Biological Activities of Pepper Alkaloids

45

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Contents

Abstract

The distinct biting quality of black pepper (*Piper nigrum*) widely used in human dietary is attributed to the alkaloid piperine. Black pepper is also used as a food preservative and as a vital component in traditional medicines in India and China. Several physiological effects of black pepper and its bioactive alkaloid piperine have been reported in recent decades. By stimulating the digestive enzymes of pancreas, piperine enhances the digestive capacity. Piperine has been documented to enhance the bioavailability of a number of therapeutic drugs as well as phytochemicals by its inhibitory influence on drug transformation reactions in liver and intestine. It strongly inhibits hepatic and intestinal aryl hydrocarbon hydroxylase and glucuronyl transferase. Piperine's bioavailability

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enhancing property is also partly attributed to increased absorption as a result of its effect on the ultrastructure of intestinal brush border. Piperine has been evidenced to have antidiarrheal property and an effect on intestinal motility and on the ultrastructure of intestinal microvilli improving absorbability of micronutrients. Piperine has been demonstrated in in vitro studies to protect against oxidative damage by inhibiting or quenching reactive oxygen species. Piperine treatment also lowers lipid peroxidation in vivo and beneficially influences antioxidant status in situations of oxidative stress. Piperine has been found to possess antimutagenic and antitumor influences.

Capsaicin, the pungent alkaloid of red pepper (*Capsicum annuum*), has been extensively studied for its biological effects which are of pharmacological relevance. These include cardioprotective influence, anti-lithogenic effect, anti-inflammatory and pain-relieving effect, thermogenic influence, and effects on gastrointestinal system. The involvement of neuropeptides, substance P, serotonin, and somatostatin in the pharmacological actions of capsaicin has been extensively investigated. Tropical application of capsaicin is proved to alleviate pain in arthritis, postoperative neuralgia, diabetic neuropathy, psoriasis, etc. Toxicological studies on capsaicin administered by different routes are documented. Capsaicin inhibits acid secretion and stimulates alkali and mucus secretion and particularly gastric mucosal blood flow which helps in prevention and healing of gastric ulcers. Antioxidant and anti-inflammatory properties of capsaicin are established in a number of studies. Chemopreventive potential of capsaicin is evidenced in cell line studies. The health beneficial hypocholesterolemic influence of capsaicin besides being cardioprotective has other implications, namely, prevention of cholesterol gallstones and protection of the structural integrity of erythrocytes under conditions of hypercholesterolemia. Beneficial influences of capsaicin on gastrointestinal system include digestive stimulant action and modulation of intestinal ultrastructure so as to enhance permeability to micronutrients.

Keywords

Alkaloids • biological activities • capsaicin • *capsicum annuum* • piperine • *piper nigrum*

1 Piperine of Black Pepper (*Piper nigrum*)

1.1 Introduction

Black pepper (*Piper nigrum*) – the dried berries of the plant belonging to the family Piperaceae – is one of the most widely used among spices, valued for its distinct biting quality. Black pepper has been used as a spice in India since prehistoric times; it is known to Indian cooking since at least 2000 BC [1]. Black pepper is produced from the green unripe berries of the pepper plant by briefly cooking in hot water [2]. White pepper which is commonly found in Western countries is produced

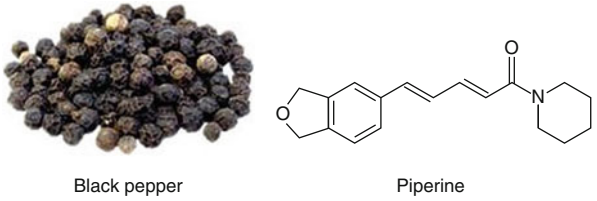

Fig. 45.1 Black pepper and piperine

by soaking ripe pepper berries in water for about a week, during which the flesh of the fruit softens and decomposes; rubbing off the skin would result in the naked seed which is then dried [2] (Fig. 45.1).

Black pepper is historically used also in traditional medicines and home remedies in India [3]. Black pepper figures in remedies in *Ayurveda*, *Siddha*, and *Unani* medicine in India for such illnesses as constipation, diarrhea, earache, gangrene, heart disease, hernia, hoarseness, indigestion, insect bites, insomnia, joint pain, liver problems, lung disease, oral abscesses, sunburn, tooth decay, and toothaches. Black pepper was relied upon to treat specific conditions such as diarrhea and fevers, but it appears that the extensive, generalized use was to enhance the effects of many herbal remedies [3].

Long pepper (*Piper longum*) and black pepper (*Piper nigrum*) (both of which are now known to contain piperine) have been prescribed in *Ayurvedic* system of medicine in India for thousands of years, a practice which may have enhanced pharmacological actions of other compounds in traditional herbal medicines. Black pepper is a component of *trikatu* (three acrids) along with long pepper and ginger (*Zingiber officinale*) in equal proportions; *trikatu* is widely used in combination with other *Ayurvedic* medications. Black pepper is specifically cited in *Ayurveda* to internally treat fevers, gastric and abdominal disorders, and urinary problems [4]. Medicinal external treatments with black pepper include treatments for rheumatism, neuralgia, and boils [4]. Possible uses of black pepper in Indian folk medicine include the treatment of respiratory diseases, dysentery, pyrexia, and insomnia [4]. Black pepper is part of a herbal, folk remedy relied to treat diarrhea [5]. Black pepper is also a constituent of traditional Chinese medicine for stimulation of digestion and relieving diarrhea [6]. Black pepper has been used in China as a folk remedy for epilepsy.

The spiciness of black pepper which is characterized by a distinct biting quality is due to the alkaloid compound piperine, which is found both in the outer fruit and in the seed [2]. Refined piperine is about 1 % as hot as the capsaicin of red chili pepper. The bioactive and pungent ingredient of black pepper was identified as piperine and isolated in 1820 by the Dutch chemist Hans Christian Oersted [2].

1.2 Biological Effects of Piperine

Many health beneficial physiological effects of black pepper or its bioactive alkaloid – piperine – have been reported in recent decades and been reviewed [7].

1.2.1 Inhibition of Drug-Metabolizing Enzyme System

In the context of piperine having been reported to enhance drug bioavailability, Atal et al. studied the interaction of piperine with hepatic drug biotransformation reactions in vitro and in vivo [8]. Piperine inhibited hydroxylation of aryl hydrocarbon, *N*-demethylation of ethylmorphine, *O*-deethylation of 7-ethoxy-coumarin, and glucuronidation of 3-hydroxybenzo (α-) pyrene (3-OH-BP) in rat liver in vitro in a dose-dependent manner. Piperine inhibited hepatic microsomal aryl hydrocarbon hydroxylase (AHH) noncompetitively from the untreated and 3-methylcholanthrene-treated rats with a K_i of 30 μM. Similarly, the kinetics of inhibition of ethylmorphine-*N*-demethylase from control rat liver exhibited noncompetitive inhibition with a K_m of 0.8 mM and K_i of 35 μM. These studies demonstrated that piperine is a nonspecific inhibitor of drug metabolism which shows little discrimination between different cytochrome P_{450} forms. Oral administration of piperine in rats strongly inhibited the hepatic AHH and UDP-glucuronyl transferase activities. Pretreatment with piperine prolonged hexobarbital sleeping time and zoxazolamine paralysis time in mice. These observations suggest that piperine is a potent inhibitor of drug metabolism. The mechanism of inhibition of glucuronidation by piperine has been explored by examining the rate of glucuronidation of 3-OH-BP and UDP-glucuronic acid (UDPGA) concentration in isolated epithelial cells of the guinea pig small intestine [9], and it was found that glucuronidation of 3-OH-BP was dependent on duration of incubation, cellular protein, and endogenous UDPGA concentration. Piperine caused a concentration-related decrease in UDPGA content and the rate of glucuronidation in these cells. Piperine noncompetitively inhibited hepatic UDP-glucuronyltransferase with Ki of 70 μM. The study demonstrated that piperine modifies the rate of glucuronidation by lowering the endogenous UDPGA concentration and also by inhibiting the transferase activity (Table 45.1).

Twenty-four hours following intragastric administration of piperine (800 mg/kg) in adult rats, a significant decrease in the hepatic levels of cytochrome P_{450}, benzphetamine *N*-demethylase, aminopyrine *N*-demethylase, and aniline hydroxylase has been observed [13]. An *i.p.* administration of rats with piperine (100 mg/kg) produced a significant decrease in hepatic cytochrome P_{450} and activities of benzphetamine *N*-demethylase, aminopyrine *N*-demethylase, and aniline hydroxylase 1 h after the treatment [15]. Twenty-four hours later, these parameters along with cytochrome b_5 and NADPH-cytochrome-C reductase remained depressed in piperine-treated rats. Piperine noncompetitively inhibited aromatic hydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin deethylase activities in lung microsomes of rats and guinea pigs in vitro [10]. Piperine given at a dose of 25 mg/kg to rats caused a maximal inhibition of both the enzymes at 1 h. Similarly, upon daily treatment of piperine (15 mg/kg) to rats for 7 days, deethylase activity was consistently inhibited, while AHH showed faster recovery. Piperine thus appeared to cause differential inhibition of two forms of cytochrome P_{450} and thus would accordingly affect the steady-state level of those drugs metabolized by these pulmonary forms of cytochrome P_{450}.

Table 45.1 Inhibitory effects of piperine on drug-metabolizing enzyme system in vitro and in vivo

System	Observed effect	References
In vitro	(a) Inhibition of aryl hydroxylation, *N*-demethylation, *O*-deethylation, and glucuronidation in vitro by piperine	[8]
	(b) Decreased UDP-glucuronic acid concentration and rate of glucuronidation in isolated epithelial cells of guinea pig small intestine by piperine	[9]
	(c) Inhibition of aryl hydroxylase and *O*-deethylase activities by piperine in vitro in pulmonary microsomes	[10]
	(d) Suppression of aryl hydroxylation in cell culture is mediated by direct interaction of piperine with cytochrome P_{450} and not by downregulation of its gene expression	[11]
	(e) Piperine decreased the activities of liver microsomal aryl hydroxylase, *N*-demethylase and UDP-glucuronosyl transferase, and cytochrome P_{450}	[12]
Rats	(a) Lower aryl hydroxylase and UDP-glucuronyl transferase activities, prolonged hexobarbital sleeping time in piperine-treated rats	[8]
	(b) Inhibition of aryl hydroxylase and *O*-deethylase activities by piperine in vivo in pulmonary microsomes	[10]
	(c) Decreased activities of hepatic microsomal cytochrome P_{450}, *N*-demethylase, aryl hydroxylase by intragastric/intraperitoneal piperine	[13]
	(d) Inhibition of UDP-glucose dehydrogenase and UDP-glucuronyl transferase in liver and intestine by piperine	[14]
	(e) Lowered activity of *N*-demethylase, UDP-glucuronosyl transferase, and NADPH-cytochrome-C reductase as a result of piperine feeding	[12]
Guinea pig	(a) Inhibition of UDP-glucose dehydrogenase and UDP-glucuronyl transferase in liver and intestine by piperine	[14]

Piperine caused a concentration-related strong noncompetitive inhibition of UDP-glucose dehydrogenase (UDPGDH) reversibly and equipotently in rat and guinea pig liver and intestine [14]. However, the UDPGA contents were decreased less effectively by piperine in isolated rat hepatocytes compared with enterocytes of guinea pig small intestine. Data on UDPGA content and rate of glucuronidation suggested that piperine is a potent inhibitor of UDPGDH and it exerts stronger effects on intestinal glucuronidation than in rat liver. The effect of dietary piperine (0.02 %) on the activities of the liver drug-metabolizing enzyme system has been examined in rats [12]. Piperine significantly stimulated the activity of aryl hydroxylase, while the activities of *N*-demethylase, UDP-glucuronyl transferase, and NADPH-cytochrome-C reductase were lowered as a result of piperine feeding. Piperine also significantly decreased the activities of liver microsomal AHH, *N*-demethylase and UDP-glucuronosyl transferase, and cytochrome P_{450} in vitro when included at 1×10^{-6} mol/L.

A study of the modulation of benzo(α-)pyrene metabolism and regulation of cytochrome CYP1A1 gene expression by piperine in 5 L cells in culture revealed that piperine-mediated inhibition of AHH activity and consequent suppression of the procarcinogen activation results from direct interaction of piperine with

cytochrome P_{450}1A1-protein and not because of downregulation of its gene expression [11]. Piperine was evaluated for beneficial effects in Alzheimer's disease by studying the potential for herb-drug interactions involving cytochrome P_{450}, UDP-glucuronosyl transferase, and sulfotransferase enzymes. Piperine was a relatively selective noncompetitive inhibitor of CYP3A with less effect on other enzymes evaluated [16]. Piperine inhibited recombinant CYP3A4 much more potently than CYP3A5.

1.2.2 Enhancing Effect on the Bioavailability of Drugs and Phytochemicals

Piperine is now established as a bioavailability enhancer of various structurally and therapeutically diverse drugs and other substances. Potential of piperine to increase the bioavailability of drugs in humans is of great clinical significance. Most of the clinical trials have shown that piperine increases levels of medications: phenytoin (used in epilepsy), propranolol (used for hypertension), rifampicin (used in tuberculosis), theophylline (lung medication), and coenzyme Q_{10}. This effect is due to the inhibitory interaction of piperine with cytochrome P_{450} enzymes of the liver and small intestine that are involved in drug metabolism, namely, CYP1A2, CYP1A1, CYP2D6, CYP3A4, and P-glycoprotein [17] (Table 45.2).

The scientific basis of the use of acrids (long pepper, black pepper, and ginger as constituents of *trikatu*) in a large number of medications in the indigenous *Ayurvedic* system of medicine in India has been evaluated by Atal et al. [18]. The observed >200 % increase in the blood levels of the test drug vasicine by *Piper longum* and of test drug sparteine by >100 % under the influence of piperine in a clinical study suggested that these acrids have the capacity to increase the bioavailability of certain drugs. These authors concluded that the *trikatu* group of drugs increases bioavailability of drugs either by promoting rapid absorption from the gastrointestinal tract, or by protecting the drug from being metabolized in its first passage through the liver after being absorbed, or by a combination of these two mechanisms. The effect of piperine on the bioavailability of propranolol and theophylline has been examined in a crossover study, wherein subjects received a single oral dose of propranolol (40 mg) or theophylline (150 mg) alone or in combination with piperine (20 mg/day for 7 days) [19]. An enhanced systemic availability of oral propranolol and theophylline was evidenced as a result of piperine treatment.

A pharmacokinetic study has examined the effect of piperine, a known inhibitor of hepatic and intestinal glucuronidation on the bioavailability of curcumin, the bioactive ingredient of the spice turmeric administered with piperine in healthy human volunteers [20]. After a dose of 2 g curcumin taken without piperine, serum levels were very low, while concomitant administration of piperine (20 mg) produced 2,000 % higher concentrations from 0.25 to 1 h post-drug. The study shows that in the dosages used, piperine enhances the serum concentration, extent of absorption, and bioavailability of curcumin in humans. When curcumin was given alone at 2 g/kg to rats, moderate serum concentrations were achieved over a period of 4 h [20]. Concomitant administration of piperine (20 mg/kg) increased

Table 45.2 Modulation of bioavailability of drugs, phytochemicals, and carcinogens by piperine

System	Remarks	References
Humans	(a) Increased bioavailability of vasicine and sparteine as a result of *Piper longum*/piperine treatment	[18]
	(b) Enhanced systemic availability of propranolol and theophylline as a result of piperine treatment	[19]
	(c) Increased serum concentration of curcumin by concomitant administration of piperine	[20]
	(d) Increased plasma levels of coenzyme Q_{10} by co-administration of piperine	[21]
	(e) Increased plasma concentration of phenytoin when co-administered along with piperine	[22]
	(f) Increased plasma concentration of antiretroviral agent nevirapine when co- administered along with piperine	[23]
Rats	(a) Decreased metabolic activation of fungal toxin aflatoxin B_1 and hence its increased accumulation in plasma	[24]
	(b) Enhanced bioavailability of β-lactam antibiotics – amoxicillin trihydrate and cefotaxime – by co-administration of piperine	[25]
	(c) Enhanced bioavailability of curcumin when administered concomitantly with piperine	[26]
Mice	(a) Delayed elimination of antiepileptic drug – phenytoin – by treatment of piperine	[27]
	(b) Increased plasma levels and delayed excretion of epigallocatechin-3-gallate from green tea as a result of intragastric co-treatment with piperine	[28]

the serum curcumin concentration for a short period of 1–2 h post-drug, and the bioavailability was increased by 154 %. Enhanced bioavailability of curcumin was evidenced when the same was orally administered concomitant with piperine in rats [26]. Intestinal absorption of curcumin was relatively higher when administered concomitantly with piperine, and it stayed significantly longer in the body tissues. This assumes importance in the context of diverse medicinal properties of curcumin.

