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EFFECT OF *Piper longum* Linn. (PIPPALI) IN MONOSODIUM GLUTAMATE TOXICITY IN RATS

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Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

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Kerala Agricultural University, Thrissur

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I hereby declare that this thesis, entitled **"EFFECT OF *Piper longum* Linn. (PIPPALI) IN MONOSODIUM GLUTAMATE TOXICITY IN RATS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Introduction

INTRODUCTION

Monosodium glutamate (MSG) is the sodium salt of glutamic acid, a non-essential amino acid that forms 20% of dietary protein. It is used as a flavor enhancer all over the world especially in Chinese and Japanese foods. The flavor enhancing property of MSG was first noted in 1908 by Ikeda, a Japanese chemist and it became commercially available in the early 1950s. Since that time it has been used increasingly in food. Twenty thousand tons of MSG are manufactured and used in the United States of America each year. Although there is no strict dosage for MSG, a minimum of 1 g per serving has been suggested (Schaumburg *et al*., 1969). But as much as 4-6 g may be ingested in a highly seasoned restaurant meal (Allen *et al*., 1987). Nowadays there has been increased consumption of ready to eat, canned, Chinese and Japanese foods, all containing MSG.

Although it may appear difficult to fault a substance that is one of the building blocks of proteins, MSG is not a wholly innocuous substance. It is proposed as the cause of Chinese restaurant syndrome in humans which is characterized by head ache, burning sensation along the back of the neck, chest tightness, nausea and sweating (Schaumburg *et al*., 1969, Kenney and Tidball, 1972). In addition to the Chinese restaurant syndrome, MSG is known to elicit other toxic effects such as, impairment in memory retention, damage in the hypothalamic neurons and alterations in mitochondrial lipid peroxidation and antioxidant status in different brain regions namely, cerebral hemispheres, cerebellum, brain stem and diencephalons (Park *et al*., 2000, Singh *et al*., 2003). Furthermore, MSG administration has been shown to induce hyperphagia and increase energy intake leading to obesity (Diemen *et al*., 2006). The asthma provoking potential of MSG in sensitive individuals is also proved (Allen *et al*., 1987).

Alterations in the level of certain biochemical indices, such as carbohydrates, lipids and proteins in rats treated with MSG are also well documented. These reports are suggestive of the fact that MSG administration induces a shift in the carbohydrate metabolism towards lipogenesis leading to hyperlipidemia (Malik and Ahluwalia, 1994). Hyperglycemia and hyperlipidemia induced by MSG can result in lipid peroxidation of biomembranes and production of oxygen free radicals (Jain, 1989). Chronic administration of MSG at dose rates of 4mg/g bodyweight and above induced oxidative stress in the erythrocytes, liver, kidney, heart, brain and lungs of experimental animals (Ahluwalia *et al*., 1996, Choudhary *et al*., 1996, Bopanna *et al*., 1999 and Onyema *et al*., 2006).

Oxidative stress is a state of imbalance between generation of reactive oxygen species (ROS) like hydroxyl and superoxide radicals, and the level of antioxidant defense system. It results in the damage of biopolymers including nucleic acids, proteins, polyunsaturated fatty acids and carbohydrates. ROS and free radical mediated processes have been implicated in the pathogenesis of a wide variety of diseases like Alzheimer's disease, Parkinson's disease, atherosclerosis, cancer, rheumatoid arthritis, immunological incompetence, neurodegenerative disorders and damage to liver and kidney.

It is proposed that MSG induced oxidative stress is initiated by high concentrations of extracellular glutamate that prevent cystine uptake into the cells, followed by the depletion of intracellular cysteine which results in decreased level of reduced glutathione (Hong and Liu, 2004). Another possible mechanism of MSG toxicity is due to excessive stimulation of glutamate receptors resulting in increased intracellular calcium ion concentration. The deregulation of calcium homeostasis results in mitochondrial overload with a subsequent production of ROS (Singh *et al*., 2003). Presence of glutamatergic receptors has been demonstrated in central nervous

system and peripheral organs such as liver, kidney, lungs, spleen and testicles (Hinoi *et al*., 2004). This suggests that MSG is capable of exhibiting a strong and diffuse damaging effect probably in all the organs and systems.

