on iron-induced hepatic toxicity in rats. *Toxicology.* **107**:39–45.

Rompelberg, C. J., Evertz, S. J., Bruijntjes-Rozier, G. C., Van den Heuvel, P. D. and Verhagen, H. (1996). Effect of eugenol on the genotoxicity of established mutagens in the liver. *Food Chem. Toxicol.* **34**:33–42.

Ruby, A. J., Kuttan, G., Babu, K. D., Rajasekharan, K. N. and Kuttan, R. (1995). Anti-tumour and antioxidant activity of natural curcuminoids. *Cancer Lett.* **94**:79–83.

Rukkumani, R., Aruna, K., Varma, P. S., Rajasekaran, K. N. and Menon, V. P. (2004). Comparative effects of curcumin and an analog of curcumin

on alcohol and PUFA induced oxidative stress. *J. Pharm. Pharm. Sci.* **7**: 274–283.

Salimath, B. P., Sundaresh, C. S. and Srinivas, L. (1986). Dietary components inhibit lipid peroxidation in erythrocyte membrane. *Nutr. Res.* **6**: 1171–1178.

Sambaiah, K., Ratankumar, S., Kamanna, V. S., Satyanarayana, M. N. and Rao, M. V. L. (1982). Influence of turmeric and curcumin on growth, blood constituents and serum enzymes in rats. *J. Food Sci. Technol.* **19**: 187–190.

Selvendiran, K., Senthilnathan, P., Magesh, V. and Sakthisekaran, D. (2004). Modulatory effect of Piperine on mitochondrial antioxidant system in benzo(α)pyrene-induced experimental lung carcinogenesis. *Phytomedicine*. **11**:85–89.

Sengupta, A., Ghosh, S. and Das, S. (2003). Tomato and garlic can modulate azoxymethane-induced colon carcinogenesis in rats. *Eur. J. Cancer Prev.* **12**:195–200.

Sharma, O. P. (1976). Antioxidant activity of curcumin and related compounds. *Biochem. Pharmacol.* **25**:1811–1812.

Shukla, P. K., Khanna, V. K., Khan, M. Y. and Srimal, R. C. (2003). Protective effect of curcumin against lead neurotoxicity in rat. *Hum. Exp. Toxicol.* **22**:653–658.

Soni, K. B. and Kuttan, R. (1992). Effect of oral curcumin administration on serum peroxides and cholesterol levels in human volunteers. *Indian J. Physiol. Pharmacol.* **36**: 273–275.

Soudamini, K. K., Unnikrishnan, M. C., Soni, K. B. and Kuttan, R. (1992). Inhibition of lipid peroxidation and cholesterol levels in mice by curcumin. *Indian J. Physiol. Pharmacol.* **36**:239–243.

Sreejayan, N. and Rao, M. N. (1994). Curcuminoids as potent inhibitors of lipid peroxidation. *J. Pharm. Pharmacol.* **46**:1013–1016.

Sreejayan, N. and Rao, M. N. (1996). Free radical scavenging activity of curcuminoids. *Arzneimittel Forschung.* **46**:169–171.

Sreejayan, N. and Rao, M. N. (1997). Nitric oxide scavenging by curcuminoids. *J. Pharm. Pharmacol.* **49**:105–107.

Srinivasan, K. (2005). Role of spices beyond food flavouring: Nutraceuticals with multiple health effects. *Food Rev. Int.* **21**:167–188.

Srinivasan, M. R., Sambaiah, K., Satyanarayana, M. N. and Rao, M. V. L. (1980). Influence of red pepper and capsaicin on growth, blood constituents and nitrogen balance in rats. *Nutr. Rep. Int.* **21**:455–467.

Srinivasan, M. R. and Satyanarayana, M. N. (1981). Effect of black pepper (*Piper nigrum*) and piperine on growth, blood constituents and organ weights in rats. *Nutr. Rep. Int.* **23**:871–876.

Stoilova, I., Krastanov, A., Stoyanova, A., Denev, P. and Gargovaa, S. (2007). Antioxidant activity of a ginger extract (*Zingiber officinale*). *Food Chemistry.* **102**:764–770.