Black pepper extract consisting of 98 % piperine has been shown to increase plasma levels of orally administered coenzyme Q_{10} in a clinical study [21]. The relative bioavailability of 90 and 120 mg of coenzyme Q_{10} administered in a single dose or for 14 and 21 days with placebo or with 5 mg of piperine was determined by comparing measured changes in plasma concentration. Supplementation of 120 mg coenzyme Q_{10} with piperine for 21 days produced a significant 30 % greater AUC than with coenzyme Q_{10} plus placebo. Piperine has been reported to enhance the oral bioavailability of phenytoin, an antiepileptic drug in human volunteers [22]. Piperine (20 mg administered along with phenytoin) increased significantly the mean plasma concentration of phenytoin in patients receiving either a 150 or 200 mg twice daily dose of phenytoin. A similar effect of piperine in altering the pharmacokinetics of phenytoin has been reported from a study on mice, where pretreatment of piperine significantly delayed the elimination of phenytoin [27]. There was a significant increase in AUC, C_{max}, and K_a. Enhanced bioavailability of

nevirapine, a potent non-nucleoside inhibitor of HIV-1 reverse transcriptase indicated for use in combination with other antiretroviral agents for the treatment of HIV-1 infection, has been evidenced when administered along with piperine [23]. Mean maximum plasma concentration, area under the plasma concentration-time curve post-dose, was increased significantly when co-administered with piperine (20 mg) in healthy male subjects.

It has been observed that intragastric co-treatment with dietary piperine enhances the bioavailability of epigallocatechin-3-gallate (EGCG) from green tea in mice [28]. Co-administration of 164 μmol/kg EGCG and 70 μmol/kg piperine to mice increased the plasma C_{max} and AUC by 1.3-fold compared to mice treated with EGCG only. Piperine appeared to increase EGCG bioavailability by inhibiting glucuronidation and gastrointestinal transit. Co-administration of piperine enhanced the bioavailability of β-lactam antibiotics amoxicillin trihydrate and cefotaxime significantly in rats [25]. The improved bioavailability is reflected in various pharmacokinetic parameters, namely, t_{max}, C_{max}, half-life, and AUC of these antibiotics. The effect of piperine on the metabolic activation and distribution of aflatoxin B_1 (AFB_1) in rats has been studied [24]. Rats pretreated with piperine accumulated considerable AFB_1 in plasma and in the tissues examined as compared to the controls.

1.2.3 Beneficial Influences on the Gastrointestinal System

Digestive Stimulant Action

While studying the effect of spices on the secretion and composition of saliva in human subjects, it has been observed that black pepper enhances the secretion of saliva and the activity of salivary amylase [29]. The digestive stimulant action of spices is exerted through (1) a beneficial stimulation of the liver to produce and secrete bile rich in bile acids, which play a very important role in fat digestion and absorption, or (2) a beneficial stimulation of the activities of enzymes of pancreas and intestine that participate in digestion [30]. Black pepper and its bioactive alkaloid piperine, examined for their effect on bile secretion as a result of dietary intake for a period of time in experimental rats, did not show any beneficial stimulatory influence on bile acid production by the liver and its secretion into bile [31]. On the other hand, oral administration of piperine as a single dose significantly increased bile acid secretion. Influence of dietary intake of piperine on the pancreatic digestive enzymes and the terminal digestive enzymes of the small intestinal mucosa has been examined in experimental rats. Significantly increased activities of pancreatic lipase, amylase, chymotrypsin, and trypsin were observed as a result of dietary intake of piperine in these experimental rats [32]. Such beneficial influence of this spice on the activity of these enzymes was not evident when administered as a single oral dose. Piperine also significantly enhanced the activity of intestinal lipase and amylase in animals given single oral dose of piperine [33] (Table 45.3).

Dietary piperine (0.02 %) was examined for its influence on bile secretion, digestive enzymes of pancreas, and absorption of dietary fat in rats fed high fat (30 %) for 8 weeks [34]. Piperine enhanced the activity of pancreatic lipase and

Table 45.3 Beneficial influences of piperine on gastrointestinal system

System	Observed effect	References
Digestive stimulant action		
Rats	(a) Stimulation of digestive enzymes of pancreas by dietary piperine	[32]
	(b) Stimulation of digestive enzymes of intestine by dietary piperine	[33]
	(c) Oral administration of piperine increases biliary bile acid secretion	[31]
	(d) Enhanced activity of pancreatic lipase, higher secretion of bile acids in high-fat-fed rats which was associated with enhanced fat absorption	[34]
Influence on intestinal motility and food transit time		
Humans	(a) Increased orocecal transit time after black pepper consumption	[41]
Rats	(a) Gastrointestinal food transit time shortened by dietary piperine	[35]
	(b) Piperine inhibited gastric emptying of solids/liquids	[36]
Mice	(a) Piperine inhibited gastrointestinal transit	[36]
	(b) Piperine dose-dependently delayed gastrointestinal motility	[37]
Effect on gastric mucosa		
Humans	(a) Black pepper caused increases in gastric parietal and pepsin secretion and increased gastric cell exfoliation in humans	[38]
Rats	(a) Black pepper increased gastric acid secretion in anesthetized rats	[39]
	(b) Piperine increased gastric acid secretion	[40]
	(c) Piperine had protective action against stress-induced gastric ulcer	[41]
	(d) Dietary piperine showed protective effect on gastric and intestinal mucosa with respect to activities of antioxidant enzymes and gastric mucin content	[42]
	(e) Dietary piperine alleviated the diminished activities of antioxidant enzymes in gastric and intestinal mucosa during ethanol-induced oxidative stress	[42]
Mice	(a) Piperine had protective action against stress-induced gastric ulcer	[41]
Antidiarrheal property		
Mice	(a) Piperine inhibited diarrhea produced by castor oil, arachidonic acid, etc.	[36]
	(b) Piperine reduced castor oil-induced intestinal fluid accumulation	[43]
Influence on absorptive function		
Rats	(a) Piperine stimulated γ-glutamyl transpeptidase activity and enhanced uptake of amino acids in isolated epithelial cells of rat jejunum	[44]
	(b) Piperine modulated membrane dynamics and permeation characteristics, increasing absorptive surface and induction of synthesis of proteins associated with cytoskeletal function	[45]
	(c) Dietary piperine induced alteration in BBM fluidity and permeability property, associated with increased microvilli length, resulting in higher absorptive surface of the small intestine	[46]
	(d) Duodenum, jejunum, and ileum portions of small intestines isolated from rats pre-fed piperine showed higher uptake of iron, zinc, and calcium	[47]
	(e) Higher in vitro absorption of β-carotene in the intestines was evidenced in piperine-fed animals	[48]
	(f) Dietary piperine improved intestinal absorption of orally administered β-carotene	[49]

caused higher secretion of biliary bile acids in high-fat-fed rats which was associated with enhanced fat absorption. Stimulation of lipid mobilization from adipose tissue was suggested by the decrease in perirenal adipose tissue weight by dietary piperine. This was also accompanied by prevention of the accumulation of triglyceride in liver and serum in high-fat-fed rats. Activities of key lipogenic enzymes in liver were reduced which was accompanied by an increased activity of hormone-sensitive lipase. Thus, dietary piperine enhances fat digestion and absorption in high-fat-fed situation through enhanced secretion of bile salts and a stimulation of the activity pancreatic lipase. At the same time, the energy expenditure is facilitated to prevent the accumulation of absorbed fat.

Influence on Absorptive Function

Piperine (25–100 μM) has been shown to significantly stimulate γ-glutamyl transpeptidase (γ-GT) activity in vitro and enhance the uptake of amino acids in freshly isolated epithelial cells of rat jejunum [44]. The kinetic behavior of γ-GT was altered in the presence of piperine, suggesting that this alkaloid may interact with the lipid environment to produce effects leading to increased permeability of the intestinal cells. It is hypothesized that piperine's bioavailability-enhancing property may be partly attributed to increased absorption [45]. Piperine also caused an increase in intestinal brush border membrane fluidity and stimulated the activities of leucine aminopeptidase and glycyl-glycine dipeptidase due to the alteration in enzyme kinetics. This suggests that piperine modulates the membrane dynamics due to its apolar nature by interacting with surrounding lipids and hydrophobic portions in the protein vicinity, which may decrease the tendency of membrane lipids to act as stearic constrains to enzyme proteins and thus modify enzyme conformation. Ultrastructural studies with piperine showed an increase in microvilli length with a prominent increase in free ribosomes and ribosomes on the endoplasmic reticulum in enterocytes, suggesting that synthesis or turnover of cytoskeletal components or membrane proteins may be involved in the observed effect. Thus, piperine may induce alterations in membrane dynamics and permeation characteristics, along with induction of the synthesis of proteins associated with cytoskeletal function, resulting in an increase in the absorptive surface, thus assisting efficient permeation through the epithelial barrier.

Beneficial influence of black pepper and its bioactive alkaloid piperine on the small intestinal ultrastructure has been revalidated in a recent study [46]. Groups of rats were maintained on dietary black pepper (0.5 %) or piperine (0.02 %) for 8 weeks. Membrane fluidity study using an apolar fluorescent probe showed increased BBM fluidity in these spice-fed animals. This was corroborated by decreased cholesterol: phospholipid ratio in jejunal and ileal regions of the intestine. The dietary spice or its bioactive compound stimulated the activities of BBM enzymes – glycyl-glycine dipeptidase, leucine aminopeptidase, and γ-glutamyl transpeptidase – in jejunal mucosa, suggesting a modulation in membrane dynamics due to the apolar spice bioactive compounds interacting with surrounding lipids and hydrophobic portions in the protein vicinity and thus modify enzyme conformation. Scanning electronic microscopy of the intestinal villi in these spice treatments

revealed alteration in the ultrastructure, especially an increase in microvilli length which would mean a beneficial increase in the absorptive surface of the small intestine, providing for an increased bioavailability of micronutrients. Thus, dietary black pepper and piperine were evidenced to induce alteration in BBM fluidity and passive permeability property, associated with the induction in the increased microvilli length, resulting in increased absorptive surface of the small intestine.

In view of dietary black pepper and piperine specifically altering the ultrastructure and permeability characteristics of small intestines, these dietary interventions have been examined for their possible influence on intestinal absorption of minerals [47]. Everted segments of duodenum, jejunum, and ileum portions of small intestines isolated from rats pre-fed this spice for 8 weeks and examined for the uptake of iron, zinc, and calcium from incubations containing digesta of finger millet showed higher absorption of these minerals. Dietary black pepper and piperine have also been studied for their possible influence on absorption of β-carotene by examining its uptake by the intestinal segments from rats pre-fed these spices [48]. Higher in vitro absorption of β-carotene in the intestines was evidenced in piperine-fed animals which was 47 % higher than control, while dietary black pepper produced 59 % increase. An animal study has also evaluated the influence of dietary piperine on the absorption of orally administered β-carotene and its conversion to vitamin A [49]. Concentration of β-carotene significantly increased in serum, liver, and intestine of piperine-fed rats 4 h after single oral administration of β-carotene suggesting improved intestinal absorption of β-carotene. Retinol concentration was not however changed in these animals suggesting that bioconversion of β-carotene to vitamin A was not similarly influenced. Activities of the two enzymes involved in the bioconversion of β-carotene to vitamin A, namely, β-carotene-15,15′-dioxygenase and retinal reductase in intestines and liver, remained unaffected by dietary piperine. Activities of these two enzymes involved in the bioconversion of β-carotene to retinal were inhibited by piperine in vitro, thus corroborating with in vivo observation.

Influence on Gastrointestinal Motility and Food Transit Time

An increase in orocecal transit time has been observed in human subjects after black pepper (1.5 g) consumption in a study of the effect on small intestinal peristalsis [50]. Piperine inhibited gastric emptying of solids/liquids in rats and gastrointestinal transit in mice in a dose- and time-dependent manner [36]. It significantly inhibited gastric emptying of solids and gastrointestinal transit at the doses extrapolated from humans (1 and 1.3 mg/kg *p.o.* in rats and mice, respectively). One week oral treatment with the same dose in rats and mice did not produce a significant change compared to single dose administration. Gastric emptying inhibitory activity of piperine is independent of gastric acid and pepsin secretion. Piperine which activates vanilloid receptors (0.5 – 20 mg/kg *i.p.*) dose-dependently delayed gastrointestinal motility in mice [37]. The inhibitory effect of piperine (10 mg/kg) was strongly attenuated in capsaicin (75 mg/kg)-treated mice. The study indicated that the vanilloid ligand piperine can reduce upper gastrointestinal motility. The effect of piperine involves capsaicin-sensitive neurons but not vanilloid receptors.

The gastrointestinal food transit time in experimental rats was significantly shortened by dietary piperine [35]. The reduction in food transit time produced by dietary piperine roughly correlated with its beneficial influence on digestive enzymes [30]. Thus, dietary piperine which has enhanced the activity of digestive enzymes also has markedly reduced the food transit time at the same level of consumption. This reduction in food transit time could probably be attributed to acceleration in the overall digestive process as a result of increased availability of digestive enzymes.

Antidiarrheal Property

Peppers are added in traditional antidiarrheal formulations of different herbs. In a study made in experimental mice, the antidiarrheal activity of piperine against diarrhea produced by castor oil, $MgSO_4$, and arachidonic acid has been evidenced at 8 and 32 mg/kg *p.o.* dose [51]. Inhibition of castor oil-induced entero pooling by piperine suggests its inhibitory effect on prostaglandins. Piperine (2.5–20 mg/kg, *i.p.*) dose-dependently reduced castor oil-induced intestinal fluid accumulation in experimental mice; piperine reduces castor oil-induced fluid secretion with a mechanism involving capsaicin-sensitive neurons [43].

Effect on Gastric Mucosa

Pungent spices have long been implicated to cause gastric mucosal injury. The effects of black pepper on the gastric mucosa were assessed using double-blind intragastric administration of the spice (1.5 g) to healthy human volunteers and found to be similar to aspirin [38]. Black pepper caused significant increases in parietal secretion, pepsin secretion, and potassium loss. Gastric cell exfoliation (as reflected in DNA loss in gastric contents) was increased after black pepper administration and mucosal micro bleeding was also observed.

On the other hand, protective action of piperine against experimental gastric ulcer has been evidenced in rats and mice wherein the gastric mucosa damage was induced by stress, indometacin, HCl, and pyloric ligation [41]. Piperine at 25 – 100 mg/kg *i.g.* protected animals from gastric ulceration in a dose-dependent manner as indicated by inhibition of the volume of gastric juice, gastric acidity, and pepsin activity. Black pepper has been reported to significantly increase gastric acid secretion in anesthetized rats [39]. Piperine has been shown to produce dose-dependent (20–142 mg/kg) increase in gastric acid secretion in rats [40]. Involvement of cholinergic receptors in the observed piperine-induced increase in gastric acid secretion is ruled out as the effect of piperine was significantly antagonized by cimetidine (1 mg/kg) but not by atropine (1 mg/kg). There is however an indication that increased acidity induced by piperine could be due to stimulation of histamine H_2 receptors by this pepper alkaloid.

