Black Pepper and its Pungent Principle-Piperine: A Review of Diverse Physiological Effects

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Black pepper (Piper nigrum) is one of the most widely used among spices. It is valued for its distinct biting quality attributed to the alkaloid, piperine. Black pepper is used not only in human dietaries but also for a variety of other purposes such as medicinal, as a preservative, and in perfumery. Many physiological effects of black pepper, its extracts, or its major active principle, piperine, have been reported in recent decades. Dietary piperine, by favorably stimulating the digestive enzymes of pancreas, enhances the digestive capacity and significantly reduces the gastrointestinal food transit time. Piperine has been demonstrated in in vitro studies to protect against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species. Black pepper or piperine treatment has also been evidenced to lower lipid peroxidation in vivo and beneficially influence cellular thiol status, antioxidant molecules and antioxidant enzymes in a number of experimental situations of oxidative stress. The most far-reaching attribute of piperine has been its inhibitory influence on enzymatic drug biotransforming reactions in the liver. It strongly inhibits hepatic and intestinal aryl hydrocarbon hydroxylase and UDP-glucuronyl transferase. Piperine has been documented to enhance the bioavailability of a number of therapeutic drugs as well as phytochemicals by this very property. Piperine's bioavailability enhancing property is also partly attributed to increased absorption as a result of its effect on the ultrastructure of intestinal brush border. Although initially there were a few controversial reports regarding its safety as a food additive, such evidence has been questionable, and later studies have established the safety of black pepper or its active principle, piperine, in several animal studies. Piperine, while it is non-genotoxic, has in fact been found to possess anti-mutagenic and anti-tumor influences.

Keywords   black pepper, piperine, antioxidant effect, bioavailability enhancing effect, anti-mutagenic, anti-cancer influence

INTRODUCTION

Black pepper (Piper nigrum) is one of the most widely used among spices. It is valued for its distinct biting quality attributed to piperine and its isomers (Govindarajan, 1977). Black pepper is used not only in human dietaries but also for other purposes such as medicinal, as a preservative, in perfumery, and even as an insecticide. Black pepper is considered as the king of spices, as it fetches the highest return as judged from the volume of international trade. The solvent extracted pepper oleoresin, containing the essential oil contributing to the aroma of pepper and piperine, the alkaloid contributing to the pungency, has many advantages such as convenience of commercial handling and free from microbial contamination and biodeterioration, and hence sometimes preferred to pepper in processed foods. Many phys-

iological effects of black pepper, its extracts or its major active principle, piperine, have been reported in recent decades.

SAFETY OF BLACK PEPPER CONSUMPTION

Though pepper has been in use for a long time as a food additive, there are controversial reports regarding its safety as a food additive (Buchanan, 1978; Concon, et al., 1979). These reports point to the possible carcinogenicity of an ethanolic extract containing piperine among several other constituents having methylenedioxy benzene as part of the molecule. Concon et al. (1979) observed the incidence of malignant and multiple tumors in mice cutaneously administered with pepper. Piperine (Fig. 1), the major active principle of black pepper, is closely related in structure to the known natural carcinogens—safrole, estragole, and methyleugenol which are also widely distributed in spices and plant oils (Ames, 1983). Namiki et al. (1984) have

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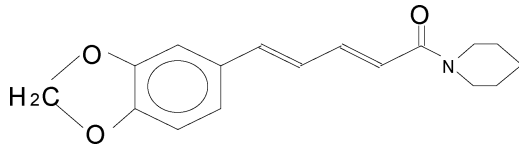
Figure 1 Structure of piperine.

reported that among several spices treated with sodium nitrite, pepper exhibited the strongest mutagenic activity as indicated by the Ames test. Epstein and Swartz (1984) consider that evidence for the carcinogenicity of pepper is inadequate as it is based on the results of single questionable study of Concon et al. (1979).

In acute toxicity studies, LD_{50} values for a single i.v., i.p., s.c., i.g., and i.m. administration of piperine to adult male mice were 15.1, 43, 200, 330, and 400 mg/kg body wt, respectively (Piyachaturawat et al.,1983). The i.p. LD_{50} value was higher viz., 60 mg/kg body weight in adult females and 132 mg/kg body weight in weanling male mice. In adult female rats, the i.p. LD_{50} value was 33.5 mg/kg body weight whereas the i.g. LD_{50} value was 514 mg/kg body weight. Most animals given a lethal dose died of respiratory paralysis within 3–17 min. In subacute toxicity studies, the rats died within 1–3 days after treatment. Histopathologic changes included severe hemorrhagic necrosis and edema in the gastrointestinal tract, the urinary bladder, and the adrenal glands. Death of these animals may be attributable to multiple dysfunctions in their organs.

Earlier studies in our laboratory indicated that no adverse effect was caused by feeding black pepper or piperine at levels equivalent to normal human intake or as much as 250 times as indicated by growth, organ weights, and blood constituents (Srinivasan and Satyanarayana, 1981). Black pepper, its oleoresin, or its active principle piperine, fed to rats at doses 5–20 times normal human intake did not cause any adverse effect on growth, food efficiency ratio and organ weights, blood cell counts, and the levels of blood constituents like hemoglobin, total serum proteins, albumin, globulin, glucose and cholesterol, activities of serum aminotransferases and phosphatases, fat, and nitrogen balance (Bhat and Chandrasekhara, 1986).

The non-genotoxic nature of piperine was evidenced in latter studies using four different test systems, namely, Ames test using Salmonella typhimurium, micronucleus test, sperm shape abnormality test and dominant lethal test using Swiss albino mice (Karekar et al., 1996). In the Ames test, six different doses of piperine, in the range of 0.005–10 μmol/plate, did not induce his+ revertants, with or without metabolic activation, indicating its non-mutagenic nature. In the bone narrow micronucleus test using two doses (10 and 20 mg/kg body weight), piperine itself was non-mutagenic. Like in somatic cells, piperine (10 and 50 mg/kg body weight) failed to induce mutations in male germ cells of mice as assessed by using the sperm shape abnormality and dominant lethal tests. Piperine thus appears to be a non-genotoxic chemical. The immuno-toxicological effects of piperine were investigated in Swiss mice, gavaged at a dose of 1.12, 2.25, or 4.5 mg/kg body weight for five consecutive days

(Dogra et al., 2004). All these dose levels had no overt toxic effect, while the lowest dose had no immunotoxic effect.

INFLUENCE OF BLACK PEPPER OR PIPERINE ON DIGESTION

Spices, by virtue of their pungent principles and by imparting flavor to foodstuffs, enhance salivary and gastric secretions. Glatzel, studying the effect of spices on the secretion and composition of saliva in human subjects, observed that black pepper and other spices enhance the secretion of saliva and the activity of salivary amylase (Glatzel, 1968). The digestive stimulant action of spices is probably exerted through 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. Several commonly used spices including black pepper and its active principle, piperine, have been examined for their effect on bile secretion in our laboratory using experimental rats (Bhat and Chandrasekhara, 1987). In these animal models, bile has been systematically collected by cannulating the common biliary-pancreatic duct following the spice treatment. Spices have been examined for their influence on bile as a result of both a continued intake through the diet for a period of time and as a one-time exposure orally. The results of these studies revealed that dietary black pepper or piperine which have no hypocholesterolemic influence, had no beneficial stimulatory influence on bile acid production by the liver and its secretion into bile (Bhat and Chandrasekhara, 1987). On the other hand, the oral administration of piperine as a single dose significantly increases bile acid secretion. Stimulation in bile acid secretion (μmol/h) was to an extent of about 30% over the control.

Exhaustive animal studies have been carried out in our laboratory to examine the influence of spices on the activities of enzymes that participate in digestion. The influence of dietary intake and single dose administration of several commonly used spices or their active principles including piperine on the pancreatic digestive enzymes and the terminal digestive enzymes of the small intestinal mucosa has been reported by us (Platel and Srinivasan, 1996, 2000). In these studies, piperine was fed to animals at levels corresponding to about five times the average human dietary intake of black pepper. The levels were based on calculated dietary intake of spices in the form of curry powder and on a dietary survey conducted in India (Thimmayamma et al., 1983). Dietary intake of piperine significantly increased pancreatic lipase activity and piperine stimulated lipase activity up to 30% of control. In contrast to the beneficial stimulation of pancreatic lipase by piperine as a result of continued intake, a single, oral dose consumption of the same failed to exert a stimulatory effect. The pancreatic amylase activity is observed to be elevated by dietary piperine (to an extent of 87%). These studies also reveal that piperine when incorporated in the diet, stimulates trypsin activity by as much as 150%. Chymotrypsin was also significantly higher in animals fed piperine. Such a beneficial influence of this spice on the activity of proteases was not

Table 1 Antioxidant influence of black pepper and piperine

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

evident when administered as a single oral dose. Piperine prominently enhanced the activity of intestinal lipase. The stimulation of this enzyme activity was more than 100% of the control in spice principle-treated groups. An appreciable increase in intestinal lipase activity was observed in animals given single oral doses of piperine and so also the activity of intestinal amylase (Platel and Srinivasan, 1996).