Herbal medicine is one of the oldest forms of treatment known and used by all races and people. About 35,000 to 70,000 plant species are being used world wide in health care systems by 70% of the world's population. Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. Evidences suggest that Ayurveda developed in India is, perhaps, the earliest medical system that describes many herbal alternatives having little side effects as compared to the synthetic modern drugs.

Piper longum (Indian long pepper/ Pippali) is an important medicinal plant, which finds use in Ayurvedic and Unani systems of medicine. It is an important culinary spice throughout the Indian subcontinent, Sri Lanka, Middle East countries and in the American continent. The plant is a slender aromatic perennial climber with fruits having a warm taste. The fruits are considered to have stimulant, carminative, laxative and stomachic properties. They are useful in asthma, bronchitis, tumors, spleen disorders, inflammation, leprosy, insomnia and jaundice. The roots are used as laxative and anthelmintic (Kirtikar and Basu, 1975).

Fruits of *Piper longum* contain an alkaloid, piperine, which can enhance the bioavailability of other drugs. There are also evidences for the antiulcer, antidiarrhoeal, immunomodulatory, hepatoprotective, antioxidant, fungicidal, insecticidal and acaricidal effects of the plant. However, *in vivo* studies on the antioxidant potential of *Piper longum* are limited. The present study is aimed to evaluate the following effects of MSG and *Piper longum* in experimental rats.

1. Effect of MSG on the biochemical and morphological aspects of vital organs such as liver and kidney.
2. Hepato and nephro protective effect of ethanolic extract of fruits of *Piper longum* on MSG toxicity.
3. Effect of ethanolic extract of fruits of *Piper longum* as a herbal medicine to treat MSG toxicity. .

Review of literature

2. REVIEW OF LITERATURE

Current study is aimed at evaluating the toxicity caused by monosodium glutamate (ajinomoto) in rats, especially in the vital organs such as, liver and kidney and the effect of *Piper longum* extract to treat the toxic effects as well as to protect the vital organs from the induction of MSG toxicity.

Liver has an essential role in a myriad of body functions such as, nutrient digestion, metabolism, detoxification of drugs and xenobiotics, excretion of hydrophobic metabolites and synthesis of most plasma proteins. Major role of kidney is to eliminate the metabolic wastes from the body by the process of excretion. Several biochemical tests are useful to detect, diagnose and evaluate the involvement of liver and kidney in many disease conditions. These tests also help to monitor therapy and assess the prognosis. Determining the level of various metabolites such as, serum total cholesterol, triacylglycerol, bilirubin, urea, creatinine, total protein, albumin and enzymes such as, aminotransferases (ALT, AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT), arginase, sorbitol dehydrogenase and glutamate dehydrogenase give an idea about the normal functioning or extent of damage or dysfunction of these organs.

Decrease in the level of total protein and albumin occurs when 80 percent or more of the functional capacity of liver is lost. Hypoalbuminemia can also arise by other mechanisms notably protein losing enteropathies, hepatopathies, nephropathies, redistribution of albumin into peritoneal and plural effusions and accelerated degradation (Evans, 1988).

The measurement of non protein nitrogenous metabolites such as urea and creatinine in serum is used to assess kidney function. Excess of urea and creatinine in blood (azotemia) is the terminal clinical expression of kidney failure and results from the failure of the kidneys to maintain adequate excretory, regulatory and endocrine functions (Newman, 2001).

In most species, death occurs within a week after total cessation of renal function. Partial loss of renal function results in variable deviations from normal, depending on the quantity of functional tissue remaining (Finco, 1997)

2.1. *Piper longum*

The ripe fruits of *Piper longum* are useful in bronchitis, asthma, abdominal complaints, tumours, jaundice and diseases of spleen (Kirtikar and Basu, 1975).