In a recent study, the protective effect of dietary black pepper (0.5 %) and piperine (0.02 %) with respect to activities of antioxidant enzymes in gastric and intestinal mucosa was examined [42]. These dietary interventions significantly enhanced the activities of antioxidant enzymes – superoxide dismutase, catalase, glutathione reductase, and glutathione-*S*-transferase – in both gastric and intestinal mucosa, suggesting a gastrointestinal protective role for black pepper and piperine. In a separate study, these were found to alleviate the diminished activities of

antioxidant enzymes in gastric and intestinal mucosa under conditions of ethanol-induced oxidative stress. The gastro protective effect was also reflected in their positive effect on mucosal glycoproteins, thereby lowering mucosal injury. The amelioration of the ethanol-induced decrease in the activities of antioxidant enzymes in gastric and intestinal mucosa by dietary spices suggests their beneficial gastrointestinal protective role.

1.2.4 Antioxidant Effects

Piperine has been demonstrated in in vitro experiments to protect against oxidative damage by quenching free radicals and reactive oxygen species and inhibiting lipid peroxidation [52]. Piperine is reported to have marginal inhibitory effects on ascorbate/Fe^{2+}-induced lipid peroxidation in rat liver microsomes even at high concentrations (600 μM) when compared to the beneficial inhibition of lipid peroxidation by antioxidants – vitamin E, *t*-butylhydroxy toluene and *t*-butylhydroxy anisole [53]. Both water and ethanol extract of black pepper exhibited strong total antioxidant activity and significant inhibition of peroxidation of linoleic acid emulsion [54]. Piperine is shown to be an effective antioxidant and offers protection against oxidation of human low-density lipoprotein (LDL) [55]. The aqueous extract of black pepper and piperine have been examined for their effect on human PMNL 5-lipoxygenase (5-LO), the key enzyme involved in biosynthesis of leukotrienes [56]. The formation of 5-LO product was significantly inhibited in a concentration-dependent manner. Thus, piperine might exert an antioxidant physiological role by modulating 5-LO pathway (Table 45.4).

Piperine treatment (10 mg/kg/day, *i.p.* for 14 days) has been assessed for protection against diabetes-induced oxidative stress in streptozotocin-induced diabetic rats [58]. Treatment with piperine reversed the diabetic effects on glutathione concentration in brain, on renal glutathione peroxidase and superoxide dismutase activities, and on cardiac glutathione reductase activity and lipid peroxidation. Thus, treatment with piperine for 14 days is only partially effective as an antioxidant in diabetes. The ability of piperine to reduce the oxidative changes induced by chemical carcinogens (7,12-dimethyl benzanthracene, dimethyl aminomethyl azobenzene, and 3-methyl cholanthrene) has been investigated in rat intestinal model [57]. A protective role of piperine against the oxidative alterations by these carcinogens was indicated by the observed inhibition of TBARS, a significant increase in the glutathione levels and restoration in γ-glutamyl transpeptidase and Na^+, K^+-ATPase activity in intestinal mucosa. Oral supplementation of piperine (50 mg/kg) effectively suppressed lung carcinogenesis by benzo (α)pyrene as revealed by a decrease in the extent of mitochondrial lipid peroxidation and concomitant increase in the activities of enzymatic antioxidants and nonenzymatic antioxidant levels when compared to lung carcinogenesis-bearing mice [61]. This suggests that piperine may extend its chemopreventive effect by modulating lipid peroxidation and augmenting antioxidant defense system.

The effect of supplementation of black pepper or piperine for a period of 10 weeks on tissue lipid peroxidation and enzymic and nonenzymic antioxidants has been examined in rats fed a high-fat diet (20 % coconut oil and 2 % cholesterol),

Table 45.4 Antioxidant effects of piperine

System	Observed effect	Reference
In vitro	(a) Inhibition/quenching of superoxides and hydroxyl radicals by piperine; inhibition of lipid peroxidation	[52]
	(b) Marginal inhibitory effect of piperine on ascorbate-Fe^{++}-induced lipid peroxidation in rat liver microsome	[53]
	(c) Water and ethanol extract of black pepper exhibited strong total antioxidant activity and inhibited peroxidation of linoleic acid emulsion	[54]
	(d) Piperine protects Cu^{++}-induced lipid peroxidation of human LDL	[55]
	(e) Black pepper aqueous extract and piperine inhibit human PMNL 5-lipoxygenase	[56]
Rats	Piperine treatment protected against oxidative stress induced in intestinal lumen by carcinogens	[57]
Streptozotocin-diabetic rats	*i.p.* administration of piperine for 2 weeks partially protected against diabetes-induced oxidative stress	[58]
High-fat-fed rats	Dietary black pepper/piperine reduces high-fat diet-induced oxidative stress by lowering lipid peroxidation, restoring activities of antioxidant enzymes and GSH	[59]
Mice	Piperine treatment decreased mitochondrial lipid peroxidation and augmented antioxidant defense system during benzo(α) pyrene- induced lung carcinogenesis	[60]

and it was observed that these can reduce high-fat diet-induced oxidative stress [59]. Simultaneous supplementation with black pepper or piperine lowered TBARS and conjugated diene levels and maintained antioxidant enzymes and glutathione levels in the liver, heart, kidney, intestine, and aorta near to those of control rats.

1.2.5 Antimutagenic and Tumor Inhibitory Effects

Black pepper has been shown to be effective in reducing the mutational events induced by the promutagen ethyl carbamate in *Drosophila* melanogaster [62]. Suppression of metabolic activation or interaction with the active groups of mutagens could be mechanism by which this spice exerts its antimutagenic action. While studying piperine for its immunomodulatory and antitumor activity, piperine was found to be cytotoxic toward Dalton's lymphoma ascites (DLA) and Ehrlich ascites carcinoma (EAC) cells at 250 μg/mL [63]. Piperine was also found to be cytotoxic toward L929 cells in culture at a concentration of 50 μg/mL. Administration of piperine (1.14 mg/animal) could inhibit the solid tumor development in mice induced with DLA cells and increase the life span of mice-bearing Ehrlich ascites carcinoma tumor (Table 45.5).

The effect of piperine on the cytotoxicity and genotoxicity of aflatoxin B_1 (AFB_1) has been studied in rat hepatoma cells H4IIEC3/G-(H4IIE) using cellular growth and formation of micronuclei as endpoints [64]. Piperine markedly reduced the toxicity of aflatoxin. That is, AFB_1-induced formation of micronuclei in a concentration-dependent manner probably by suppressing cytochromes P_{450} mediated bioactivation

Table 45.5 Antimutagenic and cancer preventive effects of piperine

System	Observed effect	References
In vitro and cell lines	(a) Black pepper is effective in reducing mutational events induced by procarcinogen – ethylcarbamate in *Drosophila*	[62]
	(b) Piperine markedly reduced the AFB_1-induced formation of micronuclei in H4IIE cells in a concentration-dependent manner	[64]
	(c) Piperine counteracts $CYP_{450}2B1$-mediated toxicity of AFB_1 in Chinese hamster cells and therefore has chemopreventive effect against procarcinogens activated by $CYP_{450}2B1$	[65]
Rats	(a) Piperine administration effectively reduced cyclophosphamide-induced chromosomal aberrations in bone marrow cells	[66]
	(b) Dietary black pepper was evidenced to suppress colon carcinogenesis induced by the procarcinogen 1,2-dimethylhydrazine	[67]
Mice	(a) Tumor inhibitory activity of black pepper in mice implanted with Ehrlich ascites tumor	[68]
	(b) Piperine inhibited tumor development in mice induced with Dalton's lymphoma cells and increased the life span of afflicted mice	[63]
	(c) Anti-metastatic activity of piperine on lung metastasis induced by melanoma cells	[69]
	(d) Chemopreventive effect of piperine on benzo(α)pyrene induced experimental lung cancer	[61, 70, 71]

of the mycotoxin. The potential of piperine for inhibiting the activity of cytochrome $P_{450}2B1$ and protecting against AFB_1 has been investigated in Chinese hamster r2B1 cells engineered for the expression of rat $CYP_{450}2B1$ [65]. Piperine at 60 μM completely counteracted cytotoxicity and formation of micronuclei by 10 μM AFB_1 and reduced the toxic effects of 20 μM AFB_1 by > 50 %. The results suggest that (1) piperine is a potent inhibitor of rat $CYP_{450}2B1$ activity, (2) AFB_1 is activated by r2B1 cells to cytotoxic and genotoxic metabolites, and (3) piperine counteracts $CYP_{450}2B1$-mediated toxicity of AFB_1 in the cells and might, therefore, offer a potent chemopreventive effect against procarcinogens activated by $CYP_{450}2B1$.

The antimutagenic effect of piperine has been studied with respect to its influence on chromosomes in rat bone marrow cells [66]. Wistar rats orally administered piperine (100, 400, and 800 mg/kg) were challenged with cyclophosphamide (*i.p.* 50 mg/kg). Piperine (100 mg/kg) produced significant reduction in cyclophosphamide-induced chromosomal aberrations, suggesting that it may have antimutagenic potential. Black pepper extracts have been demonstrated to possess tumor inhibitory activity [72]. Tumor-reducing activity of orally administered extracts of black pepper was studied in mice transplanted *i.p.* with Ehrlich ascites tumor, wherein life span was increased in these mice by 65 % [68]. The antimetastatic activity of piperine has been demonstrated by the inhibition of lung metastasis induced by B16F-10 melanoma cells in C57BL/6 mice [69].

The cytoprotective effect of piperine on benzo(α-)pyrene-induced lung cancer has been investigated in mice and observed that piperine may extend its

chemopreventive effect by modulating lipid peroxidation and augmenting antioxidant defense system [70]. Oral administration of piperine (100 mg/kg) effectively suppressed this experimental lung cancer. The protective role of piperine was examined during experimental lung carcinogenesis with reference to its effect on DNA damage and detoxification enzyme system [73]. The activities of detoxifying enzymes such as glutathione transferase, quinone reductase, and UDP-glucuronyl transferase were decreased while the hydrogen peroxide level was increased in the lung cancer-bearing animals. Supplementation of piperine (50 mg/kg) enhanced these detoxification enzymes and reduced DNA damage. These results explain the association between anti-peroxidative effect of piperine and ultimately the capability of piperine to prevent cancer. A significant suppression in the micronuclei formation induced by benzo(α-)pyrene and cyclophosphamide following oral administration of piperine at doses of 25, 50, and 75 mg/kg in mice has been reported [74].

Piperine has been evidenced to show chemopreventive effects when administered orally on lung cancer-bearing animals [61]. The beneficial effect of piperine is primarily exerted during initiation phase and post-initiation stage of benzo(α-)pyrene-induced lung carcinogenesis, via beneficial modulation of lipid peroxidation and membrane-bound ATPase enzymes. The ability of piperine to prevent lung carcinogenesis induced by benzo(α-)pyrene in mice and its effects on cell proliferation has been studied [60, 71]. Administration of piperine significantly decreased the levels of lipid peroxidation, protein carbonyls, nucleic acid, and polyamine synthesis that were found to be increased in lung cancer-bearing animals. Piperine could effectively inhibit benzo(α-)pyrene-induced lung carcinogenesis in albino mice by offering protection from protein damage and also by suppressing cell proliferation. Dietary (0.5 %) black pepper has been evidenced to suppress colon carcinogenesis induced by 1,2-dimethylhydrazine in rats [67].

1.2.6 Negative Influence on Reproductive System

Black pepper is used as contraceptive in folk medicine in parts of India. The reproductive toxicity of piperine has been studied in albino mice with respect to the effect on estrous cycle, toxicity to male germ cells, fertilization, implantation, and growth of pups [75]. Piperine (10 and 20 mg/kg) increased the period of the diestrous phase resulting in decreased mating performance and fertility. Postpartum litter growth was not affected by the piperine treatment, and sperm shape abnormalities were not induced at doses up to 75 mg/kg. Considerable anti-implantation activity was recorded after 5 days post-mating oral treatment with piperine. These results suggest that piperine interferes with several crucial reproductive events in a mammalian model. The effect of piperine on the fertilization of eggs with sperm has been investigated in female hamsters intragastrically treated with piperine at doses of 50 or 100 mg/kg from day 1 through day 4 of the estrous cycle [76]. During piperine treatment, these females were superovulated and artificially inseminated (AI) with spermatozoa from untreated male hamsters at 12 h after hCG injection. Administration of piperine to the superovulated animals markedly enhanced the percent fertilization at 9 h after AI (Table 45.6).

Table 45.6 Other biological effects of piperine

System	Observed effect	References
Effect on reproductive system		
In vitro	(a) Piperine decreased fertilizing ability of hamster sperms and degree of polyspermia in vitro	[77]
Rats	(b) Continued oral intake of piperine produced reduction in weights of testis, fall in sperm concentration, decrease in intra-testicular testosterone	[78]
Mice	(c) Oral intake of piperine decreased fertility due to interference with crucial reproductive events in albino mice	[75]
Anti-inflammatory activity		
Rats	Anti-inflammatory activity of piperine in experimental models: carrageenan- induced rat paw edema, cotton pellet granuloma, croton oil-induced granuloma pouch	[79]
Hepatoprotective activity		
Mice	Piperine exerted protection against *t*-butyl hydroperoxide and carbon tetrachloride in hepatotoxicity by reducing lipid peroxidation	[80]
Melanocyte stimulation		
In vitro	Growth stimulatory activity of black pepper extract in cultured melanocytes	[81]
Neuropharmacological activity		
Rats	(a) Piperine-administered animals possessed antidepression-like activity and cognitive-enhancing effect	[82]
	(b) Antidepressant-like effects of chronically administered piperine depend on the augmentation of the neurotransmitter synthesis	[83]
Anticonvulsant effects		
Human	Piperine treatment reduced the number of seizures in epileptic children	[84]

Piperine administration (10 mg/kg for 30 days) in mature male albino rats caused a significant reduction in the weights of testis and accessory sex organs [78]. Histological studies revealed that piperine caused severe damage to the seminiferous tubule, decrease in seminiferous tubular and Leydig cell nuclear diameter, and desquamation of spermatocytes and spermatids. The effect of piperine on the fertilizing ability of hamster sperm was investigated in vitro [77]. Addition of $0.18 - 1.05$ mM piperine reduced both the percentage of eggs fertilized and the degree of polyspermia in a dose-dependent manner. Rats orally administered piperine at doses of 1, 10, and 100 mg/kg for 30 consecutive days showed a decrease in the activity of antioxidant enzymes and sialic acid levels in the epididymis and thereby increased reactive oxygen species levels that could damage the epididymal environment and sperm function [85].

1.2.7 Other Physiological Effects

Anti-inflammatory activity of piperine has been reported in rats employing different experimental models like carrageenan-induced rat paw edema, cotton pellet granuloma, and croton oil-induced granuloma pouch [79]. Piperine acted significantly on early acute changes in inflammatory processes and chronic granulative changes. Pungent principles of dietary spices including piperine have been reported to induce a warming action via adrenal catecholamine secretion [86].