ANTIOXIDANT EFFECT OF PIPERINE

Oxygen radical injury and lipid peroxidation have been suggested as major causes of atherosclerosis, cancer, and the aging process. Reactive oxygen species and reactive metabolic intermediates generated from various chemical carcinogens are known to play an important role in cell damage and in the initiation and progression of carcinogenesis. Many radical scavengers, interestingly naturally occurring antioxidants, have been found to be effective in inhibiting the induction of carcinogenesis by a wide variety of chemical carcinogens. Studies have also indicated that various spice principles form an important group as antioxidants. Piperine has been demonstrated in *in vitro* experiments to protect against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species and inhibit lipid peroxidation (Mittal and Gupta, 2000). Piperine was found to act as a hydroxyl radical scavenger at low concentrations, but at higher concentrations, it activated the Fenton reaction resulting in increased generation of hydroxyl radicals. Whereas it acts as a powerful superoxide scavenger with an IC_{50} of 1.82 mM, a 52% inhibition of lipid peroxidation was observed at a dose of 1.4 mM with an IC_{50} of 1.23 mM. However, Krishnakantha and Lokesh (1993) have observed that piperine failed to scavenge superoxide anions while investigating the effect of various spice principles on scavenging of superoxide anion as measured by nitroblue-tetrazolium reduction in xanthine-xanthine oxidase system. Reddy and Lokesh (1992) have reported that piperine had only 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 (Table 1).

Piperine is shown to be an effective antioxidant and offers protection against the oxidation of human low density lipoprotein (LDL) as evaluated by copper ion-induced lipid peroxidation of human LDL by measuring the formation of thiobarbituric acid reactive substance and relative electrophoretic mobility of LDL on agarose gel (Naidu and Thippeswamy, 2002). The aqueous extract of black pepper as well as piperine have been examined for their effect on human PMNL 5-lipoxygenase (5-LO), the key enzyme involved in biosynthesis of leukotrienes (Prasad et al., 2004). The formation of 5-LO product 5-HETE was significantly inhibited in a concentration-dependent manner with IC_{50} values of 0.13 mg for aqueous extracts of pepper and 60 μM for piperine. Thus, piperine of black pepper might exert an antioxidant physiological role by modulating 5-LO pathway.

Using diabetes mellitus as a model of oxidative damage, Rauscher et al. (2000) investigated whether piperine treatment (10 mg/kg/day, i.p. for 14 days) would protect against diabetes-induced oxidative stress in streptozotocin-induced diabetic Sprague-Dawley rats. All tissues from diabetic animals exhibited disturbances in antioxidant defense when compared with normal controls. 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. Piperine treatment did not reverse the effects of diabetes on hepatic antioxidant status. Thus, subacute treatment with piperine for 14 days is only partially effective as an antioxidant in diabetes.

Khajuria et al. (1998) have investigated whether piperine is able to inhibit or reduce the oxidative changes induced by chemical carcinogens in a rat intestinal model. Carcinogenesis was initiated in intestinal lumen of rats with 7,12-dimethyl benzanthracene, dimethyl aminomethyl azobenzene, and 3-methyl cholanthrene. Oxidative alterations were assessed by determining thiobarbituric acid reactive substances (TBARS) as a measure of lipid peroxidation, thiol status and expression of γ-glutamyl transpeptidase (γ-GT), and Na^+,K^+-ATPase activity in intestinal mucosa. Data indicated that carcinogen induced

glutathione depletion with substantial increase in TBARS and enzyme activities. A protective role of piperine against the oxidative alterations by the carcinogen was indicated by the observed inhibition of TBARS, a significant increase in the glutathione levels and restoration in γ-GT and Na^+,K^+-ATPase activity.

Selvendiran et al. (2004) have recently investigated the impact of piperine on alterations of mitochondrial antioxidant system and lipid peroxidation in benzo(α)pyrene (B(α)p) induced experimental lung carcinogenesis. Oral supplementation of piperine (50 mg/kg body weight) effectively suppressed lung carcinogenesis by B(α)p in mice as revealed by a decrease in the extent of mitochondrial lipid peroxidation and concomitant increase in the activities of enzymatic antioxidants (superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic antioxidant (reduced glutathione, vitamin E, and vitamin C) levels when compared to lung carcinogenesis bearing animals. This suggests that piperine may extend its chemopreventive effect by modulating lipid peroxidation and augmenting antioxidant defense system.

Vijayakumar et al. (2004) have recently examined the effect of supplementation of black pepper or piperine on tissue lipid peroxidation, enzymic, and non-enzymic antioxidants in rats fed a high-fat diet and observed that these spices can reduce high-fat diet induced oxidative stress. Groups of Wistar rats were fed a high-fat diet (20% coconut oil, 2% cholesterol and 0.125% bile salts), a high-fat diet plus black pepper (0.25 g or 0.5 g/kg body weight), a high-fat diet plus piperine (0.02 g/kg body weight) for a period of 10 weeks. Significantly elevated levels of TBARS, conjugated dienes (CD) and significantly lowered activities of super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and reduced glutathione (GSH) in the liver, heart, kidney, intestine, and aorta were observed in rats fed the high fat diet as compared to the control rats. Simultaneous supplementation with black pepper or piperine lowered TBARS and CD levels and maintained SOD, CAT, GPx, GST, and GSH levels near to those of control rats.

INFLUENCE OF BLACK PEPPER ON LIPID METABOLISM

There were no significant changes in serum free and total cholesterol and liver total cholesterol in rats fed a normal diet supplemented with 0.02, 0.15, 0.5, 2.0, and 5% black pepper or 0.05% piperine (Srinivasan and Satyanarayana, 1981) or pepper oleoresin at 11, 22, and 44 mg% levels in the diet for 8 weeks (Bhat and Chandrasekhara, 1986). In contrast, Cho and Lee (1983) reported that the feeding of pepper at a 5% level in the diet to rats for 8 weeks led to a significant increase in serum cholesterol. Dietary black pepper, which did not affect the serum and the liver cholesterol concentration, also did not affect the activity of hepatic cholesterol-7α-hydroxylase, the rate limiting enzyme in the conversion of cholesterol to bile acids (Srinivasan and Sambaiah, 1991).

INFLUENCE OF PIPERINE ON DRUG METABOLIZING ENZYME SYSTEM

In the context of piperine having been reported to enhance drug bioavailability, Atal et al. (1985) studied the interaction of piperine with drug biotransforming reactions in hepatic tissue in vitro and in vivo. Piperine inhibited aryl hydrocarbon hydroxylation, ethylmorphine-N-demethylation, 7-ethoxy-coumarin-O-deethylation, and 3-hydroxybenzo(α) pyrene (3-OH-BP) glucuronidation in rat liver post-mitochondrial supernatant in vitro in a dose-dependent manner. Piperine's inhibition of these reactions in liver post-mitochondrial supernatant from 3-methylcholanthrene- and phenobarbital-treated rats was similar to the controls. Inhibition by piperine of arylhydrocarbon hydroxylase (AHH) from 3-methylcholanthrene-treated rats was comparable to that observed with 7,8-benzoflavone. Piperine caused noncompetitive inhibition of hepatic microsomal AHH from the untreated and 3-methylcholanthrene-treated rats with a K_i of 30 μM which was close to the apparent K_m of AHH observed in the controls. Similarly, the kinetics of inhibition of ethylmorphine-N-demethylase from control rat liver microsomes 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. The maximal inhibition of AHH observed within 1 h restored to a normal value in 6 h. Pretreatment with piperine prolonged hexobarbital sleeping time and zoxazolamine paralysis time in mice at half the dose of SKF-525A. These results demonstrate that piperine is a potent inhibitor of drug metabolism (Table 2).

Table 2 Influence of piperine on drug metabolizing enzyme system

Effects demonstrated	Investigators
a) Inhibition of aryl hydroxylation, N-demethylation, O-deethylation and glucuronidation in vitro by piperine	Atal et al. (1985)
b) Lower aryl hydroxylase and UDP-glucuronyl transferase activities, prolonged hexobarbital sleeping time in piperine treated rats	Atal et al. (1985)
c) Decreased UDP-glucuronic acid concentration and rate of glucuronidation in isolated epithelial cells of Guineapig small intestine by piperine	Singh et al. (1986)
d) Inhibition of aryl hydroxylase and O-deethylase activities by piperine in vitro and in vivo in pulmonary microsomes	Reen and Singh (1991)
e) Decreased activities of hepatic microsomal cytochrome P_{450}, N-demethylase, aryl hydroxylase by intragastric/intra-peritoneal piperine in Sprague-Dawley rats	Dalvi and Dalvi (1991)
f) Inhibition of UDP-glucose dehydrogenase and UDP- glucuronyl transferase in rat and guinea pig liver and intestine by piperine	Reen et al. (1993)
g) Suppression of aryl hydroxylation in cell culture is mediated by direct interaction of piperine with cytochrome P_{450} and not by down regulation of its gene expression	Reen et al. (1996)

Singh et al. (1986) further explored the basis of inhibition of glucuronidation by piperine by examining the rate of glucuronidation of 3-OH-BP and UDP-glucuronic acid (UDPGA) content in the intact isolated epithelial cells of the guinea-pig small intestine. Glucuronidation of 3-OH-BP was dependent on the duration of incubation, cellular protein, and endogenous UDPGA concentration. Piperine caused a concentration-related decrease in the UDPGA content and the rate of glucuronidation in the cells. It required much lower concentrations of piperine than D-galactosamine to diminish the endogeneous level of UDPGA. At 50 μM piperine, the rate of glucuronidation was reduced to about 50% of the basal rate. Piperine caused noncompetitive inhibition of hepatic microsomal UDP-glucuronyltransferase with Ki of 70 μM. The study demonstrated that piperine modifies the rate of glucuronidation by lowering the endogeneous UDPGA content and also by inhibiting the transferase activity.