According to Nadkarni (1976) the major chemical constituents present in *Piper longum* are resins, volatile oils, starch, gum, fatty oil, inorganic matter and an alkaloid, piperine.

Bhat and Chandrasekharan (1986) observed that upon administration of piperine by gavage to male Wistar rats, 97 percent was absorbed without undergoing any metabolic change during absorption. Three percent of the administered dose was excreted as piperine in the feces. Piperine was not detected in urine. They also suggested that scission of the methylenedioxy group of piperine, glucuronidation and sulphation are the major steps in the disposition of piperine in rats.

Chauhan *et al*. (1998) developed a reverse phase high pressure liquid chromatography method to determine piperine which is the most active ingredient in various Piper species. They estimated that the fruits and roots of *Piper longum* contain 2.02 percent and 1.2 percent piperine respectively on dry weight basis.

2.1.1. Antioxidant and hepatoprotective effects

Koul and Kapil (1993) evaluated the antihepatotoxic effect of piperine, an active alkaloidal constituent obtained from extracts of *Piper longum* and *Piper nigrum* and compared it with a known hepatoprotective drug, silymarin. They found that piperine exerted a significant protection against tert-butyl hydroperoxide and carbon tetrachloride (CCl_4) hepatotoxicity by reducing lipid peroxidation and preventing the depletion of reduced glutathione in toxicity induced mice, but the hepato protective potency was lower than that of silymarin.

Khajuria *et al*. (1997) investigated the free radical scavenging property of piperine against the oxidative agents, hydrogen peroxide and cumene hydroperoxide in rat intestinal lumen model. They observed that piperine administration along with oxidants invoked a prominent increase in non protein thiol and reduced glutathione (GSH) levels whereas, lipid peroxides and protein thiols were not affected. They suggested that piperine offered protection against oxidant induced alterations through the scavenging action of GSH.

Khajuria *et al.* (1998a) investigated the modulatory role of piperine in the oxidative changes induced by chemical carcinogens such as 7,12, dimethyl benzanthracene, dimethyl amino-methyl azobenzene and 3-methyl cholenthrene in rat intestinal model. Treatment with carcinogens induced GSH depletion with substantial increase in thiobarbituric acid reactive substances as well as the activities

of GGT and Na^+-K^+-ATPase. Piperine treatment along with carcinogens resulted in inhibition of the production of thiobarbituric acid reactive substances. It mediated a significant increase in the GSH levels and restoration in GGT and Na^+-K^+-ATPase activity.

Jalapure *et al.* (2003) screened the ethanolic extract of *Piper longum* fruits and five different crude fractions, petroleum ether, solvent ether, ethyl acetate, butanol and butanone, orally for protective activity against CCl_4 induced hepatotoxicity in adult male Wistar rats. Oral administration of ethanolic extract and butanol fraction of ethanolic extract of fruits of *Piper longum* at 300 mg/kg body weight showed a significant hepatoprotective activity when compared with that of control and Liv-52 treated rats. They observed a significant decrease in the activities of serum ALT and AST in rats treated with *Piper longum* as compared to that of CCl_4 control and also significant signs of amelioration of CCl_4 induced hepatotoxicity in histopathological sections. They suggested that the hepatoprotective action of *Piper longum* fruits in rats may be due to its ability to induce microsomal enzymes which accelerates the excretion of CCl_4 from the body.

Karthikeyan and Rani (2003) studied the antioxidant enzymes and non enzymatic antioxidants in pepper species and reported that the antioxidant enzymes include catalase, superoxide dismutase, ascorbate oxidase and glutathione peroxidase. The non enzymatic antioxidants detected were glutathione, ascorbic acid and vitamin A. The activity of catalase and superoxide dismutase was predominant in *Piper longum* while *Piper nigrum* showed highest glutathione peroxidase activity.