Piperine has been reported to exert a significant protection against *t*-butyl hydroperoxide and carbon tetrachloride-induced hepatotoxicity by reducing lipid peroxidation, by leakage of enzymes – alanine aminotransferase and alkaline phosphatase – and by preventing the depletion of glutathione and total thiols in the intoxicated mice [80]. The neuropharmacological activity of piperine-administered Wistar rats (5, 10, and 20 mg/kg once daily) was determined after single, 1, 2, 3, and 4 weeks of treatment [82]. Piperine showed antidepression-like activity and cognitive-enhancing effect at all treatment duration, suggesting its potential to improve brain function. Antidepressant-like effects of piperine have been demonstrated in two depressive models: forced swimming test and tail suspension test [83]. The results indicated that after 2 weeks of chronic administration, piperine (10 − 20 mg/kg) significantly reduced the duration of immobility in both the models. The study demonstrated that the antidepressant-like effects of piperine and antiepilepsirine might depend on the augmentation of the neurotransmitter synthesis or the reduction of the neurotransmitter reuptake.

Melanocyte stimulants are of interest as potential treatments for the depigmentary skin disorder vitiligo. Black pepper water extract and piperine promote melanocyte proliferation in vitro. Black pepper extract was found to possess growth-stimulatory activity in cultured melanocytes [81]. Its aqueous extract at 0.1 mg/mL was observed to cause nearly 300 % stimulation of the growth of a cultured mouse melanocyte line, in 8 days; hence, it is inferred that piperine is a potential repigmenting agent for the treatment of vitiligo.

A study of the in vitro effects of piperine on three bioenergetic reactions, namely, oxidative phosphorylation, ATPase activity, and calcium transport by isolated rat liver mitochondria, suggested that piperine inhibits mitochondrial oxidative phosphorylation at the level of respiratory chain [87]. Piperine did not inhibit the mitochondrial ATPase activity induced by dinitrophenol and was found to diminish calcium uptake. The influence of piperine on the enzymes and bioenergetic functions in isolated rat liver mitochondria and hepatocytes has been studied, and it was observed that piperine produces concentration-related site-specific effects on mitochondrial bioenergetics and enzymes of energy metabolism [88].

Piper longum and *Piper nigrum* are conventionally used as immuno-enhancers in Indian system of medicine. The underlying mechanism however remains unknown. Pepper has been used in China as a folk remedy for epilepsy. Piperine has been identified by researchers as having anticonvulsant effects in animal models, and antiepilepsirine, a derivative of piperine, has been used in China to treat epilepsy since 1975. A clinical trial on epileptic children-tested antiepilepsirine (10 mg/kg; two or three times a day) decreased the number of seizures in majority of subjects [84].

1.3 Absorption and Metabolism of Piperine

Tissue distribution and elimination of piperine has been examined following its oral intake in rats. Piperine administered to rats at a dose of 170 mg/kg by gavage or

85 mg/kg *i.p.* was absorbed to an extent of about 97 % [26, 89]. A maximum of 10.8 % of administered piperine was seen in tissues at 6 h. Only 3 % of the administered dose was excreted as piperine in the feces, while it was not detectable in urine. When rat intestinal segments were incubated with 100–1,000 μg of piperine, about 44–63 % of the added piperine disappeared from the mucosal side [89, 90]. Absorption of piperine in this in vitro system which was maximum at 800 μg per 10 mL was about 63 % [89]. The absorbed piperine could be traced in both the serosal fluid and in the intestinal tissue. When piperine was associated with mixed micelles, its in vitro intestinal absorption was relatively higher [90].

Highest concentration in stomach and small intestine was attained at about 6 h post-piperine dosage [91]. Only traces of piperine were detected in serum, kidney, and spleen from 30 min to 24 h. About $1 - 2.5$ % of the *i.p.*-administered piperine was detected in the liver during $0.5 - 6$ h after administration as contrasted with $0.1 - 0.25$ % of the orally administered dose. The increased excretion of conjugated uronic acids, conjugated sulfates, and phenols indicated that scission of the methylenedioxy group of piperine, glucuronidation, and sulfation appear to be the major steps in the disposition of piperine in the rat. After oral administration of piperine (170 mg/kg) to rats, the metabolites in urine were identified to be piperonylic acid, piperonyl alcohol, and piperonal and vanillic acid in the free form, whereas only piperic acid was detected in $0 - 6$ h bile [91]. Kidney appears to be the major excretion route for piperine metabolites in rats as no metabolite could be detected in feces. In a later investigation [92], a new major urinary metabolite 5-(3,4-methylenedioxy phenyl)-2,4-pentadienoic acid-*N*-(3-yl propionic acid)-amide was detected in rat urine and plasma using HPLC. This metabolite has a unique structure in that it retains methylenedioxy ring and conjugated double bonds while the piperidine ring is modified to form propionic acid group.

The absorption dynamics of piperine in intestine has been studied, and the data suggested that piperine is absorbed very fast across the intestinal barrier [93]. It may act as an apolar molecule and form apolar complex with drugs and solutes. It may modulate membrane dynamics due to its easy partitioning thus helping in efficient permeability across the barriers. Being essentially water insoluble, piperine is presumed to be assisted by serum albumin for its transport in blood after its intestinal absorption. The binding of piperine to serum albumin has confirmed by employing steady-state and time-resolved fluorescence techniques [94]. These observations are significant in understanding the transport of piperine in blood under physiological conditions.

1.4 Conclusions

Black pepper or its main bioactive alkaloid piperine, the ingredients used in a number of ancient and folk medicines, is now demonstrated to possess diverse health beneficial physiological effects. The most far-reaching attribute of piperine has been its inhibitory influence on hepatic and intestinal drug-metabolizing system. It strongly inhibits a particular cytochrome P_{450} and hence phase-I reactions

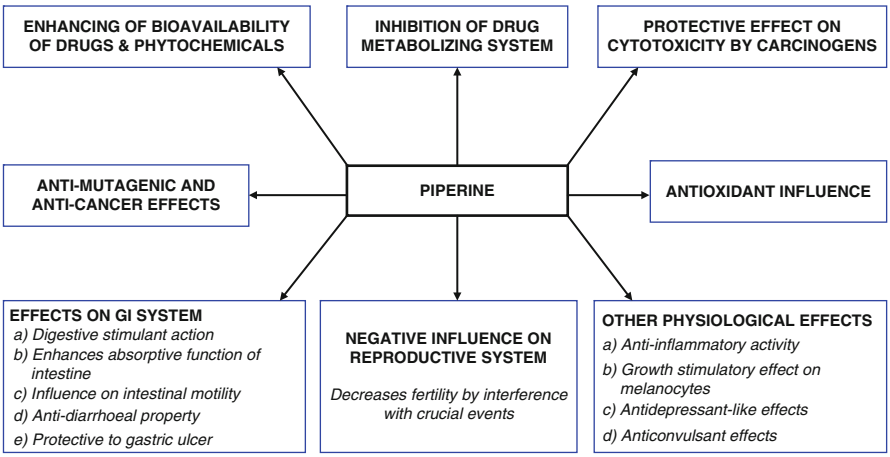

Fig. 45.2 Summary of the diverse physiological effects of piperine

mediated by the same, especially aromatic hydroxylation. It also strongly retards glucuronidation reactions of phase-II. As a result of interference with crucial drug-metabolizing reactions in the liver, piperine enhances the bioavailability of therapeutic drugs, i.e., increases their plasma half-life and delays their excretion. This particular inhibitory effect of piperine on drug metabolism and hence on drug bioavailability may be harnessed for increasing therapeutic effects. Gastrointestinal system is affected by black pepper and piperine in many ways. Both black pepper and piperine have been evidenced to have antidiarrheal property and an effect on intestinal motility and on the ultrastructure of intestinal microvilli improving absorbability of micronutrients. Piperine has been evidenced to protect against oxidative damage by inhibiting or quenching free radicals and lower lipid peroxidation and beneficially influence cellular antioxidant status in different situations of oxidative stress. Piperine also possesses cytoprotective effect by retarding the activation of certain procarcinogens by the drug-metabolizing system. Antimutagenic and antitumor properties of piperine have been evidenced in a few animal and cell line studies. Among other physiological effects piperine exerts, its potential antifertility influence on reproductive system has been clearly established in in vitro and animal systems (Fig. 45.2).

2 Capsaicin of Red Pepper (*Capsicum annuum*)

2.1 Introduction

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) is the major pungent principle of chili peppers, belonging to the genus *Capsicum*. Dihydrocapsaicin and nordihydrocapsaicin are the other two alkaloids in the order of relative abundance. Chili peppers are extensively used in food as pungent spice, particularly in tropical

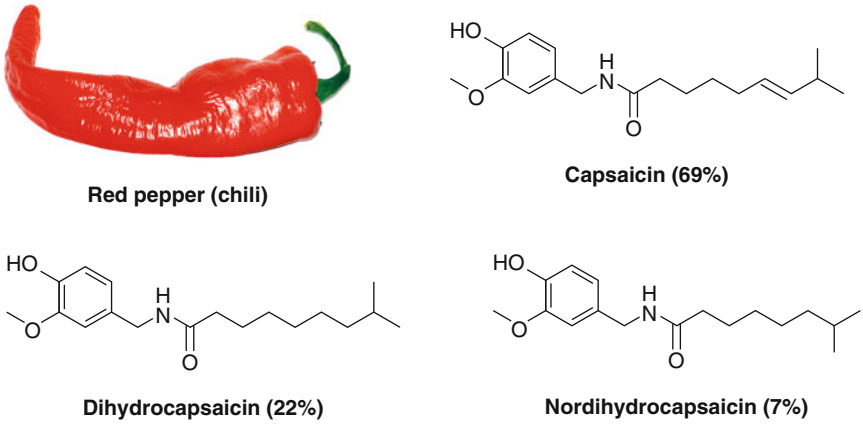

Fig. 45.3 Red pepper and capsaicinoids

countries. This alkaloid responsible for the pungency of the spice is also an irritant that produces a burning sensation with which it comes into contact. Capsaicin and several related compounds are called capsaicinoids and are produced as a secondary metabolite by chili peppers, probably as deterrents against certain herbivores and fungi. Pure capsaicin is a hydrophobic, colorless, odorless, crystalline compound. Capsaicin is the main capsaicinoid in chili peppers, followed by dihydrocapsaicin (Fig. 45.3).

Capsaicin is present in large quantities in the placental tissue (which holds the seeds), the internal membranes, and, to a lesser extent, the other fleshy parts of the fruits of Capsicum plants. The seeds themselves do not produce any capsaicin, although the highest concentration of capsaicin can be found in the white pith of the inner wall, where the seeds are attached [95]. Capsaicin is believed to be synthesized in the interlocular septum of chili peppers by addition of a branched-chain fatty acid to vanillylamine; specifically, capsaicin is made from vanillylamine and 8-methyl-6-nonenoyl Coenzyme-A [96].

2.2 Biological Effects of Capsaicin

Many health beneficial physiological effects of red pepper or its bioactive alkaloid – capsaicin – have been reported in recent decades which are summarized below:

2.2.1 Hypocholesterolemic and Hypolipidemic Effects

Red pepper, known for its characteristic pungency and its pungent principle capsaicin have been reviewed for their biological activity [97, 98] was probably the first investigator who studied the metabolic changes caused by feeding red pepper. The beneficial influence of red pepper or its pungent principle capsaicin on

lipid metabolism is documented by several investigators. While studying the influence of red pepper and capsaicin on fat absorption in rats on a choline-free high hydrogenated fat (40 %) diet, Sambaiah et al. [99] observed that 5 % red pepper or equivalent levels of capsaicin (15 mg%) included in the diet had a tendency to lower serum and liver cholesterol levels. In another investigation, Srinivasan et al. [100] have reported a reduction in serum total cholesterol levels in rats on a 10 % groundnut oil diet incorporated with 1.5, 3.0, or 15 mg% capsaicin. In yet another study, capsaicin at as low as 0.2 mg% in the diet led to a lowering of serum total cholesterol in both 10 % and 30 % fat-fed rats [101]. An increase in LDL-cholesterol and a reduction in HDL-cholesterol were also observed in the 30 % hydrogenated fat group. In a subchronic toxicity study [102], rats were administered 50 mg/kg/day of capsaicin by gavage or 0.5 g/kg/day of a crude extract of capsicum fruit for 60 days. At 30, 40, 50, and 60 days, plasma total cholesterol levels were significantly reduced along with triglycerides and phospholipids (Table 45.7).

The effect of 14 mg% capsaicin in a diet containing 30 % lard has been studied [103]. The dose of capsaicin fed to rats was reported to be related to that commonly ingested by the Thai people. At the end of the 10-day isocaloric feeding period, serum cholesterol and pre-β-lipoprotein levels were not altered. The influence of capsaicin has also been studied in sucrose-induced hypertriglyceridemia in rats [112]. Capsaicin was fed at 0.15, 1.5, and 15 mg% levels in the diet (the lowest dose is comparable to human intake) for a period of 1 week. Total cholesterol and HDL-cholesterol were either significantly elevated.

The efficacy of capsaicin as a hypocholesterolemic agent has also been investigated in animals fed cholesterol in their diets. Sambaiah and Satyanarayana [104] have reported that the serum cholesterol levels in rats on a 1 % cholesterol + 5 % red pepper diet were lower than those not fed with red pepper. Liver cholesterol was lower in the red pepper- as well as capsaicin (an equivalent level of 15 mg%)-fed groups. Fecal excretion of free cholesterol and of bile acids was enhanced in animals fed the spice and capsaicin. The anti-hypercholesterolemic efficacy of dietary capsaicin has been evidenced in rats fed an atherogenic high-cholesterol diet, and such an influence also resulted in countering of the changes in membrane lipid profile in the erythrocytes [105]. In streptozotocin-induced diabetic situation however, dietary capsaicin did not show any beneficial hypolipidemic property [106].

Intubation of rabbits with 8 mg capsaicin/rabbit (body wt of about 850 g/day for 35 days) did not have any effect with regard to plasma cholesterol, triglyceride, and HDL-cholesterol when they were on a normal diet [108]. In contrast, in rabbits on a 0.5 % cholesterol diet, capsaicin had a beneficial effect in that the plasma cholesterol, triglycerides, and total cholesterol: HDL-cholesterol ratio were significantly lower than in animals fed cholesterol only. Turkeys on a 2–3 mg capsaicin/kg feed for 9 days along with 0.5 % cholesterol had lower total serum cholesterol than the controls [109]. Hypercholesterolemia was produced by feeding a 0.2 % cholesterol-supplemented diet, and capsaicin and dihydrocapsaicin were administered daily via the buccal route at dose of 4 mg per bird for 6 weeks [110]. In

Table 45.7 Hypolipidemic effects of capsaicin and red pepper in animal models

Animal model	Effect demonstrated	References
Rats on 40 % fat	5 % red pepper or 0.015 % capsaicin lowered serum and liver cholesterol	[99]
Rats	1.5, 3, and 15 mg% capsaicin reduced serum cholesterol	[100]
Rats on 10 % or 30 % fat	0.2 % capsaicin effectively lowered serum cholesterol	[101]
Rats	Subchronic levels of capsaicin (50 mg/kg for 60 days) lowered cholesterol and triglycerides	[102]
Rats on 30 % lard	14 mg% capsaicin produced hypocholesterolemic effect	[103]
Rats on 1 % cholesterol	Effective hypocholesterolemic effect; higher excretion of fecal sterols and bile acids	[104]
Rats on 0.5 % cholesterol	15 mg% capsaicin produced anti-hypercholesterolemic effect	[105]
Diabetic rats	15 mg% dietary capsaicin did not reverse hypercholesterolemia and hypertriglyceridemia	[106]
Hypercholesterolemic rats	Dietary capsaicin stimulated activity of hepatic cholesterol-7α-hydroxylase	[107]
Hypercholesterolemic rabbits	Reduced blood cholesterol, triglycerides	[108]
Hypercholesterolemic turkeys	Reduced blood cholesterol; ameliorated aortic atherosclerotic lesions by capsaicin	[109, 110]
Gerbils	75 mg oleoresin/kg decreased blood cholesterol and triglycerides; prevented lipid accumulation in liver and aorta	[111]

animals on a normal diet, total cholesterol, LDL-cholesterol, and HDL-cholesterol concentrations in plasma were increased, whereas VLDL-cholesterol was significantly decreased. Plasma total and LDL-cholesterol were significantly lower in birds on the cholesterol diet administered dihydrocapsaicin. Both the compounds brought about a reduction in VLDL-cholesterol and an increase in HDL-cholesterol in the cholesterol-fed group. Dihydrocapsaicin was more effective than capsaicin. The effect of capsicum oleoresin on dietary hypercholesterolemia was observed in gerbils at a dose of 75 mg/kg body weight/day [111]. The oleoresin reduced serum cholesterol and triglycerides as well as liver cholesterol and triglycerides. Capsaicin oleoresin feeding prevented the accumulation of cholesterol and triglycerides in the liver and aorta. The fecal excretion of cholesterol and triglycerides was significantly increased in oleoresin-fed gerbils.