Dalvi and Dalvi (1991) have examined the influence of intragastrically administered piperine (100 mg/kg) on hepatic mixed function oxygenase system in adult Sprague-Dawley rats. An increase in hepatic microsomal cytochrome P_{450} and cytochrome b_5, NADPH-cytochrome-C reductase, benzphetamine N-demethylase, aminopyrine N-demethylase, and aniline hydroxylase was observed 24 h following treatment. On the other hand, a 10 mg/kg dose given i.p. exhibited no effect on the activities of the aforementioned parameters of the hepatic drug-metabolizing enzyme system. However, when the intragastric and intraperitoneal doses were increased to 800 mg/kg and 100 mg/kg, respectively, piperine produced a significant decrease in the levels of cytochrome P_{450}, benzphetamine N-demethylase, aminopyrine N-demethylase, and aniline hydroxylase 24 h after treatment. 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 (Dalvi and Dalvi, 1991a). Twenty-four h later, these parameters along with cytochrome b_5 and NADPH-cytochrome-C reductase remained depressed in piperine-treated rats. This suggested that the effect of piperine on hepatic mixed-function oxidases is monophasic.

In vitro and in vivo modulation of drug metabolizing enzymes by piperine has been investigated in pulmonary microsomes of rats and guinea pigs (Reen and Singh, 1991). Piperine caused concentration related non-competitive inhibition in vitro (50% at 100 μM) of AHH and 7-ethoxycoumarin deethylase (7ECDE) activities, which were comparable in control and 3-methylcholanthrene (3MC) treated rats. In guinea pig microsomes however, piperine caused strong inhibition at lower concentrations (35% at 10 μM) and relatively much lesser inhibition with further increase in concentrations. In vivo, piperine given at a dose of 25 mg/kg body weight to rats caused a maximal inhibition at 1 h of both the enzymes, while only AHH returned to normal value within 4 h. Similarly, upon daily treatment of piperine (15 mg/kg body wt) to rats for 7 days, 7ECDE was consistently inhibited, while AHH showed faster recovery. Piperine thus appeared to cause a 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}.

The modifying potential of black pepper on the hepatic biotransformation system has been assessed in mice fed on a diet containing 0.5, 1, and 2 % black pepper (w/w) for 10 and 20 days (Singh and Rao, 1993). Data revealed a significant and dose-dependent increase in glutathione S-transferase and sulfhydryl content in the experimental groups except the one maintained on 0.5% black pepper diet for 10 days. Elevated levels of cytochrome b_5 and cytochrome P_{450} were also significant and dose-dependent. The level of melondialdehyde (MDA) was lowered in the group fed on a 2% black pepper diet for 20 days. Being a potential inducer of the detoxication system, the possible chemopreventive role of black pepper in chemical carcinogenesis is suggested.

The effects of piperine on UDP-glucose dehydrogenase (UDP-GDH) and glucuronidation potentials of rat and guinea pig liver and intestine were studied by Reen et al. (1993). Piperine caused a concentration-related strong non-competitive inhibition of UDP-GDH (50% at 10 μM) reversibly and equipotently, in both tissues. Data from structure-activity comparisons of piperine analogs indicated that the presence of conjugated double bonds in the side chain of the molecule is a factor in piperine inhibition. However, the UDPGA contents were decreased less effectively by piperine in isolated rat hepatocytes compared with enterocytes of guinea pig small intestine. Piperine at 50 μM caused a marginal decrease of UDPGA in hepatocytes when the rate of glucuronidation of 3-OH-BP decreased by about 40%. UDP-glucuronyltransferase (UGT) activities towards 3-OH-BP and 4-OH-biphenyl were also determined. Piperine did not affect the rate of glucuronidation of 4-OH-biphenyl in rat liver, whereas that of 3-OH-BP was impaired significantly. In a guinea pig small intestine, both these activities were inhibited significantly requiring less than 25 μM piperine to produce a more than 50% inhibition of UGT(s). The results suggested that piperine is a potent inhibitor of UDP-GDH, by virtue of conjugated double bonds in the molecule, and it exerts stronger effects on intestinal glucuronidation than in rat liver.

By studying the modulation of B(α)p metabolism and regulation of cytochrome CYP1A1 gene expression by piperine in 5L cells in culture, it is observed that piperine mediated the inhibition of the AHH activity and the consequent suppression of the procarcinogen activation is the result of direct interaction of piperine with cytochrome P4501A1-protein and not because of down regulation of its gene expression (Reen et al., 1996).

INFLUENCE OF PIPERINE ON BIOAVAILABILITY OF DRUGS

Piperine, the alkaloidal constituent of black and long peppers, 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

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

System	Effect demonstrated	Investigator
Humans	Increased bioavailability of vasicine and sparteine as a result of Piper longum/piperine treatment	Atal et al. (1981)
Humans	Enhanced systemic availability of propranolol and theo-phylline as a result of piperine treatment	Bano et al. (1991)
Rats	Decreased metabolic activation of fungal toxin aflatoxin B_1 and hence its increased accumulation in plasma	Allamesh et al. (1992)
Humans	Increased serum concentration of curcumin by concomitant administration of piperine	Shobha et al. (1998)
Humans	Increased plasma levels of coenzyme Q_{10} by coadministration of piperine	Badmaev et al. (2000)
Mice	Delayed elimination of anti-epileptic drug – phenytoin by treatment of piperine	Velpandian et al. (2001)
Rats	Enhanced bioavailability of β-lactam antibiotics – Amoxi-cillin trihydrate and Cefotaxime by coadministration of piperine	Hiwale et al. (2002)
Mice	Increased plasma levels and delayed excretion of epigallo-catechin-3-gallate from green tea as a result of intragastric cotreatment with piperine	Lambert et al. (2004)

humans is of great clinical significance. A concise mechanism responsible for its bioavailability enhancing action is poorly understood. Atal et al. (1981) have evaluated the scientific basis of the use of the trikatu group of acrids (long pepper, black pepper, and ginger) in the large number of prescriptions in the indigenous Ayurvedic system of medicine. Piper longum (long pepper) increased the blood levels of the test drug, vasicine, by nearly 233%. Under the influence of piperine, blood levels of the test drug, sparteine, increased more than 100%. The results suggest that these acrids have the capacity to increase the bioavailability of certain drugs. The authors concluded that the trikatu group of drugs increases the 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 (Table 3).

Piperine has been reported by several researchers to have an effect on the activation and deactivation of exogenous substances. (-)-Epigallocatechin-3-gallate (EGCG) from green tea (Camellia sinensis) has demonstrated chemopreventive activity in animal models of carcinogenesis. Lambert et al. (2004) have observed that cotreatment with dietary piperine enhances the bioavailability of EGCG in mice. Intragastric coadministration of 163.8 μmol/kg EGCG and 70.2 μmol/kg piperine to male CF-1 mice increased the plasma C(max) and area under the curve (AUC) by 1.3-fold compared to mice treated with EGCG only. Piperine appeared to increase EGCG bioavailability by inhibiting glucuronidation and gastrointestinal transit. Piperine (100 μM) inhibited EGCG glucuronidation in mice small intestine (by 40%). EGCG appearance in the colon and the feces of piperine-cotreated mice was slower than in mice treated with EGCG alone. The effect of piperine on the bioavailability and pharmacokinetics of propranolol and theophylline has been examined in a crossover study, wherein subjects received a single oral dose of propranolol (40 mg) or theophylline (150 mg) alone or in combination with piperine (20 mg/day for 7 days) (Bano et al., 1991). An enhanced systemic availability of oral propranolol and theophylline was evidenced as a result of piperine treatment.

Velpandian et al. (2001) have reported from a study on mice a similar effect of piperine in altering the pharmacokinetics of phenytoin, an anti-epileptic drug. Pretreatment of piperine significantly delayed the elimination of phenytoin.

Co-administration of piperine enhanced the bioavailability of β-lactam antibiotics, amoxycillin trihydrate, and cefotaxime significantly in rats (Hiwale et al., 2002). The improved bioavailability is reflected in various pharmacokinetic parameters viz. the tmax, the Cmax, the half-life, and AUC, of these antibiotics and was attributed to the effect of piperine on microsomal metabolizing enzymes.

Black pepper extract consisting of 98% piperine has been evidenced to increase plasma levels of orally supplemented coenzyme Q_{10} in a clinical study using a double-blind design (Badmaev et al., 2000). The relative bioavailability of 90 mg and 120 mg of coenzyme Q_{10} administered in a single-dose experiment or in separate experiments 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, approx. 30% greater, AUC, than was observed during supplementation with coenzyme Q_{10} plus placebo. It is inferred that the bioenhancing mechanism of piperine to increase the plasma levels of supplemental coenzyme Q_{10} is nonspecific.