Lee *et al*. (2006) assessed the effect of the alkaloid, piperine against the toxicity of 1-methyl-4-phenylpyridinium (MPP^+) in differentiated PC12 cells. Piperine treatment revealed a differential effect on the cytotoxicity of MPP^+

depending on concentration and had its maximum inhibitory effect at 1 μM level. The addition of piperine (0.5–10 μM) significantly reduced the MPP^+ induced nuclear damage, mitochondrial membrane permeability changes, formation of reactive oxygen species and depletion of GSH. In contrast, piperine at 50–100 μM showed cytotoxicity and exhibited additive effect on the MPP^+ toxicity.

Vijayakumar and Nalini (2006) found that piperine supplementation markedly protected erythrocytes from oxidative stress by improving antioxidant status in high fat diet and antithyroid drug induced hyperlipidemic rats. Piperine normalized erythrocyte osmotic fragility, reduced lipid peroxidation, lowered the cholesterol/ phospholipids ratio and improved the status of superoxide dismutase, catalase, glutathione peroxidase, vitamin E and GSH in erythrocytes and vitamin C in plasma.

Wakade *et al*. (2008) investigated the anti oxidant potential of methanolic extract of *Piper longum* fruits against adriamycin induced myocardial oxidative stress in rats. They observed that pretreatment with *Piper longum* extract at the dose rates of 250 mg/kg and 500 mg/kg body weight by gavage for 21 days prevented the increase in serum ALT, AST, LDH and creatine kinase activities induced by i.p. injection of adriamycin on day 21. *Piper longum* pretreatment also lowered the lipid peroxide level in heart, restored the GSH content and augmented the activities of myocardial antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase.

2.1.2. Bioavailability enhancement

Atal *et al*. (1985) investigated the biochemical basis of enhanced bioavailability of drugs by piperine and found that oral administration of piperine in

rats strongly inhibited the aryl hydrocarbon hydroxylase and UDP glucuronyl transferase activities.

Bano *et al*. (1987) studied the effect of piperine on pharmacokinetics of phenytoin in healthy volunteers. They observed that a single daily dose of piperine (20 mg) for 7 days decreased the absorption half life, prolonged the elimination half life and produced a higher area under the drug concentration curve in comparison to phenytoin alone.

Piperine inhibited liver microsomal enzyme system and thereby potentiated the pentobarbitone sleeping time in rats in a dose dependent manner with peak effect at 30 min (Mujumdar *et al*., 1990).

The studies conducted by Dhuley *et al.* (1993) revealed that pretreatment with piperine reduced the liver lipid peroxidation, acid phosphatase and oedema induced by carrageenan during experimental inflammation in rats.

Singh and Reen (1994) conducted *in vitro* studies to evaluate the effect of piperine on drug metabolizing enzymes in the microsomes. They reported that treatment of hepatoma cells in culture with piperine for a larger period of 5 days resulted in a biphasic influence on the aryl hydrocarbon hydroxylase (AHH) activity, an initial inhibitory phase followed by an induction phase. The activity of AHH was induced by four and three fold on day 3 and day 5 of treatment.

Gupta *et al.* (1998) stated that piperine inhibited the biotransformation and metabolism of nimesulide leading to significantly higher level of the drug in the systemic circulation and showed a dose dependent synergistic effect on nimesulide induced anti-nociception in mice.

Karan *et al.* (1998) reported that co-administration of piperine with isoniazid failed to inhibit the metabolism of isoniazid and thus reduced its bioavailability in rabbits.

Khajuria *et al.* (1998b) evaluated the permeability characteristics of piperine on oral absorption in intestinal everted sacs. They found that piperine is a weak base, highly lipophilic in nature which is absorbed very fast across the intestinal barrier through transcellular pathway. They also found that piperine formed apolar complex with drugs and solutes with which it was compounded and also modulated the membrane dynamics which increased the absorptive area resulting in efficient permeability of drugs through membranes and enhancement of their bioavailability.