The possible mechanism of action of capsaicinoids is the net effect of decreased cholesterol absorption and increased excretion of cholesterol and bile acids in the feces which may lead to a decrease in plasma LDL-cholesterol concentration by induced expression of hepatic LDL receptors [110]. These authors also have discussed the differences in response between normal and cholesterol-fed animals to possible hypocholesterolemic compounds. It has been demonstrated that dietary capsaicin stimulates hepatic conversion of cholesterol to bile acids through a stimulation of the activity of cholesterol-7α-hydroxylase, an important pathway

for elimination of cholesterol from the body [107]. However, simultaneous stimulation of cholesterol synthesis as well through the activity of HMG-CoA reductase by this spice principle suggests that there may not be any significant contribution of the stimulation of bile acid biosynthesis to the hypocholesterolemic action of this spice principle, and the latter action may solely be due to interference with exogenous cholesterol absorption.

Heat processing of red pepper results in a significant loss of active principle [113, 114]. The hypocholesterolemic potency of raw and pressure-cooked red pepper was evaluated in experimental rats rendered hypercholesterolemic by feeding cholesterol-enriched diet and maintained for 8 weeks on 5 % spice diet [115]. The results suggested that although heat processing of red pepper by pressure cooking resulted in a considerable loss of capsaicin, the hypolipidemic potency of the parent spice was not significantly compromised.

2.2.2 Influence of Capsaicin on Biliary Cholesterol and Bile Acids: Anti-lithogenic Influence

Feeding of 7.5 and 15 mg% capsaicin to rats led to a significant increase in biliary total bile acids [116]. One of the implications of hypocholesterolemic influence is anti-lithogenic potential. Since capsaicin, besides being hypocholesterolemic agent, also enhances bile secretion and influences its composition, its influence on gallstone formation has been examined. Dietary capsaicin (0.015 %) caused a significant reduction in the formation of gallstones in mice and hamsters maintained on a lithogenic diet [117, 118]. Further, capsaicin effected a marked regression of preestablished gallstones in mice [119]. Increased cholesterol saturation index, cholesterol: Phospholipid ratio, and cholesterol: bile acid ratio in the bile caused by lithogenic diet was countered by dietary capsaicin. The anti-lithogenic influence of this spice compound was attributable to the cholesterol-lowering effect of these in blood and liver and their ability to lower cholesterol saturation index by altering the bile composition. When a combination of capsaicin and curcumin were given during experimental induction of cholesterol gallstone (CGS) in mice, there was no additive influence in reducing the incidence of CGS; nevertheless, the combination was more beneficial in reducing the oxidative stress in lithogenic situation [120]. The antilithogenecity of capsaicin has been considered to be due not merely to their ability to lower cholesterol saturation index but also to their influence on biliary proteins [121].

2.2.3 Protective Effect on Erythrocyte Integrity

Hyperlipidemic conditions are believed to affect the fluidity of red blood cells [122]. Hypolipidemic spice compound capsaicin in the diet might offer beneficial protective influence on the integrity of erythrocyte membranes, which are presumably altered in hyperlipidemic situation. In rats rendered hypercholesterolemic by feeding a cholesterol-enriched diet for 8 weeks, erythrocyte membranes were relatively enriched in cholesterol, resulting in elevated cholesterol: phospholipid ratio of their membranes affecting their structural integrity [105]. Inclusion of capsaicin (0.015 %) along with high cholesterol in the diet produced not only the

hypolipidemic effect but also countered this altered lipid profile of erythrocyte membranes and thus corrected the increased osmotic fragility of erythrocytes [105]. Dietary capsaicin partially countered the changes in erythrocytes of hypercholesterolemic rats, namely, fatty acid profile of the membranes, phospholipid composition of the membrane bilayer, and reduced Ca^{2+}, Mg^{2+}-ATPase [123]. ESR spectra and fluorescence anisotropy parameters also revealed altered fluidity of erythrocytes in hypercholesterolemic rats which was significantly reversed by dietary capsaicin. In rats rendered hypertriglyceridemic by maintaining them on a high (30 %)-fat diet for 8 weeks, the lipid profile of erythrocyte membranes was not affected, but the erythrocytes displayed a resistance to osmotic lysis [124]. Inclusion of capsaicin (0.015 %) along with high fat in the diet which produced the hypotriglyceridemic effect appeared to beneficially correct this altered osmotic fragility of erythrocytes.

2.2.4 Antioxidant Effects

Lipid peroxidation in human erythrocyte membranes was found to be inhibited by capsaicin [125]. The antioxidant property of capsaicin in terms of inhibiting lipid peroxidation in rat liver [53] and in soybean phosphatidylcholine liposomal biomembrane has been reported [126]. Capsaicin is observed to inhibit copper ion-induced lipid peroxidation of human LDL [55]. The data suggested that capsaicin is an effective antioxidant and offers protection against oxidation of human LDL. Capsaicin inhibited the lipid peroxidation in rat liver mitochondria induced by ADP/Fe^{2+} significantly, more than the well-known antioxidant α-tocopherol [127]. Capsaicin was also found to scavenge 1,1′-diphenyl-2-picrylhydrazyl (DPPH) radicals in membranes. Capsaicin was found to scavenge radicals 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-lipoxygenase (5-LO) in human PMNL cells, the key enzyme involved in the biosynthesis of leukotrienes [56] (Table 45.8).

Wistar rats administered capsaicin (*i.p.* 3 mg/kg body weight) for three consecutive days showed a reduction of oxidative stress measured as malondialdehyde in the liver, lung, kidney, and muscle [128]. From this study, it is hypothesized that capsaicin can be a potent antioxidant even when consumed for a short period. The influence of capsaicin on the antioxidant status of red blood cells and liver tissue in hyperlipidemic rats is reported [129]. Capsaicin (0.015 %) in the diet which produced the hypotriglyceridemic effect was also effective in reducing the oxidant stress, which was indicated by countering of the depleted antioxidant molecules and antioxidant enzymes in erythrocytes and liver, and decreasing of the elevated lipid peroxide content. The beneficial influence of capsaicin on the antioxidant status of red blood cells and liver in induced hypercholesterolemic rats is also evidenced [130]. The depletion in intracellular thiols and GSH in red blood cells under hypercholesterolemic situation was effectively countered by dietary (0.015 %)

Table 45.8 Antioxidant influence of capsaicin in in vitro and in vivo systems

Animal model	Effect demonstrated	References
Human erythrocyte membranes	Lipid peroxidation was inhibited by capsaicin	[125]
Rat liver microsomes	Ascorbate-Fe^{++}-induced lipid peroxidation was inhibited by capsaicin	[53]
Soybean phospholipid liposomal membrane	Inhibition of oxidation of methyl linoleate micelles by capsaicin	[126]
Rat liver mitochondria	Inhibition of lipid peroxidation induced by ADP/Fe^{2+} and scavenging of DPPH radicals by capsaicin	[127]
Human low-density lipoprotein	Inhibition of Cu^{2+} induced lipid peroxidation by capsaicin	[55]
Human PMNL cells	Inhibition of 5-lipoxygenase	[56]
Rats	Capsaicin administration reduced oxidative stress in the liver, lung, kidney, and muscle	[128]
High-fat-fed rats	Beneficial influence of dietary capsaicin on antioxidant status of red blood cells	[129]
Hypercholesterolemic rats	Beneficial influence of dietary capsaicin on antioxidant status of red blood cells	[130]

capsaicin. Glutathione reductase activity that was lowered in hypercholesterolemic conditions was completely countered by the dietary spice principle. Decreased hepatic total thiols in the hypercholesterolemic situation were partially corrected by dietary capsaicin treatment. Similarly, the lowered activities of hepatic antioxidant enzymes – GSH-reductase, GSH-transferase, catalase, and superoxide dismutase – in hypercholesterolemic rats were effectively countered by the dietary capsaicin.

2.2.5 Anti-inflammatory Property

With increasing interest in alternatives to nonsteroidal anti-inflammatory agents in the management of chronic inflammation, the use of food-based approaches is emerging. Lipid peroxides play a crucial role in arthritis and other inflammatory diseases. Both in vitro and in vivo animal studies have documented the anti-inflammatory potential of capsaicin (of red pepper). Animal studies have revealed that capsaicin also lowers the incidence and severity of paw inflammation and also delays the onset of adjuvant-induced paw edema in rats [131, 132]. This spice principle inhibited the formation of arachidonate metabolites (PgE_2, leukotrienes) and increased the secretion of lysosomal enzymes – elastase, collagenase, and hyaluronidase – by macrophages. It is noteworthy that the levels of 6-keto PgF_{1a} – a vasodilator – increased [133].

Natural anti-inflammatory compound capsaicin appears to operate by inhibiting one or more of the steps linking pro-inflammatory stimuli with COX activation, such as the blocking by capsaicin of NF-kB translocation into the nucleus [134].

It has been shown recently that the natural anti-inflammatory compounds such as capsaicin were as effective as indomethacin (a nonsteroidal anti-inflammatory drug) in inhibiting aberrant crypt foci in the rat.

2.2.6 Chemopreventive Potential

This phytochemical has been found to interact with microsomal xenobiotic-metabolizing enzymes in rodents. Capsaicin has been proposed to inactivate cytochrome P-450 HE1 by irreversibly binding to the active sites of the enzyme [135]. Besides cytochrome P-450 HE1, other isoforms of the P-450 super family are also reported to be inhibited by capsaicin. The inhibition by capsaicin of microsomal monooxygenases involved in carcinogen activation implies its chemopreventive potential.

Studies suggest that capsaicin is able to kill prostate cancer cells by causing them to undergo apoptosis [136]. The studies were performed on tumors formed by human prostate cancer cell cultures grown in mouse models, and showed tumors treated with capsaicin were about one fifth the size of the untreated tumors. There have been several clinical studies conducted in Japan and China that showed capsaicin directly inhibits the growth of leukemic cells [137]. Another study suggests capsaicin is able to trigger apoptosis in human lung cancer cells as well. An epidemiological study has found significantly higher rates for stomach and liver cancer in counties inhabited by groups with high consumption of capsaicin-rich foods [138].

2.2.7 Antidiabetic Potential

Substance P, a neuropeptide released by capsaicin, has been shown to reverse diabetes in mice [139], but the effects to insulin secretion seem to be species dependent. In humans, substance P seems to decrease insulin release and cause fluctuations in blood sugar levels [140]. Capsaicin is also being explored as a possible prophylaxis for type 1 diabetes. Capsaicin was injected subcutaneously in neonatal diabetes-prone NOD mice to permanently remove a prominent subset of pancreatic sensory neurons, which express the transient vanilloid receptor protein (TRPV1). Insulin resistance and β cell stress of prediabetic NOD mice are prevented when TRPV1+ neurons are eliminated. In other words, mice which were genetically predisposed to type 1 diabetes were prevented from developing type 1 diabetes via removal of these neurons, which are thought to attract pathogenic T cells to attacking pancreatic β cells thus causing type 1 diabetes [141].

2.2.8 Thermogenic and Weight-Reducing Influence

According to animal and human studies, the oral intake of capsaicin increases the production of heat by the body for a short time. Dietary red pepper or its pungent principle capsaicin affects satiety and has a promising thermogenic influence that could play an important role in the prevalence and severity of obesity [103], although more data are required to substantiate this benefit. The use of this spice to displace fats and salt in the diet (to make the food palatable) may reduce cardiovascular risk. Although there is no evidence showing that weight loss is

directly correlated with ingesting capsaicin, there is a positive correlation between ingesting capsaicin and a decrease in weight regain. Capsaicin is said to cause a shift in substrate oxidation from carbohydrate to fat oxidation [142] which leads to a decrease in appetite as well as a decrease in food intake. Both oral and gastrointestinal exposure to capsaicin increase satiety and reduce energy as well as fat intake [143]. Oral exposure proves to yield stronger reduction suggesting that capsaicin has sensory effects. Short-term studies suggest that capsaicin aids in the decrease of weight regain. However, long-term studies are limited because of the pungency of capsaicin [144]. Another recent study has suggested that the ingestion of capsaicinoids can increase levels of brown adipose tissue through an increase in energy expenditure and oxidation caused by the capsaicin [145]. In yet another recent study, the beneficial effects of dietary tender cluster beans (*Cyamopsis tetragonoloba*) in checking the weight gain and adverse changes in lipid profile in high-fat-fed condition were potentiated by co-administration of capsaicin in rats [146].

2.2.9 Antiulcer Activity

In recent years, infection of the stomach with *Helicobacter pylori* which disrupts the normal inhibitory control for acid secretion resulting in excess acid destroying the mucosal barrier has been understood to be the main cause of gastric ulcers [147]. Excessive acid secretion in the stomach and reduction in gastric mucosal blood flow are considered responsible for ulcer formation. The colonization of *H. pylori* in the stomach is associated with a phospholipase likely to damage the protective layer of stomach. *H. pylori* thrives in the stomach by producing the enzyme urease.

In view of its irritant and likely acid-secreting nature, persons with ulcers were being advised to avoid consumption of red pepper (chili). However, recent studies have revealed that capsaicin of red pepper is not the cause for ulcer formation but a benefactor. Numerous studies suggest that eating hot peppers regularly is protective against stomach cancer [148]. Detailed studies have revealed that capsaicin per se does not stimulate but inhibits acid secretion, stimulates alkali and mucus secretions and gastric mucosal blood flow which help in disposing of acid from the stomach, thus prevention and healing of ulcers [149]. Capsaicin acts by stimulating afferent neurons in the stomach and signals for protection against injury causing agents. An epidemiological study has found three times higher peptic ulcer incidence among Chinese population in Singapore as compared to Malaysians and Indians who are in the habit of consuming more pungent chili in their daily diets [150].

Interestingly, capsaicin has been found to specifically inhibit the growth of *H. pylori*. Capsaicin inhibits also the release of gastrin and stimulates that of somatostatin, the physiological inhibitor of acid secretion. It is also a potent inhibitor of NF-kB whose activation may lead to various pathological conditions and reactive oxygen species. Phosphodiesterase inhibitors are powerful vasodilatory agents and their likely increase of cAMP levels has an antiulcer effect. Capsaicin is a phosphodiesterase inhibitor and may exert its protective effect in this way besides its stimulation of gastric mucosal blood flow. Numerous studies have substantiated

the protective role of capsaicin. Seminal to these studies is the discovery about the selective sensitization and desensitization of unmyelinated neurons by capsaicin [151]. Reactive oxygen species are known to be involved in the pathogenesis of gastritis, gastric ulcers, and gastric cancer. Capsaicin has proved to be an antioxidant protecting cellular membranes, cardiac and skeletal muscles, etc., against reactive oxygen species. Capsaicin inhibited lipid peroxidation induced by ethanol in the gastric mucosa [152].