The effect of piperine, a known inhibitor of hepatic and intestinal glucuronidation on the bioavailability of curcumin was evaluated in rats and healthy human volunteers (Shoba et al., 1998). When curcumin was given alone at 2 g/kg to rats, moderate serum concentrations were achieved over a period of 4 h. Concomitant administration of piperine 20 mg/kg increased the serum concentration of curcumin for a short period of 1–2 h post drug. Time to maximum was significantly increased while plasma half-life and clearance significantly decreased, and the bioavailability was increased by 154%. On the other hand, in humans, after a dose of 2 g curcumin alone, serum levels were either undetectable or very low. Concomitant administration of piperine 20 mg produced much higher concentrations from 0.25 to 1 h post-drug, the increase in bioavailability was 2000%. The study shows that in the dosages used, piperine enhances the serum concentration, the extent of absorption and the bioavailability of curcumin in both rats and humans. This assumes importance in the context of diverse medicinal properties of curcumin of Curcuma longa.

The effect of piperine on the metabolic activation and distribution of [3H]-aflatoxin B_1 (AFB$_1$) in rats has been studied by Allamesh et al. (1992). Piperine markedly inhibited liver microsome-catalysed AFB$_1$ binding to calf thymus DNA

Table 4 Influence of black pepper and piperine on gastrointestinal system

Effects demonstrated	Investigators
Digestive stimulant action:	
a) Stimulation of digestive enzymes of Pancreas by dietary piperine	Platel and Srinivasan (2000)
b) Stimulation of digestive enzymes of Intestine by dietary piperine	Platel and Srinivasan (1996)
c) Oral administration of piperine increases biliary bile acid secretion	Bhat and Chandrasekhara (1987)
Influence on intestinal motility and food transit time:	
a) Gastrointestinal food transit time shortened by prolonged dietary piperine in rats	Platel and Srinivasan (2001)
b) Increased orocecal transit time after black pepper consumption in humans	Vazquez-Olivencia et al. (1992)
c) Piperine inhibited gastric emptying of solids/liquids in rats; inhibited gastrointestinal transit in mice	Bajad et al. (2001)
d) Piperine dose-dependedly delayed gastrointestinal motility in mice	Izzo et al. (2001)
Effect on gastric mucosa:	
a) Black pepper caused increases in gastric parietal and pepsin secretion and increased gastric cell exfoliation in humans	Myers et al. (1987)
b) Black pepper increased gastric acid secretion in anesthetized rats	Vasudevan et al. (2000)
c) Piperine increased gastric acid secretion in albino rats	Ononiwu et al. (2002)
d) Piperine showed protective action against gastric ulcer in rats and mice induced by stress, indometacin, HCl	Bai and Xu (2000)
Anti-diarrhoral property:	
a) Piperine inhibited diarrhea produced by castor oil, $MgSO_4$, arachidonic acid in mice	Bajad et al. (2001)
b) Piperine reduced castor oil induced intestinal fluid accumu-lation in mouse intestine.	Capasso et al. (2002)
Influence on absorptive function:	
a) Piperine stimulated γ-glutamyl transpeptidase activity and enhanced uptake of amino acids in isolated epithelial cells of rat jejunum	Johri et al. (1992)
b) Piperine modulated the membrane dynamics and permea-tion characteristics, increasing the absorptive surface and induction of synthesis of proteins associated with cyto-skeletal function	Khajuria et al. (2002)

in vitro, in a dose-dependent manner. Rats pretreated with piperine accumulated considerable AFB_1 radioactivity in plasma and in the tissues examined as compared to the controls. However, piperine had no influence on hepatic AFB_1-DNA binding in vivo, which could possibly be due to the null effect of piperine on liver cytosolic glutathione transferase activity. Piperine-treated rat liver microsomes demonstrated a tendency to enhance AFB_1 binding to calf thymus DNA in vivo.

INFLUENCE ON THE GASTROINTESTINAL SYSTEM (TABLE 4)

Effect on Gastric Mucosa

While pungent spices have long been implicated as a cause of gastric mucosal injury, very few studies have been reported on their long-term effect on the gastric mucosa. Myers et al. (1987) assessed the effects of black pepper on the gastric mucosa using double-blind intragastric administration of the spice (1.5 g) to healthy human volunteers, with aspirin (655 mg) as positive control. Serial gastric washes were performed after the administration and gastric contents were analyzed for DNA, pepsin, blood, sodium, potassium, parietal cell secretion, and nonparietal cell secretion. 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. Mucosal microbleeding was seen after spice administration. The effect of black pepper was similar to aspirin in any parameter studied. The long-term result of daily pepper ingestion is unknown.

Vasudevan et al. (2000) have studied the effect of various spices on gastric acid secretion in anesthetized rats, and report that black pepper significantly increased gastric acid secretion. Piperine was studied for its effect on gastric acid secretion in white albino rats (Ononiwu et al., 2002). Increasing the dose from 20 mg/kg to 142 mg/kg produced dose dependent significant increase in gastric acid secretion. The effect of piperine was significantly antagonized by cimetidine (1 mg/kg) but not by atropine (1 mg/kg). Any involvement of cholinergic receptors in the observed piperine-induced increase in gastric acid secretion is thus excluded. There is however an indication that stimulation of histamine H2 receptors by piperine is likely to be involved in the increased acidity induced by piperine.

On the other hand, Bai and Xu (2000) have evidenced protective action of piperine against experimental gastric ulcer in rats and mice. The gastric mucosa damage was induced by stress, indometacin, HCl, and pyloric ligation in rats or mice. The number of gastric ulcers, the volume and acidity of gastric juices, and pepsin activity were monitored. Piperine at 25, 50, and 100 mg/kg i.g. protected animals from gastric ulceration in a dose-dependent manner. The inhibitory rates were 16.9, 36.0, and 48.3% in stress ulcers; 4.4, 51.1, and 64.4% in indometacin ulcers; 19.2, 41.5, and 59.6% in HCl ulcers; 4.8, 11.9, and 26.2% in pyloric ligation ulcers, respectively; Piperine inhibited the volume of gastric juice, gastric acidity, and pepsin activity.

Antidiarrhoeal Property

Peppers are added in traditional antidiarrhoeal formulations of different herbs. A study has been made in experimental mice to determine the rationale, if any, for its use in traditional antidiarrhoeal formulations (Bajad et al., 2001), where the antidiarrhoeal activity of piperine against castor oil, $MgSO_4$ and arachidonic acid was examined. It significantly inhibited diarrhoea produced

by these cathartics at 8 and 32 mg/kg p.o. dose. Inhibition of castor oil induced the enteropooling by piperine suggests its inhibitory effect on prostaglandins. Capasso et al. (2002) investigated the effect of piperine on castor oil-stimulated fluid accumulation in the small intestine of mice. Piperine (2.5–20 mg/kg, i.p.) dose-dependently reduced castor oil-induced intestinal fluid accumulation. It was further understood that piperine reduces castor oil-induced fluid secretion with a mechanism involving capsaicin-sensitive neurons, but not capsazepine-sensitive vanilloid receptors.

Influence on Absorptive Function

The effect of piperine on the absorptive function of the intestine has been studied in in vitro experiments which showed that piperine (25–100 μM) significantly stimulated γ-glutamyl transpeptidase (γ-GT) activity, enhanced the uptake of radiolabelled amino acids and increased the lipid peroxidation in freshly isolated epithelial cells of rat jejunum (Johri et al., 1992). The kinetic behavior of γ-GT towards substrate and acceptor altered in the presence of piperine.This suggested that piperine may interact with the lipid environment to produce effects leading to increased permeability of the intestinal cells. It is hypothesized that piperine's bioavailability-enhancing property may be attributed to increased absorption, which may be due to alteration in membrane lipid dynamics and change in the conformation of enzymes in the intestine (Khajuria et al., 2002). The results of membrane fluidity studies using an apolar fluorescent probe, pyrene (which measures the fluid properties of hydrocarbon core), showed an increase in intestinal brush border membrane fluidity. Piperine also stimulated Leucine amino peptidase and Glycyl-glycine dipeptidase activity, due to the alteration in enzyme kinetics. This suggests that piperine could modulate the membrane dynamics due to its apolar nature by interacting with surrounding lipids and hydrophobic portions in the protein vicinity, which may decrease the tendency of membrane lipids to act as stearic constrains to enzyme proteins and thus modify enzyme conformation. Ultra-structural 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, it is suggested that piperine may induce alterations in membrane dynamics and permeation characteristics, along with the induction of the synthesis of proteins associated with cytoskeletal function, resulting in an increase in the small intestine absorptive surface, thus assisting efficient permeation through the epithelial barrier.