Karan *et al.* (1999) studied the effect of Trikatu (an ayurvedic medicine containing *Piper longum*, *Piper nigrum* and *Zingiber officinalis* in the ratio of 1:1:1; w/w) on the pharmacokinetics of rifampicin in rabbits and reported that co-administration of Trikatu with rifampicin reduced its rate of bioavailability and antibacterial efficacy.

In Carrageenan induced rat paw oedema model of inflammation, nimesulide co-administered with piperine at increasing doses produced significantly higher anti-inflammatory effects as compared to nimesulide alone. Acute toxicity studies on mice revealed a reduction in lethal dose of the combination as compared with nimesulide alone (Gupta *et al*., 2000).

The pharmacokinetic study on interaction of Trikatu with diclofenac sodium was carried out by Lala *et al*. (2004) in rabbit and reported that Trikatu significantly decreased serum levels of diclofenac.

Singh *et al*. (2005) studied the pharmacokinetic profile of orally administered oxytetracycline, 7 days post oral treatment of *Piper longum* at a dose rate of 15 mg/kg body weight in white leghorn hens. Prior treatment of *Piper longum* significantly reduced the elimination rate and total body clearance whereas elimination half life and total duration of pharmacological effect were increased. The treatment with *Piper longum* also reduced the loading and maintenance dose of oxytetracycline enhancing the therapeutic efficacy of the drug in poultry.

2.1.3. Other effects of *Piper longum*:

Cole (1985) studied the effect of piperine on the response of gastrointestinal tract smooth muscle to transmural nerve stimulation in guinea pigs and reported that piperine blocked the contractions of the intestine and produced a spasmolytic effect.

Shoji *et al*. (1986) isolated an amide, the dehydropipernonaline from the fruits of *Piper longum,* which possessed coronary vasodilating activity.

Ghoshal *et al*. (1996) studied the *in vitro* antiamoebic effect of ethanolic extract, hexane fraction and n-butanol soluble fraction of *Piper longum* at the dose rate of 1000 µg/ml and the chloroform fraction at 500 µg/ml against the trophozoites of *Entamoeba hystolytica* and observed amoebicidal action. Trials with ethanolic extract at a dose rate of 900 mg/kg in rats cured 90 percent of the animals with experimental caecal amoebiasis.

Reen *et al*. (1997) investigated the potential of piperine for inhibiting the activity of cyt P-4502B1 and protecting against aflatoxin B_1 toxicity in V79 Chinese hamster cells engineered for the expression of rat cyt P-4502B1. They found that

piperine counteracted cyt P-450 enzyme mediated toxicity of aflatoxin B1 in the cells and offered a potent chemopreventive effect against procarcinogens activated by the cyt P-450 enzyme.

Acute and chronic oral toxicity studies on the ethanolic extract of *Piper longum* fruits were carried out in mice by Shah *et al*. (1998). Acute dosages were 0.5, 1.0 and 3.0 g/kg while the chronic dosage was 100 mg/kg/day. The results showed that the drug did not cause any significant mortality in acute or chronic groups and any change in the haematological parameters. It did not show any significant change in the pre and post treatment body weight, but a significant increase in the weight of lungs and spleen were noticed. There was a significant increase in the reproductive organ weight and sperm mortality but failed to elicit any spermatotoxic effect.

Lin *et al*. (1999) found that piperine at the concentrations of 0.1 μM and 1.0 μM stimulated the growth of a cultured mouse melanocyte line, melan-a, in 8 days. It also induced morphological alterations in melan-a cells. The augmentation of growth by piperine was effectively inhibited by a selective protein kinase-C inhibitor suggesting that protein kinase-C signalling is involved in its activity.