2.2.10 Capsaicin in Pain Relief

Capsaicin has received considerable attention as a pain reliever. In two trials with 70 and 21 patients with osteoarthritis and rheumatoid arthritis, topical application of creams containing 0.025 % or 0.075 % capsaicin was an effective and safe alternative to analgesics employed in systemic medications which are often associated with potential side effects [153, 154]. Capsaicin has also been suggested for the initial management of neuralgia consequent to herpes infection [155].

Capsaicin has been shown to be useful in diabetic neuropathy. In a study involving 219 patients, topical application of 0.075 % capsaicin cream was effective in pain management [156]. Capsaicin is currently used in topical ointments, as well as a high-dose dermal patch (under the trade name *Qutenza*), to relieve the pain of peripheral neuropathy such as post-herpetic neuralgia caused by shingles [157]. It may be used in concentrations of between 0.025 % and 0.075 % as a cream for the temporary relief of minor aches and pains of muscles and joints associated with arthritis, simple backache, strains, and sprains, often in combination with other rubefacients [157]. Capsaicin creams are used to treat psoriasis as an effective way to reduce itching and inflammation [158, 159]. Capsaicin is also the key ingredient in the experimental drug *Adlea*, which is in Phase 2 trials as a long-acting analgesic to treat postsurgical and osteoarthritis pain [157]. Moreover, it reduces pain resulted from rheumatoid arthritis [160] as well as joint or muscle pain from fibromyalgia.

2.2.11 Beneficial Influences on Gastrointestinal System

Beneficial Modulation of Small Intestinal Ultrastructure

The beneficial influence of dietary capsaicin has been examined in experimental rats with respect to (i) the membrane fluidity of intestinal brush-border membranes (BBM), (ii) the activity of intestinal membrane-bound enzymes, and (iii) the ultrastructural alterations in the intestinal epithelium [46]. In this study, Wistar rats were maintained on dietary red pepper (3·0 %) and its bioactive compound capsaicin (0·01 %) for 8 weeks. A membrane fluidity study using an apolar fluorescent probe showed increased BBM fluidity in the spice-fed or capsaicin-fed animals. This was corroborated by decreased cholesterol: phospholipid ratio in the jejunal and ileal regions of the intestine. These dietary spices stimulated the activities of BBM enzymes (glycyl-glycine dipeptidase, leucine amino peptidase, and γ-glutamyl transpeptidase) in the jejunal mucosa, suggesting a modulation in membrane dynamics due to the apolar spice bioactive compound interacting with surrounding lipids and hydrophobic portions in the protein vicinity, which may decrease the tendency of membrane lipids to act as steric constraints to enzyme

proteins and thus modify enzyme conformation. Scanning electronic microscopy of the intestinal villi in these spice treatments revealed alterations in the ultrastructure, especially an increase in microvilli length which would mean a beneficial increase in the absorptive surface of the small intestine, providing for an increased bioavailability of micronutrients. Thus, dietary red pepper or capsaicin was evidenced to induce alterations in BBM fluidity and passive permeability property, associated with the induction of an increased microvilli length, resulting in an increased absorptive surface of the small intestine.

Digestive Stimulant Action

The digestive stimulant action of spices is probably exerted through stimulation of the liver to produce and secrete bile rich in bile acids, which play a very important role in fat digestion and absorption. Capsaicin has been examined for its effect on bile secretion in rats, after dietary intake for a period of time or as a one-time oral intake [116]. The hypocholesterolemic spice compound capsaicin stimulated bile acid production by the liver and its secretion into bile. The influence of dietary intake and single-dose administration of capsaicin on the pancreatic digestive enzymes and the terminal digestive enzymes of the small intestinal mucosa has been reported [32, 33]. Dietary intake of capsaicin stimulated pancreatic lipase activity significantly. In contrast to the continued intake, single oral dose consumption of capsaicin failed to exert a stimulatory effect on pancreatic lipase. Pancreatic amylase activity was elevated by dietary capsaicin (72 %) as well as single-dose administration of capsaicin. Capsaicin when incorporated in the diet, stimulated trypsin activity by over 100 %. Chymotrypsin was also significantly higher in animals fed capsaicin. Similar influence of the spice compound on the activity of proteases was not evident when administered as a single oral dose. Capsaicin prominently enhanced the activity of intestinal lipase. The stimulation of this enzyme activity was more than 100 % of the control in spice principle-treated group. Similarly, dietary capsaicin significantly increased the activity of intestinal amylase. Dietary capsaicin moderately stimulated the activities of intestinal disaccharidases.

Based on the evidences from animal studies, the well-recognized digestive stimulant action of red pepper or its active compound capsaicin may be considered to be mediated through two possible modes: (1) stimulation of the liver to secrete more bile enriched in bile acids and (2) stimulation of enzyme activities that participate in digestion, both of pancreatic and intestinal origin. Such stimulation of bile secretion and of the activities of digestive enzymes leads to an accelerated overall digestive process, resulting in a significant reduction in the duration of passage of food through the gastrointestinal tract [35].

Since capsaicin is known to stimulate secretion of bile with higher amount of bile acids which play a major role in digestion and absorption of dietary lipids, capsaicin has been studied to verify if it enables efficient digestion and absorption during high-fat intake [34]. In this context, dietary capsaicin (0.015 %) has been examined for its influence on bile secretion, digestive enzymes of pancreas, and absorption of dietary fat in high-fat (30 %)-fed rats for 8 weeks. Dietary capsaicin enhanced the activity of pancreatic lipase, amylase, trypsin, and chymotrypsin and

enhanced dietary fat absorption. It also increased bile secretion with higher bile acid content. Stimulation of lipid mobilization from adipose tissue was suggested by the decrease in perirenal adipose tissue weight by dietary capsaicin. This was also accompanied by prevention of the accumulation of triglyceride in liver and serum in high-fat-fed rats. Activities of key lipogenic enzymes in liver were reduced which was accompanied by an increased activity of hormone-sensitive lipase. Thus, dietary capsaicin enhances fat digestion and absorption in high-fat-fed situation through enhanced secretion of bile salts and a stimulation of the activity pancreatic lipase. At the same time, the energy expenditure is facilitated by this spice compound to prevent the accumulation of absorbed fat.

Enhanced Absorption of Micronutrients

Since dietary pungent spices may alter the ultrastructure and permeability characteristics of intestines, capsaicin has been examined for a possible influence on intestinal absorption of iron, zinc, and calcium by examining their uptake by the intestines from rats pre-fed spice compound for 8 weeks [47]. Everted segments of duodenum, jejunum, and ileum portions of small intestines isolated from these rats were examined for ex vivo uptake of iron, zinc, and calcium from incubations containing digesta of finger millet. Higher in vitro absorption of iron, zinc, and calcium in the intestines was evidenced in capsaicin-fed animals. The positive influence of dietary capsaicin on the mineral uptake by the intestinal segments was highest for calcium. The positive influence of dietary capsaicin was more pronounced on zinc uptake as compared to that of iron. These pungent spices alter permeation characteristics presumably by increasing absorptive surface and thereby enhance intestinal absorption of micronutrients.

Dietary red pepper and capsaicin which alter the ultrastructure and permeability characteristics of intestines are also reported to favorably enhance the intestinal uptake of β-carotene in vitro [48]. In an animal study conducted to evaluate the influence of dietary spice compounds on the absorption of orally administered β-carotene and its conversion to vitamin A, hepatic β-carotene was significantly increased in capsaicin-fed rats suggesting improved absorption of β-carotene [49]. Retinol concentration was not however changed in these animals suggesting that bioconversion of β-carotene to vitamin A was not similarly influenced. Among the two enzymes involved in the bioconversion of β-carotene to vitamin A, activity of intestinal and hepatic β-carotene-15,15′-dioxygenase was lowered in capsaicin treatment, while the activity of intestinal and hepatic retinal reductase was unaffected. Activity of intestinal and hepatic β-carotene-15,15′-dioxygenase was also inhibited by capsaicin in vitro, thus corroborating with in vivo observation.

2.3 Mechanism of Burning and Painful Sensation of Capsaicin

Capsaicin causes a burning sensation when it comes in contact with mucous membranes. The burning and painful sensations associated with capsaicin result from its chemical interaction with sensory neurons. Capsaicin, as a member of the

vanilloid family, binds to a vanilloid receptor (TRPV1) [161], an ion channel-type receptor that resides on the membranes of pain and heat sensing neurons [162]. VR1, which can also be stimulated with heat and physical abrasion, permits cations to pass through the cell membrane and into the cell when activated. The resulting depolarization of the neuron stimulates it to signal the brain. By binding to the VR1 receptor, capsaicin produces the same sensation that excessive heat or abrasive damage would cause.

The TRPV1 ion channel has been shown to be a member of the super family of TRP ion channels. There are a number of different TRP ion channels that have been shown to be sensitive to different ranges of temperature. Thus, capsaicin does not actually cause a chemical burn, or indeed any direct tissue damage at all, consequent to exposure to chili peppers. The inflammation resulting from exposure to capsaicin is believed to be the result of the body's reaction to nerve excitement. TRPV1 is a heat-activated calcium channel, which opens between 37 °C and 45 °C. When capsaicin binds to TRPV1, it causes the channel to open at temperature below 37 °C (normal human body temperature), which is why capsaicin is linked to the sensation of heat. Prolonged activation of these neurons by capsaicin depletes presynaptic substance P, one of the body's neurotransmitters for pain and heat. Neurons that do not contain TRPV1 are unaffected. Thus, capsaicin mimics a burning sensation, the nerves being overwhelmed by the influx. Capsaicin will be unable to evoke pain for an extended period of time since with chronic exposure, neurons are depleted of neurotransmitters, leading to reduction in sensation of pain and blockade of neurogenic inflammation. If capsaicin is removed, the neurons recover [163, 164]. The mode of action of capsaicin in inducing bronchoconstriction is thought to involve stimulation of C fiber culminating in the release of neuropeptides [165]. Essentially, the body inflames tissues as if it has undergone a burn or abrasion, and the resulting inflammation can cause tissue damage in cases of extreme exposure.

2.4 Absorption and Metabolism of Capsaicin

Capsaicin fed to rats was rapidly absorbed from the stomach, with 85 % of a 3-mg dose absorbed within 3 h [166]. Doses of 5.12 mg/mouse/week led to maximum plasma concentrations of 51.5 ng/mL and 84.8 ng/mL in male and female mice, respectively [167]. Little absorption of capsaicin also occurs across the skin. When 0.8 g of gel containing 0.075 % of capsaicin was applied to the skin of human volunteers, the average absorbed dose after 8 h of exposure was 22.7 μg/cm^2 [168]. Topical application of pure capsaicin to the skin of mice resulted in peak plasma concentrations occurring 4–12 h later, and capsaicin was detectable in the blood 24 h after dosing [167].

Rats injected intravenously accumulated capsaicin primarily in the brain and spinal cord 3 min after dosing, with lower levels found in the liver and blood, while 10 min after dosing, the greatest concentrations remained in the spinal cord [169]. Subcutaneously injected capsaicin was detected in all tissues of rat 10 min

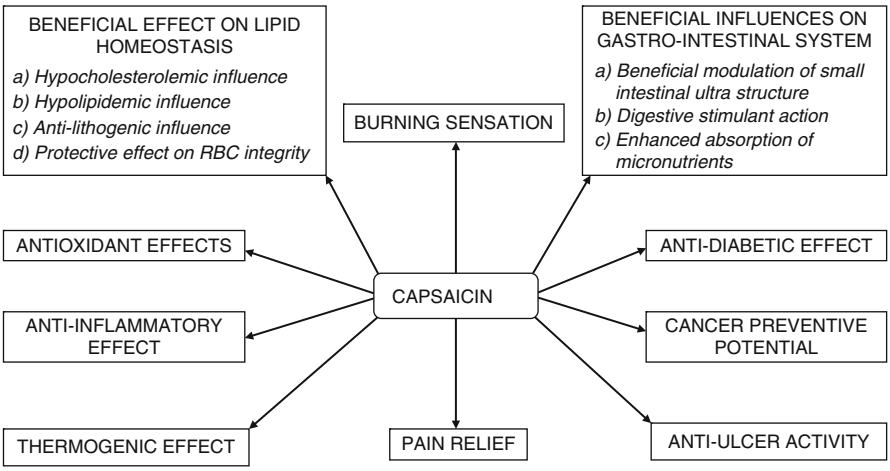

Fig. 45.4 Summary of the diverse physiological effects of capsaicin

following dosing, but residues were undetectable in any tissues 17 h later. Blood concentrations peaked 5 h following dosing, and brain and spinal cord tissue concentrations were somewhat lower. Kidneys contained the greatest concentrations and liver concentrations were low presumably due to metabolic breakdown of the capsaicin [169]. Tissue distribution and elimination of capsaicin has been examined following its oral intake (30 mg capsaicin/kg body weight) in rats [26]. Maximum distribution of 24.4 % of administered capsaicin was seen at 1 h, while no intact capsaicin was detectable after 4 days. Absorption of capsaicin was about 94 % and very rapid.

Metabolism of capsaicin occurs primarily in the liver in the rat [170, 171]. Although the same metabolites were produced, the relative amounts of each metabolite were species dependent. Metabolism of capsaicin by P_{450} enzymes may follow a number of pathways and produce a variety of metabolites, some of which may be associated with increased toxicity [172]. Less than 10 % of an oral dose of capsaicin given to rats was excreted unchanged 48 h after dosing [166].

2.5 Conclusions

The pungent principle of red pepper (hot chili) capsaicin is endowed with several biological activities which are of pharmacological relevance. These include thermogenic influence, effects on gastrointestinal system, cardioprotective influence, anti-lithogenic effect, and anti-inflammatory and pain-relieving effect. The involvement of neuropeptide substance P, serotonin, and somatostatin in the pharmacological actions of capsaicin has been extensively investigated. Tropical application of capsaicin has been proved to alleviate pain in arthritis, postoperative neuralgia, diabetic neuropathy, psoriasis, etc. Contrary to the general belief,

capsaicin inhibits acid secretion, stimulates alkali and mucus secretion, and stimulates gastric mucosal blood flow, all of which help in prevention and healing of gastric ulcers. Antioxidant and anti-inflammatory properties of capsaicin are established in a number of in vitro and in vivo studies. Chemopreventive potential of capsaicin has been evidenced in a few animal and cell line studies. The hypocholesterolemic influence of capsaicin has additional implications in the prevention of cholesterol gallstone disease and protection of the structural integrity of erythrocytes under conditions of hypercholesterolemia. Beneficial influences of capsaicin on gastrointestinal system include digestive stimulant action and modulation of small intestinal ultrastructure so as to enhance permeability to micronutrients (Fig. 45.4).