Influence on Gastrointestinal Motility and Food Transit Time

Vazquez-Olivencia et al. (1992) have evaluated the effects of black pepper on small intestinal peristalsis measuring orocecal transit time utilizing the lactulose hydrogen breathe test. The lactulose hydrogen breath test was done on healthy subjects on different days with or without black pepper (1.5 g) given in gelatin capsules. An increase in orocecal transit time was observed after black pepper consumption (90 ± 51 min to 122 ± 88 min). Bajad et al. (2001) report that piperine inhibits gastric emptying (GE) of solids/liquids in rats and gastrointestinal transit (GT) in mice in a dose and time dependent manner. It significantly inhibited GE of solids and GT at the doses extrapolated from humans (1 mg/kg and 1.3 mg/kg p.o. in rats and mice, respectively). However, at the same dose the effect was insignificant for GE of liquids. One week oral treatment of 1 mg/kg and 1.3 mg/kg in rats and mice, respectively, did not produce a significant change in activity as compared to single dose administration. GE inhibitory activity of piperine is independent of gastric acid and pepsin secretion. Izzo et al. (2001) have studied the effect of piperine, which activates vanilloid receptors, on upper gastrointestinal motility in mice. Piperine (0.5–20 mg/kg i.p.) dose-dependently delayed gastrointestinal motility. The inhibitory effect of piperine (10 mg/kg) was strongly attenuated in capsaicin (75 mg/kg in total, s.c.)-treated mice. The study indicated that the vanilloid ligand piperine can reduce upper gastrointestinal motility. The effect of piperine involves capsaicin-sensitive neurones, but not vanilloid receptors.

The gastrointestinal food transit time was significantly shortened by dietary piperine (Platel and Srinivasan, 2001). The reduction in food transit time produced by dietary piperine roughly correlates with its beneficial influence either on digestive enzymes or on bile secretion (Platel and Srinivasan, 2001). Thus, the 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.

ANTIMUTAGENIC AND TUMOR INHIBITORY EFFECTS

El Hamss et al. (2003) have shown that black pepper is effective in reducing the mutational events induced by the promutagen-ethyl carbamate in Drosophila melanogaster using the wing Somatic Mutation and Recombination Test. Suppression of metabolic activation or interaction with the active groups of mutagens could be mechanism by which the spice exerts its antimutagenic action. Black pepper extracts have been demonstrated to possess tumor inhibitory activity (Loder et al., 1969). The tumor reducing activity of orally administered extracts of black pepper was studied in mice transplanted i.p. with Ehrlich ascites tumor (Unnikrishnan and Kuttan, 1990). The life span was increased in these mice by 65% indicating the potential use of the spice as anti-cancer agents as well as anti-tumor promoters.

The alcoholic extract of Piper longum fruits and its component piperine was studied for their immunomodulatory and antitumor activity (Sunila and Kuttan, 2004). The alcoholic extract of the fruits was 100% toxic at 500 μg/ml to Dalton's lymphoma

ascites (DLA) cells and 250 μg/ml to Ehrlich ascites carcinoma (EAC) cells. Piperine was found to be cytotoxic towards DLA and EAC cells at 250 μg/ml. The alcoholic extract and piperine was also found to produce cytotoxicity towards L929 cells in culture at a concentration of 100 and 50 μg/ml, respectively. The administration of alcoholic extract of Piper longum (10 mg/animal) as well as 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 to 37 and 59%.

Pradeep and Kuttan (2002) have recently demonstrated the antimetastatic activity of piperine by studying the effect of piperine on the inhibition of lung metastasis induced by B16F-10 melanoma cells in C57BL/6 mice. A simultaneous administration of the compound with tumor induction produced a significant reduction (95%) in tumor nodule formation. The elevated levels of serum sialic acid and serum γ-GT activity in the untreated animals was significantly reduced in the animals treated with piperine. Piperine-treated animals survived the 90 days experiment. Histopathology of the lung tissue also correlated with the lifespan of the drug-treated animals.

The cytoprotective effect of piperine on B(α)p-induced experimental 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 (Selvendiran et al., 2003). Oral administration of piperine (100 mg/kg body weight) effectively suppressed lung cancer initiated with B(α)p as revealed by the decrease in the extent of lipid peroxidation with concomitant increase in the activities of enzymatic antioxidants (superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic antioxidants (reduced glutathione, vitamin E, and vitamin C) levels when compared to lung cancer bearing animals.

Piperine has been evidenced to show chemopreventive effects when administered orally on lung cancer bearing animals (Selvendiran and Sakthisekaran, 2004). The beneficial effect of piperine is primarily exerted during the initiation phase and the post-initiation stage of B(α)p induced lung carcinogenesis, via beneficial modulation of lipid peroxidation and membrane bound ATPase enzymes. Selvendiran et al. (2004a) studied the ability of piperine to prevent lung carcinogenesis induced by B(α)p in mice and its effects on cell proliferation. Administration of piperine significantly decreased the levels of lipid peroxidation, protein carbonyls, the nucleic acid content, and polyamine synthesis that were found to be increased in lung cancer bearing animals. Piperine could effectively inhibit B(α)p-induced lung carcinogenesis in albino mice by offering protection from protein damage and also by suppressing cell proliferation.

DELETERIOUS EFFECT OF PIPERINE ON THE REPRODUCTIVE SYSTEM

The effect of piperine on the fertilizing ability of hamster sperm was investigated in vitro (Piyachaturawat et al., 1991).

Sperm were incubated in a capacitation medium for 3 h prior to co-incubation with hamster eggs in a fertilization medium for another 3 h. 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. When piperine was administered to mature male albino rats at doses of 5 and 10 mg/kg body weight, p.o., respectively, for 30 days, only the higher dose caused a significant reduction in the weights of testis and accessory sex organs (Malini et al., 1999). Histological studies revealed that piperine at a 10 mg dose, caused severe damage to the seminiferous tubule, caused a decrease in seminiferous tubular and Leydig cell nuclear diameter, and desquamation of spermatocytes and spermatids. Correlated to the structural changes, a fall in caput and cauda epididymal sperm concentrations was also evident. A 10 mg dose of piperine also caused a marked increase in serum gonadotropins and a decrease in intratesticular testosterone concentration, despite normal serum testosterone titres.

The reproductive toxicity of piperine was studied in Swiss albino mice (Daware et al., 2000) with respect to the effect on the estrous cycle, their mating behavior, the toxicity to male germ cells, fertilization, and the implantation and growth of pups. Piperine (10 and 20 mg/kg body weight) increased the period of the diestrous phase resulting in decreased mating performance and fertility. Post-partum litter growth was not affected by the piperine treatment and sperm shape abnormalities were not induced at doses up to 75 mg/kg. Considerable anti-implantation activity was recorded after five days post-mating oral treatment with piperine. These results show that piperine interferes with several crucial reproductive events in a mammalian model. The effect of piperine on the fertilization of eggs with sperm has been investigated in female hamsters intragastrically treated with piperine at doses of 50 or 100 mg/kg body weight from day 1 through day 4 of the oestrous cycle (Piyachaturawat and Pholpramool, 1997). 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.

OTHER PHYSIOLOGICAL EFFECTS

The anti-inflammatory activity of piperine has been reported in rats employing different experimental models like carrageenan-induced rat paw edema, cotton pellet granuloma, and croton oil-induced granuloma pouch (Mujumdar et al., 1990). 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 (Kawada et al., 1988). Black pepper extract was found to possess growth-stimulatory activity in cultured melanocytes (Lin et al., 1999). Its aqueous extract at 0.1 mg/ml was observed to cause nearly 300% stimulation of the growth of a cultured mouse melanocyte line, melan-a, in 8 days, hence it is inferred

Table 5 Other physiological effects of black pepper and piperine

Effects demonstrated	Investigators
Antimutagenic and tumor inhibitory effects:	
a) Black pepper is effective in reducing mutational events induced by procarcinogen—ethylcarbamate in Drosophila	El Hamss et al. (2003)
b) Tumour inhibitory activity of black pepper in mice implanted with Ehrlich ascites tumour	Unnikrishnan and Kuttan (1990)
c) Piperine inhibited tumor development in mice induced with Dalton's lymphoma cells and increased the life span of mice bearing Ehrlich ascites carcinoma	Sunila and Kuttan (2004)
d) Anti-metastatic activity of piperine on lung metastasis induced by melanoma cells in mice	Pradeep and Kuttan (2002)
e) Chemopreventive effect of piperine on benzo(α)pyrene induced experimental lung cancer in mice	Selvendiran et al. (2003) Selvendiran and Sakthisekaran, (2004); Selvendiran et al. (2004a)
Effect on reproductive system:	
a) Piperine decreased fertilizing ability of hamster sperms and degree of polyspermia in vitro	Piyachaturawat et al. (1991)
b) Continued oral intake of piperine produced reduction in weights of testis, fall sperm concentration, decrease in intra-testicular testosterone concentration in mature male rats	Malini et al. (1999)
c) Oral intake of piperine decreased fertility due to interference with crucial reproductive events in albino mice.	Daware et al. (2000)
Other effects:	
a) Thermogenic action of piperine via adrenal catecholamine secretion in rats	Kawada et al. (1988)
b) Anti-inflammatory activity of piperine in experimental models: carrageenan-induced rat paw edema, cotton pellet granuloma, croton oil induced granuloma pouch	Mujumdar et al. (1990)
c) Growth stimulatory activity of black pepper extract in cultured melanocytes	Lin et al. (1999)
d) Anti-thyroid activity of piperine and hence decreased glucose concentration in mice	Panda and Kar (2003)
e) Piperine inhibited mitochondrial oxidative phosphorylation and diminished calcium uptake in vitro	Reanmongkol et al. (1988)
f) Piperine exerted protection against t-butyl hydroperoxide and carbon tetrachloride in hepatotoxicity by reducing lipid peroxidation	Koul and Kapil (1993)
g) Piperine exerted chemopreventive effect by retarding the acti-vation of procarcinogen aflatoxin B_1 and hence protecting from its cytotoxicity and genotoxicity	Singh et al. (1994)
h) Piperine promoted cytotoxicity induced by benzo(α)pyrene in cultured lung fibroblast cells	Chu et al. (1994)
i) Piperine pretreatment potentiated hepatotoxicity of carbon tetrachloride in rats	Piyachaturawat et al. (1995)

that piperine is a potential repigmenting agent for the treatment of vitiligo (Table 5).