Subramanian, M., Sreejayan, N., Rao, M. N., Devasagayam, T. P. and Singh, B. B. (1994). Diminution of singlet oxygen-induced DNA damage by curcumin and related antioxidants. *Mutat. Res.* **311**:249–255.

Sukumaran, K. and Kuttan, R. (1995). Inhibition of tobacco-induced mutagenesis by eugenol and plant extracts. *Mutat. Res.* **343**:25–30.

Sukumaran, K., Unnikrishnan, M. C. and Kuttan, R. (1994). Inhibition of tumour promotion in mice by eugenol. *Indian J. Physiol. Pharmacol.* **38**:306–308.

Sundaresan, S. and Subramanian, P. (2003). S-allylcysteine inhibits circulatory lipid peroxidation and promotes antioxidants in N-nitrosodiethylamine-induced carcinogenesis. *Pol. J. Pharmacol.* **55**:37–42.

Surh, Y. J. (1999). Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances. *Mutat. Res.* **428**:305–327.

Surh, Y. J. (2002). Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: A short review. *Food Chem. Toxicol.* **40**:1091–1097.

Suryanarayana, P., Krishnaswamy, K. and Reddy, G. B. (2003). Effect of curcumin on galactose-induced cataractogenesis in rats. *Mol. Vis.* **9**:223–230.

Suryanarayana, P., Saraswat, M., Mrudula, T., Krishna, T. P., Krishnaswamy, K. and Reddy, G. B. (2005). Curcumin and turmeric delay streptozotocin-induced diabetic cataract in rats. *Invest Ophthalmol. Vis. Sci.* **46**:2092–2099.

Thimmayamma, B. V. S., Rao, P. and Radhaiah, G. (1983). Use of spices and condiments in the dietaries of urban and rural families. *Indian J. Nutr. Dietet.* **20**:153–162.

Thiyagarajan, M. and Sharma, S. S. (2004). Neuroprotective effect of curcumin in middle cerebral artery occlusion induced focal cerebral ischemia in rats. *Life Sci.* **74**:969–985.

Thresiamma, K. C., George, J. and Kuttan, R. (1996). Protective effect of curcumin, ellagic acid and bixin on radiation induced toxicity. *Indian J. Exp. Biol.* **34**:845–847.

Tiku, A. B., Abraham, S. K. and Kale, R. K. (2004). Eugenol as an in vivo radioprotective agent. *J. Radiat. Res.* (Tokyo). **45**:435–440.

Tirkey, N., Kaur, G., Vij, G. and Chopra, K. (2005). Curcumin, a diferuloylmethane, attenuates cyclosporine-induced renal dysfunction and oxidative stress in rat kidneys. *BMC Pharmacol.* **5**:15.

Udayasekhararao, P. (1996). Short term nutritional and safety evaluation of fenugreek. *Nutr. Res.* **16**:1495–1505.

Vidhya, N. and Devaraj, S. N. (1999). Antioxidant effect of eugenol in rat intestine. *Indian J. Exp. Biol.* **37**:1192–1195.

Vijayakumar, R. S., Surya, D. and Nalini, N. (2004). Antioxidant efficacy of black pepper (*Piper nigrum* L.) and piperine in rats with high fat diet induced oxidative stress. *Redox Rep.* **9**:105–110.

Watanabe, S. and Fukui, T. (2000). Suppressive effect of curcumin on trichloroethylene-induced oxidative stress. *J. Nutr. Sci. Vitaminol.* (Tokyo). **46**:230–234.

Wei, Z. and Lau, B. H. S. (1998). Garlic inhibits free radical generation and augments antioxidant enzyme activity in vascular endothelial cells. *Nutr. Res.* **18**:61–70.

Yamamoto, Y., Aoyama, S., Hamaguchi, N. and Rhi, G. S. (2005). Antioxidative and antihypertensive effects of Welsh onion on rats fed with a high-fat high-sucrose diet. *Biosci. Biotechnol. Biochem.* **69**:1311–1317.

Yoo, C. B., Han, K. T., Cho, K. S., Ha, J., Park, H. J., Nam, J. H., Kil, U. H. and Lee, K. T. (2005). Eugenol isolated from the essential oil of *Eugenia caryophyllata* induces a reactive oxygen species-mediated apoptosis in HL-60 human promyelocytic leukemia cells. *Cancer Lett.* **225**:41–52.