References

1. Achaya KT (1994) Indian food: a historical companion. Oxford University Press, Delhi
2. Govindarajan VS (1977) Pepper – chemistry, technology & quality evaluation. Crit Rev Food Sci Nutr 9:115–225
3. Nadkarni KM, Nadkarni AK (1976) Indian Materia Medica. Popular Prakashan Pvt. Ltd., Mumbai
4. Johri RK, Zutshi U (1992) An Ayurvedic formulation 'trikatu' and its constituents. J Ethnopharmacol 37:85–91
5. Singh MB (1994) Maternal beliefs and practices regarding the diet and use of herbal medicines during measles and diarrhea in rural areas. Indian Pediatr 31:340–343
6. Bensky D (2004) Chinese herbal medicine: materia medica, 3rd edn (trans: Bensky D, Clavey S, Stoger E, Gamble A). Eastland Press, Seattle
7. Srinivasan K (2007) Black pepper and its pungent principle – piperine: a review of diverse physiological effects. Crit Rev Food Sci Nutr 47:735–748
8. Atal CK, Dubey RK, Singh J (1985) Biochemical basis of enhanced drug bioavailability of piperine: evidence that piperine is a potent inhibitor of drug metabolism. J Pharmacol Exp Ther 232:258–262
9. Singh J, Dubey RK, Atal CK (1986) Piperine-mediated inhibition of glucuronidation activity in isolated epithelial cells of the guinea-pig small intestine: evidence that piperine lowers the endogenous UDP-glucuronic acid content. J Pharmacol Exp Ther 236:488–493
10. Reen RK, Singh J (1991) *In vitro* and *in vivo* inhibition of pulmonary cytochrome P450 activities by piperine, a major ingredient of piper species. Indian J Exp Biol 29:568–573
11. Reen RK, Roesch SF, Kiefer F, Wiebel FJ, Singh J (1996) Piperine impairs cytochrome P4501A1 activity by direct interaction with the enzyme and not by down regulation of CYP1A1 gene expression in rat hepatoma 5 L cell line. Biochem Biophys Res Commun 218:562–569
12. Suresh D, Srinivasan K (2006) Influence of curcumin, capsaicin, and piperine on the rat liver drug-metabolizing enzyme system *in vivo* and *in vitro*. Can J Physiol Pharmacol 84:1259–1265
13. Dalvi RR, Dalvi PS (1991) Comparison of the effects of piperine administered *intra gastrically* and intraperitoneally on the liver and liver mixed function oxidases in rats. Drug Metab Drug Interact 9:23–30
14. Reen RK, Jamwal DS, Taneja SC, Koul JL, Dubey RK, Wiebel FJ, Singh J (1993) Impairment of UDP-glucose dehydrogenase and glucuronidation activities in liver and small intestine of rat and guinea pig *in vitro* by piperine. Biochem Pharmacol 46:229–238

15. Dalvi RR, Dalvi PS (1991) Differences in the effects of piperine and piperonyl butoxide on hepatic drug-metabolizing enzyme system in rats. Drug Chem Toxicol 14:219–229
16. Volak LP, Ghirmai S, Cashman JR, Court MH (2008) Curcuminoids inhibit multiple human cytochromes P450, UDP-glucuronosyl transferase, and sulfotransferase enzymes, whereas piperine is a relatively selective CYP3A4 inhibitor. Drug Metab Dispos 36:1594–1605
17. Hu Z, Yang Z, Ho PC, Chan SY, Heng PW, Chan E (2005) Herb-drug interactions: a literature review. Drugs 65:1239–1282
18. Atal CK, Zutshi U, Rao PG (1981) Scientific evidence on the role of Ayurvedic herbals on bioavailability of drugs. J Ethnopharmacol 4:229–232
19. Bano G, Raina RK, Zutshi U, Bedi KL, Johri RK, Sharma SC (1991) Effect of piperine on bioavailability and pharmacokinetics of propranolol and theophylline in healthy volunteers. Eur J Clin Pharmacol 41:615–617
20. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS (1998) Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Med 64:353–356
21. Badmaev VV, Majeed M, Prakash L (2000) Piperine derived from black pepper increases the plasma levels of coenzyme Q10 following oral supplementation. J Nutr Biochem 11:109–113
22. Pattanaik S, Hota D, Prabhakar S, Kharbanda P, Pandhi P (2006) Effect of piperine on the steady-state pharmacokinetics of phenytoin in patients with epilepsy. Phytother Res 20:683–686
23. Kasibhatta R, Naidu MU (2007) Influence of piperine on the pharmacokinetics of nevirapine under fasting conditions: a randomized, crossover, placebo-controlled study. Drugs 8:383–391
24. Allameh A, Saxena M, Biswas G, Raj HG, Singh J, Srivastava N (1992) Piperine, a plant alkaloid of the piper species, enhances the bioavailability of aflatoxin B1 in rat tissues. Cancer Lett 61:195–199
25. Hiwale AR, Dhuley JN, Naik SR (2002) Effect of co-administration of piperine on pharmaco-kinetics of β-lactam antibiotics in rats. Indian J Exp Biol 40:277–281
26. Suresh D, Srinivasan K (2010) Tissue distribution and elimination of capsaicin, piperine and curcumin following oral intake in rats. Indian J Med Res 131:682–691
27. Velpandian T, Jasuja R, Bhardwaj RK, Jaiswal J, Gupta SK (2001) Piperine in food: interference in the pharmacokinetics of phenytoin. Eur J Drug Metab Pharmacokinet 26:241–247
28. Lambert JD, Hong J, Kim DH, Mishin VM, Yang CS (2004) Piperine enhances the bioavailability of the tea polyphenol (−)-epigallocatechin-3-gallate in mice. J Nutr 134:1948–1952
29. Glatzel H (1968) Physiological aspects of flavour compounds. Ind Spices 5:13–21
30. Platel K, Srinivasan K (2004) Digestive stimulant action of spices: a myth or reality? Indian J Med Res 119:167–179
31. Bhat BG, Chandrasekhara N (1987) Effect of black pepper and piperine on bile secretion and composition in rats. Nahrung 31:913–916
32. Platel K, Srinivasan K (2000) Influence of dietary spices or their active principles on pancreatic digestive enzymes in albino rats. Nahrung 44:42–46
33. Platel K, Srinivasan K (1996) Influence of dietary spices or their active principles on digestive enzymes of small intestinal mucosa in rats. Int J Food Sci Nutr 47:55–59
34. Prakash UNS, Srinivasan K (2011) Fat digestion and absorption in spice pretreated rats. J Sci Food Agric 92:503–510
35. Platel K, Srinivasan K (2001) Studies on the influence of dietary spices on food transit time in experimental rats. Nutr Res 21:1309–1314
36. Bajad S, Bedi KL, Singla AK, Johri RK (2001) Piperine inhibits gastric emptying and gastrointestinal transit in rats and mice. Planta Med 67:176–179

37. Izzo AA, Capasso R, Pinto L, Di Carlo G, Mascolo N, Capasso F (2001) Effect of vanilloid drugs on gastrointestinal transit in mice. Br J Pharmacol 132:1411–1416
38. Myers BM, Smith JL, Graham DY (1987) Effect of red pepper and black pepper on the stomach. Am J Gastroenterol 82:211–214
39. Vasudevan K, Vembar S, Veeraraghavan K, Haranath PS (2000) Influence of intra-gastric perfusion of aqueous spice extracts on acid secretion in anesthetized albino rats. Indian J Gastroenterol 19:53–56
40. Ononiwu IM, Ibeneme CE, Ebong OO (2002) Effects of piperine on gastric acid secretion in albino rats. Afr J Med Sci 31:293–295
41. Bai YF, Xu H (2000) Protective action of piperine against experimental gastric ulcer. Acta Pharmacol 21:357–359
42. Prakash UNS, Srinivasan K (2010) Gastrointestinal protective effect of dietary spices during ethanol-induced oxidant stress in experimental rats. Appl Physiol Nutr Metab 35:134–141
43. Capasso R, Izzo AA, Borrelli F, Russo A, Sautebin L, Pinto A et al (2002) Effect of piperine, the active ingredient of black pepper, on intestinal secretion in mice. Life Sci 71:2311–2317
44. Johri RK, Thusu N, Khajuria A, Zutshi U (1992) Piperine-mediated changes in the permeability of rat intestinal epithelial cells: status of γ-glutamyl transpeptidase activity, uptake of amino acids and lipid peroxidation. Biochem Pharmacol 43:1401–1407
45. Khajuria A, Thusu N, Zutshi U (2002) Piperine modulates permeability characteristics of intestine by inducing alterations in membrane dynamics: influence on brush border membrane fluidity, ultrastructure and enzyme kinetics. Phytomedicine 9:224–231
46. Prakash UNS, Srinivasan K (2010) Beneficial influence of dietary spices on the ultra structure and fluidity of intestinal brush border in experimental rats. Br J Nutr 104:31–39
47. Prakash UNS, Srinivasan K (2012) Enhanced intestinal uptake of trace minerals in spice pretreated rats. J Trace Elem Med Biol (in press)
48. Veda S, Srinivasan K (2009) Influence of dietary spices – black pepper, red pepper and ginger on the uptake of β-carotene by rat intestines. J Funct Food 1:394–398
49. Veda S, Srinivasan K (2011) Influence of dietary spices on the *in vivo* absorption of ingested β-carotene in experimental rats. Br J Nutr 105:1429–1438
50. Vazquez-Olivencia W, Shah P, Pitchumoni CS (1992) The effect of red and black pepper on orocecal transit time. J Am Coll Nutr 11:228–231
51. Bajad S, Bedi KL, Singla AK, Johri RK (2001) Antidiarrhoeal activity of piperine in mice. Planta Med 67:284–287
52. Mittal R, Gupta RL (2000) *In vitro* antioxidant activity of piperine. Exp Clin Pharmacol 22:271–274
53. Reddy ACP, Lokesh BR (1992) Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. Mol Cell Biochem 111:117–124
54. Gülçin I (2005) The antioxidant and radical scavenging activities of black pepper (*Piper nigrum*) seeds. Int J Food Sci Nutr 56:491–499
55. Naidu KA, Thippeswamy NB (2002) Inhibition of human low density lipoprotein oxidation by active principles from spices. Mol Cell Biochem 229:19–23
56. Prasad NS, Raghavendra R, Lokesh BR, Naidu KA (2004) Spice phenolics inhibit human PMNL 5-lipoxygenase. Prostagl Leukotr Essent Fatty Acids 70:521–528
57. Khajuria A, Thusu N, Zutshi U, Bedi KL (1998) Piperine modulation of carcinogen induced oxidative stress in intestinal mucosa. Mol Cell Biochem 189:113–118
58. Rauscher FM, Sanders RA, Watkins JB (2000) Effects of piperine on antioxidant pathways in tissues from normal and streptozotocin-induced diabetic rats. J Biochem Mol Toxicol 14:329–334
59. Vijayakumar RS, Surya D, 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

60. Selvendiran K, Senthilnathan P, Magesh V, Sakthisekaran D (2004) Modulatory effect of Piperine on mitochondrial antioxidant system in benzo(α)pyrene-induced experimental lung carcinogenesis. Phytomedicine 11:85–89
61. Selvendiran K, Sakthisekaran D (2004) Chemopreventive effect of piperine on modulating lipid peroxidation and membrane bound enzymes in benzo(α)pyrene induced lung carcinogenesis. Biomed Pharmacother 58:264–267
62. Hamss E, Idaomar M, Alonso-Moraga A, Munoz-Serrano A (2003) Antimutagenic properties of bell and black peppers. Food Chem Toxicol 41:41–47
63. Sunila ES, Kuttan G (2004) Immunomodulatory and antitumor activity of *Piper longum* Linn. and piperine. J Ethnopharmacol 90:339–346
64. Singh J, Reen RK, Wiebel FJ (1994) Piperine, a major ingredient of black and long peppers, protects against AFB1-induced cytotoxicity and micronuclei formation in H4IIEC3 rat hepatoma cells. Cancer Lett 86:195–200
65. Reen RK, Wiebel FJ, Singh J (1997) Piperine inhibits aflatoxin B_1-induced cytotoxicity and genotoxicity in V79 Chinese hamster cells genetically engineered to express rat cytochrome P4502B1. J Ethnopharmacol 58:165–173
66. Wongpa S, Himakoun L, Soontornchai S, Temcharoen P (2007) Antimutagenic effects of piperine on cyclophosphamide-induced chromosome aberrations in rat bone marrow cells. Asian Pac J Cancer Prev 8:623–627
67. Nalini N, Manju V, Menon VP (2006) Effect of spices on lipid metabolism in 1,2-dimethyl hydrazine-induced rat colon carcinogenesis. J Med Food 9:237–245
68. Unnikrishnan MC, Kuttan R (1990) Tumour reducing and anticarcinogenic activity of selected spices. Cancer Lett 51:85–89
69. Pradeep CR, Kuttan G (2002) Effect of piperine on the inhibition of lung metastasis induced B16F-10 melanoma cells in mice. Clin Exp Metastasis 19:703–708
70. Selvendiran K, Singh JP, Krishnan KB, Sakthisekaran D (2003) Cytoprotective effect of piperine against benzo[α]pyrene induced lung cancer with reference to lipid peroxidation and antioxidant system in Swiss albino mice. Fitoterapia 74:109–115
71. Selvendiran K, Banu SM, Sakthisekaran D (2004) Protective effect of piperine on benzo (α) pyrene-induced lung carcinogenesis in Swiss albino mice. Clin Chim Acta 350:73–78
72. Loder JW, Moorhouse A, Russel GV (1984) Tumor inhibitory plants: amides of pepper novaehollandiae. Aust J Chem 22:1531–1538
73. Selvendiran K, Banu SM, Sakthisekaran D (2005) Oral supplementation of piperine leads to altered phase II enzymes and reduced DNA damage and DNA-protein cross links in Benzo (a)pyrene induced experimental lung carcinogenesis. Mol Cell Biochem 268:141–147
74. Selvendiran K, Padmavathi R, Magesh V, Sakthisekaran D (2005) Preliminary study on inhibition of genotoxicity by piperine in mice. Fitoterapia 76:296–300
75. Daware MB, Mujumdar AM, Ghaskadbi S (2000) Reproductive toxicity of piperine in Swiss albino mice. Planta Med 66:231–236
76. Piyachaturawat P, Pholpramool C (1997) Enhancement of fertilization by piperine in hamsters. Cell Biol Int 21:405–409
77. Piyachaturawat P, Sriwattana W, Damrongphol P, Pholpramool C (1991) Effects of piperine on hamster sperm capacitation and fertilization *in vitro*. Int J Androl 14:283–290
78. Malini T, Manimaran RR, Arunakaran J, Aruldhas MM, Govindarajulu P (1999) Effects of piperine on testis of albino rats. J Ethnopharmacol 64:219–225
79. Mujumdar AM, Dhuley JN, Deshmukh VK, Raman PH, Naik SR (1990) Anti-inflammatory activity of piperine. Jpn J Med Sci Biol 43:95–100
80. Koul IB, Kapil A (1993) Evaluation of the liver protective potential of piperine, an active principle of black and long peppers. Planta Med 59:413–417
81. Lin Z, Hoult JR, Bennett DC, Raman A (1999) Stimulation of mouse melanocyte proliferation by *Piper nigrum* fruit extract and its main alkaloid – piperine. Planta Med 65:600–603