Piperine was evaluated for its thyroid hormone and glucose regulatory efficacy in adult Swiss albino mice (Panda and Kar, 2003). Its daily oral administration (2.5 mg/kg) for 15 days lowered the serum levels of both the thyroid hormones, thyroxin (T_4) and triiodothyronine (T_3) as well as glucose concentrations with a concomitant decrease in hepatic 5′D enzyme and glucose-6-phospatase (G-6-Pase) activity. The decrease in T_4, T_3 concentrations and in G-6-Pase were comparable to that of a standard antithyroid drug, Proylthiouracil. It is suggested that a higher dose of piperine may inhibit thyroid function and serum glucose concentration in euthyroid individuals.

The in vitro effects of piperine on three bioenergetic reactions namely, oxidative phosphorylation, ATPase activity and calcium transport by isolated rat liver mitochondria have been investigated (Reanmongkol et al., 1988). The study suggested that piperine inhibits mitochondrial oxidative phosphorylation at the level of respiratory chain. Piperine did not inhibit the mitochondrial ATPase activity induced by dinitrophenol, but by itself stimulated activity of this enzyme. Piperine was also found to diminish calcium uptake and to facilitate the release of accumulated calcium by the mitochondria incubated with succinate or ATP. The effect of piperine on calcium transport is likely to be consequential to the effects of this compound on the mitochondrial respiratory chain and ATPase activity. 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 (Jamwal and Singh, 1993).

Piperine was evaluated for its antihepatotoxic potential in order to validate its use in traditional therapeutic formulations (Koul and Kapil, 1993). It exerted a significant protection against t-butyl hydroperoxide and carbon tetrachloride hepatotoxicity by reducing both in vitro and in vivo lipid peroxidation, leakage of enzymes—alanine aminotransferase (AlAT) and alkaline phosphatase and by preventing the depletion of glutathione and total thiols in the intoxicated mice.

Piperine pretreatment potentiated the hepatotoxicity of CCl_4 in a dose-dependent manner in rats (Piyachaturawat et al., 1995). The maximum potentiation occurred at a dose of 100 mg/kg BW was intragastrically administered 4 h prior to an intraperitoneal injection of CCl_4, at which time the activities of plasma AlAT and aspartate aminotransferase (AsAT) were elevated by 70–80%. Concurrent with the rise in AlAT and AsAT activities, the accumulation of hepatic triglyceride increased whereas the plasma level of triglyceride decreased. Piperine pretreatment also potentiated CCl_4-induced lipid peroxidation in the liver. In the in vitro system in which the tissue was preincubated with piperine and CCl_4 was added into the incubation medium, piperine also exhibited a concentration dependent potentiation on CCl_4-induced lipid peroxidation and on the activity of NADPH-cytochrome C-reductase. The results indicated that piperine potentiated CCl_4-induced hepatotoxicity by interacting with liver cells and increased the activity of

NADPH-cytochrome C reductase. The increase in activity of this enzyme accelerated the biotransformation of CCl_4, thereby increasing lipid peroxidation and enhancing hepatotoxicity.

Piperine was found to promote DNA damage and cytotoxicity induced by B(α)p in cultured V-79 lung fibroblast cells (Chu et al., 1994). The V-79 cells were treated with a non-toxic dose of piperine (1–20 μM) plus 10 μM B(α)p, or pretreated with piperine for 30 min or 2 h prior to the administration of 10 μM B(α)p. B(α)p cytotoxicity was potentiated significantly by piperine under each experimental condition. The study also suggested that the promotion by piperine of B(α)p-induced cytotoxicity in V-79 lung fibroblast cells is due to mechanisms that decrease the activities of GST and UDP-GTase and increase the formation of a B(α)p-DNA adduct.

Singh et al. (1994) studied the effect of piperine on the cytotoxicity and genotoxicity of aflatoxin B_1 (AFB$_1$) in rat hepatoma cells H4IIEC3/G-(H4IIE) using cellular growth and the formation of micronuclei as endpoints. AFB$_1$ inhibited the growth of H4IIE cells with an ED$_{50}$ of 15 nM. Piperine markedly reduced the toxicity of the mycotoxin. Thus, at 100 μM piperine largely restored the rate of growth of the cells. Likewise, piperine reduced the AFB$_1$-induced formation of micronuclei in a concentration-dependent manner. Piperine itself was not toxic to the cells up to a concentration of almost 100 μM. The results suggest, that piperine is capable of counteracting AFB$_1$ toxicity by suppressing cytochromes P$_{450}$ mediated bioactivation of the mycotoxin. Reen et al. (1997) have investigated the potential of piperine for inhibiting the activity of cytochrome P4502B1 and protecting against AFB$_1$ in V79MZr2B1 (r2B1) cells (Chinese hamster cells) engineered for the expression of rat CYP4502B1. Piperine inhibited 7-methoxycoumarin demethylase in preparations of r2B1 cells with an IC$_{50}$ of approximately 10 μM. Piperine at 60 μM completely counteracted the 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: (i) Piperine is a potent inhibitor of rat CYP4502B1 activity; (ii) AFB$_1$ is activated by r2B1 cells to cytotoxic and genotoxic metabolites; and (iii) piperine counteracts CYP4502B1 mediated toxicity of AFB$_1$ in the cells and might, therefore, offer a potent chemopreventive effect against procarcinogens activated by CYP4502B1.

ABSORPTION AND METABOLISM OF PIPERINE

Upon administration of piperine to male albino rats at a dose of 170 mg/kg by gavage or 85 mg/kg intraperitoneally, about 97% was absorbed irrespective of the mode of dosing (Bhat and Chandrasekhara, 1986). 3% of the administered dose was excreted as piperine in the feces, while it was not detectable in the urine. When everted sacs of rat intestines were incubated with 200–1000 μg of piperine, about 47–64% of the added piperine disappeared from the mucosal side (Bhat and Chandrasekhara, 1986). Only piperine was present in the serosal fluid and also the intestinal tissue, indicating that piperine did not undergo any metabolic change during absorption. The examination of the passage of piperine through the gut indicated that the highest concentration in the stomach and small intestine was attained at about 6 h. Only traces (less than 0.15%) of piperine were detected in the serum, the kidney, and the spleen from 30 min to 24 h. About 1–2.5% of the intraperitoneally 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 sulphates, and phenols indicated that scission of the methylenedioxy group of piperine, glucuronidation and sulphation appear to be the major steps in the disposition of piperine in the rat. After oral administration of piperine (170 mg/kg) to rats, the metabolites in urine (0–96 h) were identified to be piperonylic acid, piperonyl alcohol, piperonal, and vanillic acid in the free form, whereas only piperic acid was detected in 0–6 h bile (Bhat and Chandrasekhara, 1987). The kidney appears to be the major excretion route for piperine metabolites in rats as no metabolite could be detected in feces. Based on these results, a pathway for the biotransformation of piperine in rats has been proposed (Fig. 2). In a recent investigation (Bajad et al., 2003), to further study the reported differences in its metabolism in rats and humans, a new major urinary metabolite was detected in rat urine and plasma using HPLC and characterized as 5-(3,4-methylenedioxy phenyl)-2,4-pentadienoic acid-N-(3-yl propionic acid)-amide. This metabolite has a unique structure in that it retains the methylenedioxy ring and conjugated double bonds while the piperidine ring is modified to form propionic acid group.

Khajuria et al. (1998) have made an effort to understand the absorption dynamics of piperine in the intestine on oral absorption. Using intestinal everted sacs and cycloheximide treatment and exclusion of Na$^+$ the salts from the incubating medium as the variables used, the absorption half-life, the absorption rate, the absorption clearance, and apparent permeability co-efficient were computed. Data suggested that piperine is absorbed very fast across the intestinal barrier. 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.

CONCLUSIONS

Black pepper or its active principle piperine has been experimentally demonstrated by a number of independent investigators to possess diverse physiological effects (Fig. 3). Piperine has been evidenced to protect against oxidative damage by inhibiting or quenching free radicals and lower lipid peroxidation and beneficially influence cellular thiols, antioxidant molecules and antioxidant enzymes in different situations of oxidative stress. The most far-reaching attribute of piperine has been its inhibitory influence on hepatic, pulmonary, and intestinal drug metabolizing system. It strongly inhibits a particular cytochrome P$_{450}$ and hence phase-I reactions mediated by the same, especially aromatic hydroxylation. It also strongly retards glucuronidation reactions of phase-II. As a result of interference with crucial
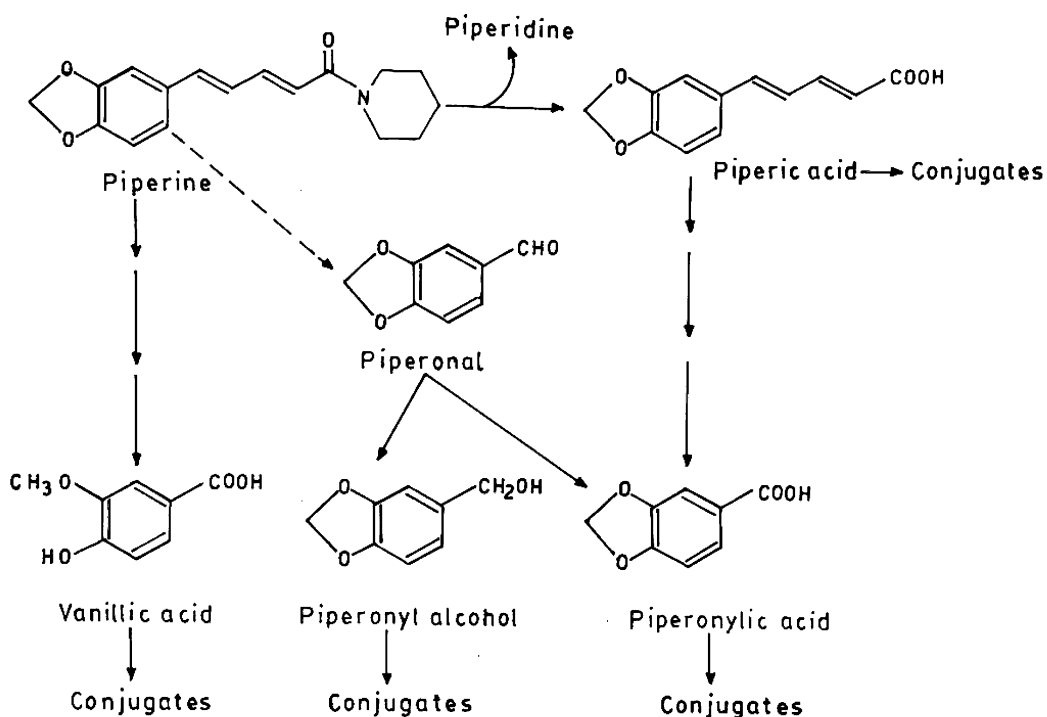
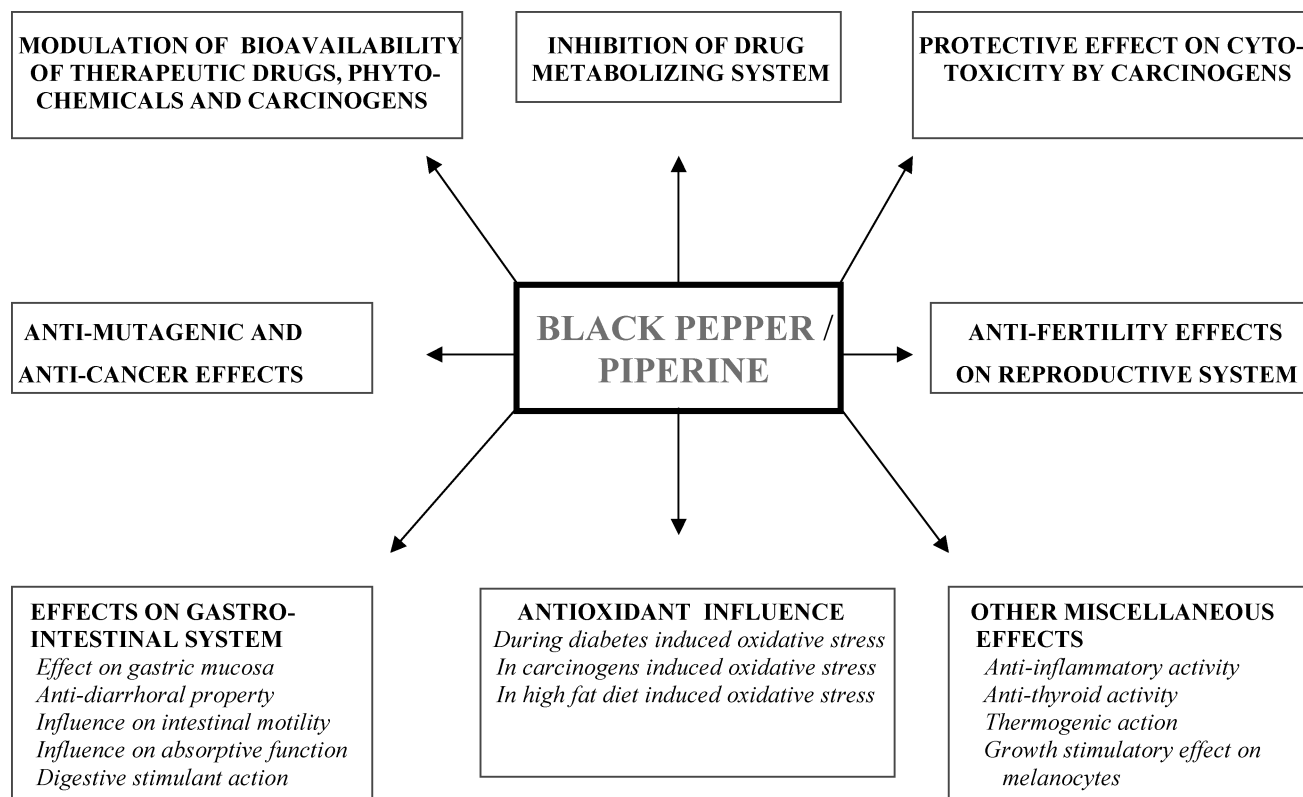**Figure 2**   Biotransformation of piperine.

Figure 3   Summary of diverse physiological effects of black pepper and piperine.

drug metabolizing reactions in the liver, piperine enhances the bioavailability of therapeutic drugs, i.e., increases their plasma half-life, and delays their excretion. Piperine also possesses a cytoprotective effect by retarding the activation of certain procarcinogens by the drug metabolizing system. The gastro-intestinal system is affected by black pepper and piperine in many ways. Both black pepper and piperine have been evidenced to have a definite effect on intestinal motility, the antidiarrhoral property, and on the ultrastructure of intestinal microvilli improving the absorbability of nutrients. Among other physiological effects piperine exerts, its potential antifertility influence on the reproductive system has been clearly established in in vitro and animal systems. Antimutagenic and anti-tumor properties of piperine have been evidenced by a number of animal and cell line studies. Although initially there were a few controversial reports regarding the safety of black pepper or piperine as a food additive, the latter studies have established the safety of this spice in several animal studies.

REFERENCES

Allameh, A. et al. (1992). Piperine, a plant alkaloid of the piper species, enhances the bioavailability of aflatoxin B1 in rat tissues, *Cancer Lett.*, **61**:195–199.

Ames, B. N. (1983). Dietary carcinogens and anticarcinogens: Oxygen radials and degenerative diseases, *Science*, **221**:1256–1264.

Atal, C. K. et al. (1981). Scientific evidence on the role of Ayurvedic herbals on bioavailability of drugs, *J. Ethnopharmacol.*, **4**:229–232.

Atal, C. K. et al. (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.

Badmaev, V. V. et al. (2000). Piperine derived from black pepper increases the plasma levels of coenzyme Q10 following oral supplementation, *J. Nutr. Biochem.*, **11**:109–113.

Bai, Y. F., and Xu, H. (2000). Protective action of piperine against experimental gastric ulcer, *Acta Pharmacol. Sin.*, **21**:357–359.

Bajad, S. et al. (2001). Antidiarrhoeal activity of piperine in mice, *Planta Med.*, **67**:284–287.

Bajad, S. et al. (2001). Piperine inhibits gastric emptying and gastrointestinal transit in rats and mice, *Planta Med.*, **67**:176–179.

Bajad, S. et al. (2003). Characterization of a new rat urinary metabolite of piperine by LC/NMR/MS studies, *Eur. J. Pharm. Sci.*, **19**:413–421.

Bano, G. et al. (1991). Effect of piperine on bioavailability and pharmacokinetics of propranolol and theophylline in healthy volunteers, *Eur. J. Clin. Pharmacol.*, **41**:615–617.

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

Bhat, B. G., and Chandrasekhara, N. (1986). Studies on the metabolism of piperine: absorption, tissue distribution and excretion of urinary conjugates in rats, *Toxicology,* **40**:83–92.

Bhat, B. G., and Chandrasekhara, N. (1987). Metabolic disposition of piperine in the rat, *Toxicology,* **44**:99–106.

Bhat, G. B., and Chandrasekhara, N. (1987a). Effect of black pepper and piperine on bile secretion and composition in rats, *Nahrung*, **31**:913–916.

Buchanan, R. L. (1980). Toxicity of spices containing methylene dioxy benzene derivatives: A review, *J. Food Safety*, **1**:275–293.

Capasso, R. et al. (2002). Effect of piperine, the active ingredient of black pepper, on intestinal secretion in mice, *Life Sci.*, **71**:2311–2317.

Cho, S. Y., and Lee, S. H. (1983). Effect of black pepper on the composition of serum and liver in rat, *Hanguk Yongyang Siklyong Hakhoechi*, **12**:219–224.