82. Wattanathorn J, Chonpathompikunlert P, Muchimapura S, Priprem A, Tankamnerdthai O (2008) Piperine, the potential functional food for mood and cognitive disorders. Food Chem Toxicol 46:3106–3110
83. Li S, Wang C, Li W, Koike K, Nikaido T, Wang MW (2007) Antidepressant-like effects of piperine and its derivative, antiepilepsirine. J Asian Nat Prod Res 9:421–430
84. Wang L, Zhao D, Zhang Z (1999) Trial of antiepilepsirine in children with epilepsy. Brain Dev 21:36–40
85. D'cruz SC, Mathur PP (2005) Effect of piperine on the epididymis of adult male rats. Asian J Androl 7:363–368
86. Kawada T, Sakabe S, Watanabe T, Yamamoto M, Iwai K (1988) Some pungent principles of spices cause the adrenal medulla to secrete catecholamine in anesthetized rats. Proc Soc Exp Biol Med 188:229–233
87. Reanmongkol W, Janthasoot W, Wattanatorn W, Dhumma-Upakorn P, Chudapongse P (1988) Effects of piperine on bioenergetic functions of isolated rat liver mitochondria. Biochem Pharmacol 37:753–757
88. Jamwal DS, Singh J (1993) Effects of piperine on enzyme activities and bioenergetic functions in isolated rat liver mitochondria and hepatocytes. J Biochem Toxicol 8:167–174
89. Bhat BG, Chandrasekhara N (1986) Studies on the metabolism of piperine: absorption, tissue distribution and excretion of urinary conjugates in rats. Toxicology 40:83–92
90. Suresh D, Srinivasan K (2007) Studies on the *in vitro* absorption of spice principles – curcumin, capsaicin and piperine in rat intestines. Food Chem Toxicol 45:1437–1442
91. Bhat BG, Chandrasekhara N (1987) Metabolic disposition of piperine in the rat. Toxicology 44:99–106
92. Bajad S, Coumar M, Khajuria R, Suri OP, Bedi KL (2003) Characterization of a new rat urinary metabolite of piperine by LC/NMR/MS studies. Eur J Pharm Sci 19:413–421
93. Khajuria A, Zutshi U, Bedi KL (1998) Permeability characteristics of piperine on oral absorption – an active alkaloid from peppers and a bioavailability enhancer. Indian J Exp Biol 36:46–50
94. Suresh D, Mahesha HG, Rao AGA, Srinivasan K (2007) Binding of bioactive phytochemical piperine with human serum albumin: a spectrofluorometric study. Biopolymers 86:265–275
95. Govindarajan VS (1986) Capsicum – production, technology, chemistry and quality: chemistry of colour, aroma and pungency stimuli. Crit Rev Food Sci Nutr 24:245–355
96. Fujiwake H, Suzuki T, Oka S, Iwai K (1980) Enzymatic formation of capsaicinoid from vanillylamine and iso-type fatty acids by cell-free extracts of *Capsicum annuum* var. annuum. Agric Biol Chem 44:2907–2912
97. Surh YJ, Lee SS (1995) Capsaicin, a double-edged sword: toxicity, metabolism, and chemopreventive potential. Life Sci 56:1845–1855
98. Majid M, Badmaev V, Lakshmi P, Natarajan S, Gopinathan S (1997) Capsaicin, the antiarthritic phytochemical. Nutriscience Publishers, Piscataway
99. Sambaiah K, Satyanarayana MN, Rao MVL (1978) Effect of red pepper and capsaicin on fat absorption and liver fat in rats. Nutr Rep Int 18:521–529
100. Srinivasan MR, Sambaiah K, Satyanarayana MN, Rao MVL (1980) Influence of red pepper and capsaicin on growth, blood constituents and nitrogen balance in rats. Nutr Rep Int 21:455–467
101. Srinivasan MR, Satyanarayana MN (1987) Influence of capsaicin, curcumin and ferulic acid in rats fed high fat diets. J Biosci 12:143–152
102. Monsereenusorn S (1983) Subchronic toxicity studies of capsaicin and capsicum in rats. Res Comm Chem Pathol Pharmacol 41:95–100
103. Kawada T, Hagihara K, Iwai K (1986) Effects of capsaicin on lipid metabolism in rats fed a high fat diet. J Nutr 116:1272–1278
104. Sambaiah K, Satyanarayana MN (1980) Hypocholesterolemic effect of red pepper and capsaicin. Indian J Exp Biol 18:898–899
105. Kempaiah RK, Srinivasan K (2002) Integrity of erythrocytes of hypercholesterolemic rats during spices treatment. Mol Cell Biochem 236:155–161

106. Babu PS, Srinivasan K (1997) Influence of dietary capsaicin and onion on the metabolic abnormalities associated with diabetes mellitus. Mol Cell Biochem 175:49–57
107. Srinivasan K, Sambaiah K (1991) Effect of spices on cholesterol-7α-hydroxylase activity and on serum and hepatic cholesterol levels in the rat. Int J Vitam Nutr Res 61:364–369
108. Negulesco JA, Young RM, Ki P (1983) Capsaicin lowers plasma cholesterol and triglycerides of lagomorphs. Artery 12:301–311
109. Ki P, Negulesco JA, Murnane M (1982) Decreased total serum myocardial and aortic cholesterol levels following capsaicin treatment. IRCS Med Sci 10:446–447
110. Negulesco JA, Noel SA, Newman HA, Naber EC, Bhat HB, Witiak DT (1987) Effects of pure capsaicinoids (capsaicin and dihydrocapsaicin) on plasma lipids and lipoprotein concentrations of turkey poults. Atherosclerosis 64:85–90
111. Gupta RS, Dixit VP, Dobhal MP (2002) Hypocholesterolemic effect of the oleoresin of *Capsicum annum* L. in gerbils (*Meriones hurrianae* Jerdon). Phytother Res 16:273–275
112. Srinivasan MR, Satyanarayana MN (1988) Influence of capsaicin, eugenol, curcumin and ferulic acid on sucrose induced hypertriglyceridemia in rats. Nutr Rep Int 38:571–581
113. Srinivasan K, Sambaiah K, Chandrasekhara N (1992) Loss of active principles of common spices during domestic cooking. Food Chem 43:271–274
114. Suresh D, Manjunatha H, Srinivasan K (2007) Effect of heat processing of spices on the concentrations of their bioactive principles: turmeric (*Curcuma longa*), red pepper (*Capsicum annuum*) and black pepper (*Piper nigrum*). J Food Compos Anal 20:346–351
115. Manjunatha H, Srinivasan K (2008) Hypolipidemic and antioxidant potency of heat processed turmeric and red pepper in experimental rats. Afr J Food Sci 2:1–6
116. Bhat BG, Srinivasan MR, Chandrasekhara N (1984) Influence of curcumin and capsaicin on the composition and secretion of bile in rats. J Food Sci Technol 21:225–227
117. Hussain MS, Chandrasekhara N (1992) Effect of curcumin on cholesterol gallstone induction in mice. Indian J Med Res 96:288–291
118. Hussain MS, Chandrasekhara N (1993) Influence of curcumin and capsaicin on cholesterol gallstone induction in hamsters and mice. Nutr Res 13:349–357
119. Hussain MS, Chandrasekhara N (1994) Biliary proteins from hepatic bile of rats fed curcumin or capsaicin inhibit cholesterol crystal nucleation in supersaturated model bile. Indian J Biochem Biophys 31:407–412
120. Shubha MC, Reddy RLR, Srinivasan K (2011) Anti-lithogenic influence of dietary capsaicin and curcumin during experimental induction of cholesterol gallstone in mice. Appl Physiol Nutr Metab 36:201–209
121. Hussain MS, Chandrasekhara N (1994) Effect of curcumin and capsaicin on the regression of pre-established cholesterol gallstones in mice. Nutr Res 14:1561–1574
122. Cazana FJD, Puyol MR, Caballero JP, Jimenez AJ, Duarte AM (1990) Effect of dietary hyperlipidemia-hypercholesterolemia on rat erythrocytes. Int J Vitam Nutr Res 60:393–397
123. Kempaiah RK, Srinivasan K (2005) Influence of dietary spices on the fluidity of erythrocytes in hypercholesterolemic rats. Br J Nutr 93:81–91
124. Kempaiah RK, Srinivasan K (2006) Beneficial influence of dietary curcumin, capsaicin and garlic on erythrocyte integrity in high-fat fed rats. J Nutr Biochem 17:471–478
125. Salimath BP, Sundaresh CS, Srinivas L (1986) Dietary components inhibit lipid peroxidation in erythrocyte membrane. Nutr Res 6:1171–1178
126. Okada Y, Okajima H (2001) Antioxidant effect of capsaicin on lipid peroxidation in homogeneous solution, micelle dispersions & liposomal membranes. Redox Rep 6:117–122
127. Kogure K, Goto S, Nishimura M, Yasumoto M, Abe K, Ohiwa C et al (2002) Mechanism of potent antiperoxidative effect of capsaicin. Biochim Biophys Acta 1573:84–92
128. Lee CY, Kim M, Yoon SW, Lee CH (2003) Short-term control of capsaicin on blood and oxidative stress of rats *in vivo*. Phytother Res 17:454–458
129. Kempaiah RK, Srinivasan K (2004) 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

130. Kempaiah RK, Srinivasan K (2004) Antioxidant status of red blood cells and liver in hypercholesterolemic rats fed hypolipidemic spices. Int J Vitam Nutr Res 74:199–208
131. Reddy ACP, Lokesh BR (1994) Studies on anti-inflammatory activity of spice principles and dietary n-3 polyunsaturated fatty acids on Carrageenan induced inflammation in rats. Ann Nutr Metab 38:349–358
132. Joe B, Lokesh BR (1997) Prophylactic and therapeutic effects of n-3 PUFA, capsaicin & curcumin on adjuvant induced arthritis in rats. J Nutr Biochem 8:397–407
133. Joe B, Lokesh BR (1997) Effect of curcumin and capsaicin on arachidonic acid metabolism and lysosomal enzyme secretion by rat peritoneal macrophages. Lipids 32:1173–1180
134. Surh YJ, Kundu JK, Na HK, Lee JS (2005) Redox-sensitive transcription factors as prime targets for chemoprevention with anti-inflammatory and antioxidative phytochemicals. J Nutr 135(suppl):2993S–3001S
135. Surh YJ, Lee RC, Park KK, Mayne ST, Liem A, Miller JA (1995) Chemoprotective effects of capsaicin and diallyl sulfide against mutagenesis or tumorigenesis by vinyl carbamate and *N*-nitrosodimethylamine. Carcinogenesis 16:2467–2471
136. Mori A, Lehmann S, O'Kelly J, Kumagai T, Desmond JC, Pervan M et al (2006) Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 mutant prostate cancer cells. Cancer Res 66:3222–3229
137. Ito K, Nakazato T, Yamato K, Miyakawa Y, Yamada T, Hozumi N et al (2004) Induction of apoptosis in leukemic cells by homovanillic acid derivative, capsaicin, through oxidative stress: implication of phosphorylation of p53 at Ser-15 residue by reactive oxygen species. Cancer Res 64:1071–1078
138. Archer VE, Jones DW (2002) Capsaicin pepper, cancer and ethnicity. Med Hypotheses 59:450–457
139. Tsui H, Razavi R, Chan Y, Yantha J, Dosch HM (2007) Sensing autoimmunity in type 1 diabetes. Trends Mol Med 13:405–413
140. Brown M, Vale W (2011) Effects of neurotensin and substance P on plasma insulin, glucagon and glucose levels. Endocrinology 98:819–822
141. Razavi R, Chan Y, Afifiyan FN, Liu XJ, Wan X, Yantha J et al (2006) TRPV1+ sensory neurons control β-cell stress and islet inflammation in autoimmune diabetes. Cell 127:1123–1135
142. Lejeune MPG, Eva M, Kovacs MR, Westerterp-Plantenga MS (2003) Effect of capsaicin on substrate oxidation and weight maintenance after modest body-weight loss in human subjects. Br J Nutr 90:651–659
143. Westerterp-Plantenga MS, Smeets A, Lejeune MPG (2004) Sensory and gastrointestinal satiety effects of capsaicin on food intake. Int J Obes 29:682–688
144. Diepvens K, Westerterp KR, Westerterp-Plantenga MS (2007) Obesity and thermogenesis related to the consumption of caffeine, ephedrine, capsaicin, and green tea. Am J Physiol Regul Integr Comp Physiol 292:R77–R85
145. Yoneshiro T, Aita S, Kawai Y, Iwanaga T, Saito M (2012) Non-pungent capsaicin analogs (capsinoids) increase energy expenditure through the activation of brown adipose tissue in humans. Am J Clin Nutr 95:845–850
146. Pande S, Srinivasan K (2012) Potentiation of hypolipidemic and weight reducing influence of dietary tender cluster bean (*Cyamopsis tetragonoloba*) by capsaicin in high-fat fed rats. J Agric Food Chem 60:8155–8162
147. López-Carrillo L, López-Cervantes M, Robles-Díaz G, Ramírez-Espitia A, Mohar-Betancourt A, Meneses-García A et al (2003) Capsaicin consumption, *Helicobacter pylori* positivity and gastric cancer in Mexico. Int J Cancer 106:277–282
148. Buiatti E, Palli D, Decarli A, Amadori D, Avellini C, Bianchi S et al (1989) A case-control study of gastric cancer and diet in Italy. Int J Cancer 44:611–616
149. Satyanarayana MN (2006) Capsaicin and gastric ulcers. Crit Rev Food Sci Nutr 46:275–328
150. Kang JY, Yeoh KG, Chia HP, Lee HP, Chia YW, Guan R, Yap I (1995) Chilli protective factor against peptic ulcer? Dig Dis Sci 40:576–579

151. Jancso G, Kiraly E, Jancso-Gabor A (1977) Pharmacologically induced selective degeneration of chemosensitive primary neurones. Nature 270:741–743
152. Park JS, Choi MA, Kim BS, Han IS, Kurata T, Yu R (2000) Capsaicin protects against ethanol-induced oxidative injury in the gastric mucosa of rats. Life Sci 67:3087–3093
153. Deal CL (1991) Effect of topical capsaicin: a double blind trial. Clin Ther 13:383–395
154. McCarthy GM, McCarthy DJ (1991) Effect of topical capsaicin in the therapy of painful osteoarthritis of the hand. J Rheumatol 19:604–607
155. Bernstein JE (1989) Treatment of chronic post-herpetic neuralgia with topical capsaicin. Am J Dermatol 21:265–270
156. The Capsaicin Study Group (1992) Effect of treatment with capsaicin on daily activities of patients with painful diabetic neuropathy. Diabet Care 15:159–165
157. Derry S, Lloyd R, Moore RA, McQuay HJ (2009) Topical capsaicin for chronic neuropathic pain in adults (Review). Cochrane Database Syst Rev, Issue 4. Art. No.: CD007393
158. Glinski W, Glinska-Ferenz M, Pierozynska-Dubowska M (1991) Neurogenic inflammation induced by capsaicin in patients with psoriasis. Acta Derm Venereol 71:51–54
159. Arnold WP, van de Kerkhof PC (1993) Topical capsaicin in pruritic psoriasis. J Am Acad Dermatol 29:438–442
160. Fraenkel L, Bogardus ST, Concato J, Wittink DR (2004) Treatment options in knee osteoarthritis: the patient's perspective. Arch Intern Med 164:1299–1304
161. Story GM, Crus-Orengo L (2007) Feel the burn. Am Sci 95:326–333
162. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824
163. Geppetti P, Nassini R, Materazzi S, Benemei S (2008) The concept of neurogenic inflammation. BJU Int 101:2–6
164. Kissin I (2008) Vanilloid-induced conduction analgesia: selective, dose-dependent, long-lasting, with a low level of potential neurotoxicity. Anesth Analg 107:271–281
165. Fuller RW, Dixon CMS, Barnes PJ (1985) Broncho-constrictor response to inhaled capsaicin in humans. J Appl Physiol 58:1080–1084
166. Toth B, Gannett P (1992) Carcinogenicity of lifelong administration of capsaicin of hot pepper in mice. In Vivo 6:59–63
167. Philip G, Baroody FM, Proud D, Naclerio RM, Togias AG (1994) The human nasal response to capsaicin. J Allergy Clin Immunol 94:1035–1045
168. Kawada T, Suzuki T, Takahashi M, Iwai K (1984) Gastrointestinal absorption and metabolism of capsaicin and dihydrocapsaicin in rats. Toxicol Appl Pharmacol 72:449–456
169. Akagi A, Sano N, Uehara H, Minami T, Otsuka H, Izumi K (1998) Non-carcinogencity of capsaicinoids in B6C3F1 mice. Food Chem Toxicol 36:1065–1071
170. Richeux R, Cascante M, Ennamany R, Sabureau D, Creppy EE (1999) Cytotoxicity and genotoxicity of capsaicin in human neuroblastoma cells SHSY-5Y. Arch Toxicol 73:403–409
171. Chanda S, Sharper VA, Hoberman AM, Bley K (2006) Developmental toxicity study of pure trans-capsaicin in rats and rabbits. Int J Toxicol 25:205–217
172. Reilly CA, Yost GS (2006) Metabolism of capsaicinoids by P450 enzymes: a review of recent findings on reaction mechanisms, bio-activation, and detoxification processes. Drug Metab Rev 38:685–706