Chu, C. Y. et al. (1994). Modulatory effect of piperine on benzo[α]pyrene cytotoxicity and DNA adduct formation in V-79 lung fibroblast cells, *Food Chem. Toxicol.*, **32**:373–377.

Concon, J. M. et al. (1979). Black pepper (*Piper nigrum*): Evidence of carcinogenicity, *Nutr. Cancer*, **1**:22–26.

Dalvi, R. R., and Dalvi, P. S. (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.

Dalvi, R. R., and Dalvi, P. S. (1991a). Differences in the effects of piperine and piperonyl butoxide on hepatic drug-metabolizing enzyme system in rats, *Drug Chem. Toxicol.*, **14**:219–229.

Daware, M. B. et al. (2000). Reproductive toxicity of piperine in Swiss albino mice, *Planta Med.*, **66**:231–236.

Dogra, R. K. et al. (2004). Immunotoxicological effects of piperine in mice, *Toxicology*, **196**:229–236.

El Hamss, R. et al. (2003). Antimutagenic properties of bell and black peppers, *Food Chem. Toxicol.*, **41**:41–47.

Epstein, S. S., and Swartz, J.B. (1984). Cancer and diet, *Science*, **224**:660–666.

Glatzel, H. (1968). Physiological aspects of flavour compounds. *Indian Spices*, **5**: 13–21.

Govindarajan, V. S. (1977). Pepper - Chemistry, technology and quality evaluation, *CRC Crit. Rev. Food Sci. Nutr.*, **9**:115–225.

Hiwale, A. R. et al. (2002). Effect of co-administration of piperine on pharmacokinetics of β-lactam antibiotics in rats, *Indian J. Exp. Biol.*, **40**:277–281.

Ioannidesn C. et al. (1981). Safrole: Its metabolism, carcinogenicity and interactions with cytochrome p-450. *Food Cosmet. Toxicol.,* **19**:657–666.

Izzo, A. A. et al. (2001). Effect of vanilloid drugs on gastrointestinal transit in mice, *Br. J. Pharmacol.*, **132**:1411–1416.

Jamwal, D. S., and 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.

Johri, R. K. et al. (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.

Karekar, V. R. et al. (1996). Assessment of genotoxic effect of piperine using *Salmonella typhimurium* and somatic and somatic and germ cells of Swiss albino mice, *Arzneimittel Forschung*, **46**:972–975.

Kawada, T. et al. (1988). Some pungent principles of spices cause the adrenal medulla to secrete catecholamine in anesthetized rats. *Proc. Soc. Exp. Biol. Med.*, **188**:229–233.

Khajuria, A. et al. (1998). Permeability characteristics of piperine on oral absorption - an active alkaloid from peppers & a bioavailability enhancer, *Indian J. Exp. Biol.*, **36**:46–50.

Khajuria, A. et al. (1998). Piperine modulation of carcinogen induced oxidative stress in intestinal mucosa, *Mol. Cell. Biochem.*, **189**:113–118.

Khajuria, A. et al. (2002). Piperine modulates permeability characteristics of intestine by inducing alterations in membrane dynamics: influence on brush border membrane fluidity, ultrastructure & enzyme kinetics, *Phytomedicine,* **9**:224–231.

Kim, H. Y. et al. (1975). Effect of spices on salivary amylase. *Iha Uihak Hoechi* (Korean), **7**:78–82.

Koul, I. B., and Kapil, A. (1993). Evaluation of the liver protective potential of piperine, an active principle of black and long peppers. *Planta Med.*, **59**:413–417.

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

Lambert, J. D. et al. (2004). Piperine enhances the bioavailability of the tea polyphenol (-)-epigallocatechin-3-gallate in mice, *J. Nutr.*, **134**:1948–1952.

Lin, Z. et al. (1999). Stimulation of mouse melanocyte proliferation by *Piper nigrum* fruit extract and its main alkaloid - piperine, *Planta Med.*, **65**:600–603.

Loder, J. W. et al. (1984). Tumor inhibitory plants: amides of pepper novaehollandiae, *Aust. J. Chem.*, **22**:1531–1538.

Malini, T. et al., (1999). Effects of piperine on testis of albino rats, *J. Ethnopharmacol.*, **64**:219–225.

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

Mujumdar, A. M. et al. (1990). Anti-inflammatory activity of piperine. *Jpn. J. Med. Sci. Biol.*, **43**:95–100.

Myers, B. M. et al. (1987). Effect of red pepper and black pepper on the stomach, *Am. J. Gastroenterol.*, **82**:211–214.

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

Namiki, K. et al. (1984). Mutagen formation by nitrite-spice reactions, *J. Agric. Food Chem.*, **32**:948–952.

Ononiwu, I. M. et al. (2002) Effects of piperine on gastric acid secretion in albino rats, *Afr. J. Med. Sci.*, **31**:293–295.

Panda, S. and Kar, A. (2003). Piperine lowers the serum concentrations of thyroid hormones, glucose and hepatic 5′D activity in adult male mice, *Horm. Metab. Res.*, **35**:523–526.

Piyachaturawat, P. et al. (1983). Acute and subacute toxicity of piperine in mice, rats and hamsters, *Toxicol. Lett.*, **16**:351–359.

Piyachaturawat, P. et al. (1991). Effects of piperine on hamster sperm capacitation and fertilization *in vitro*, *Int. J. Androl.*, **14**:283–290.

Piyachaturawat, P. et al. (1995). Potentiation of carbon tetrachloride hepatotoxicity by piperine, *Drug Chem. Toxicol.*, **18**:333–344.

Piyachaturawat, P., and Pholpramool, C. (1997). Enhancement of fertilization by piperine in hamsters. *Cell Biol. Int.*, **21**:405–409.

Platel, K., and 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.

Platel, K., and Srinivasan, K. (2000). Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats, *Nahrung*, **44**:42–46.

Platel, K., and Srinivasan, K. (2001). Studies on the influence of dietary spices on food transit time in experimental rats, *Nutr. Res.*, **21**:1309–14.

Pradeep, C. R., and 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.

Prasad, N. S. et al. (2004). Spice phenolics inhibit human PMNL 5-lipoxygenase, *Prostaglandins Leukot. Essent. Fatty Acids*, **70**:521–528.

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

Reanmongkol, W. et al. (1988). Effects of piperine on bioenergetic functions of isolated rat liver mitochondria, *Biochem. Pharmacol.*, **37**: 753–757.

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

Reen, R. K., and 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.

Reen, R. K. et al. (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.

Reen, R. K. et al. (1996). Piperine impairs cytochrome P4501A1 activity by direct interaction with the enzyme and not by down regulation of CYP1A1 gene expression in rat hepatoma 5L cell line, *Biochem. Biophys. Res. Commun.*, **218**:562–569.

Reen, R. K. et al. (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**:165–73.

Selvendiran, K. et al. (2003). Cytoprotective effect of piperine against benzo[a]pyrene induced lung cancer with reference to lipid peroxidation and antioxidant system in Swiss albino mice, *Fitoterapia*, **74**:109–115.

Selvendiran, K. et al. (2004). Modulatory effect of Piperine on mitochondrial antioxidant system in benzo(α)pyrene-induced experimental lung carcinogenesis, *Phytomedicine*, **11**:85–89.

Selvendiran, K. et al. (2004a). Protective effect of piperine on benzo(α)pyrene-induced lung carcinogenesis in Swiss albino mice, *Clin. Chim. Acta*, **350**:73–78.

Selvendiran, K., and 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.

Shoba, G. et al. (1998). Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers, *Planta Med.*, **64**:353–356.

Singh, J. et al. (1986). Piperine-mediated inhibition of glucuronidation activity in isolated epithelial cells of the guinea-pig small intestine: evidence that piperine lowers the endogeneous UDP-glucuronic acid content. *J. Pharmacol. Exp. Ther.*, **236**:488–493.

Singh, J. et al. (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.

Singh, A., and Rao, A. R. (1993). Evaluation of the modulatory influence of black pepper (*Piper nigrum* L.) on the hepatic detoxication system, *Cancer Lett.*, **72**:5–9.

Srinivasan, K., and Sambaiah, K. (1991). Effect of spices on cholesterol-7α-hydroxylase activity and on serum & hepatic cholesterol levels in the rat, *Int. J. Vitam. Nutr. Res.*, **61**:364–369.

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

Sunila, E. S., and Kuttan, G. (2004). Immunomodulatory and antitumor activity of *Piper longum* Linn. and piperine, *J. Ethnopharmacol.*, **90**:339–346.

Unnikrishnan, M.C., and Kuttan, R. (1990). Tumour reducing and anticarcinogenic activity of selected spices, *Cancer Lett.*, **51**:85–89.

Vasudevan, K. et al. (2000). Influence of intra-gastric perfusion of aqueous spice extracts on acid secretion in anesthetized albino rats, *Indian J. Gastroenterol.*, **19**:53–56.

Vazquez-Olivencia, W. et al. (1992). The effect of red and black pepper on orocecal transit time. *J. Am. Coll. Nutr.*, **11**:228–231.

Velpandian, T. et al. (2001). Piperine in food: interference in the pharmacokinetics of phenytoin, *Eur. J. Drug Metab. Pharmacokinet.*, **26**:241–247.

Vijayakumar, R. S. et al. (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.