Tripathi *et al.* (1999) tested the antigiardial and immunostimulatory effects of *Piper longum* in experimental infection of *Giardia lamblia* in mice. On *in vitro* test, an aqueous extract of *Piper longum* fruit powder at 250 µg/ml and its ethanol extract at 125 µg/ml showed 100% giardicidal activity. At 900 mg/kg body weight, fruit powder, aqueous extract and ethanolic extract showed *in vivo* giardicidal effect with ethanolic extract having the maximum activity. They also observed that *Piper longum* possessed a demonstrable immunostimulatory activity as evident from the increased haemagglutination titer, plaque forming cell counts, macrophage migration

index and phagocytic index. A maximum effect was found at 225 mg/kg body weight in mice. The effect was marginally reduced at higher doses of 450 and 900 mg/kg or the lower dose of 112.5 mg/kg.

Piper longum (water decoction) in the dose of 50 mg/kg, p.o. 60 min prior to experiment, offered significant protection against gastric ulcers induced by 2 h cold restraint stress, aspirin (200 mg/kg, 4 h) and 4 h pylorus ligation in rats. The antiulcerogenic effect of *Piper longum* was due to the augmentation of mucin secretion and decreased cell shedding rather than acid and pepsin secretion which however were found to be increased by it (Agrawal *et al*., 2000).

Another study on gastric ulcer by Bai and Xu (2000) in rats reported that piperine possessed significant antiulcer activity due to the reduction in volume of gastric juice secretion, inhibition of gastric acidity and pepsin activity.

Pipernonaline, a piperidine alkaloid derived from *Piper longum* was found to have fungicidal activity against phytopathogenic fungi such as *Pyricularia oryzae, Rhizoctonia solani* and *Botrytis cineria.* (Lee *et al*., 2001).

Bajad *et al*. (2001a) studied the antidiarrhoeal activity of piperine against castor oil, $MgSO_4$ and arachidonic acid in mice. It significantly inhibited diarrhoea produced by these cathartics at dose rates of 8 and 32 mg/kg p.o. Inhibition of castor oil induced enteropooling by piperine suggests its inhibitory effect on prostaglandins.

Bajad *et al.* (2001b) in another study found that piperine, an alkaloid of black and long peppers, inhibited gastric emptying of solids and liquids in rats and gastrointestinal transit in mice in a dose dependent manner. Gastric emptying inhibitory activity of piperine was independent of gastric acid and pepsin secretion.

Khajuria *et al*. (2002) investigated the mechanism of enhancing the bioavailability of other drugs by piperine in rat intestinal sacs. They observed that piperine induced 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. Ultra structural studies with piperine showed an increase in microvilli length with a prominent increase in ribosomes of enterocytes. They suggested that piperine may be altering the membrane dynamics and permeation characteristics, along with induction in the synthesis of proteins associated with cytoskeletal function which resulted in an increase in the absorptive capacity of small intestine.

Pipernonaline and piperoctadecalidine derived from the fruits of *Piper longum* has insecticidal and acaricidal activity against plant pests such as *Spodoptera litura* and *Myzus persicae* (Park *et al.,* 2002).

Yang *et al.* (2002) examined the mosquito larvicidal activity of *Piper longum* fruit-derived materials against the fourth-instar larvae of *Aedes aegypti.* A crude methanol extract of *Piper longum* fruits was found to be active against the larvae, and the hexane fraction of the methanol extract showed a strong larvicidal activity of 100% mortality. The biologically active component of *Piper longum* fruits was characterized as pipernonaline by spectroscopic analyses. No activity was observed with piperettine, piperine, or piperlongumine.

When the ethanolic extract of fruits of *Piper longum* was given to different groups of mice in doses ranging from 200-3000 mg/kg, no lethality was observed in any of the groups. The mice which received extracts in doses above 2000 mg/kg exhibited ptosis and were found lethargic (Jalapure *et al.*, 2003).

Panda and Kar (2003) evaluated the role of piperine on thyroid hormone status and the regulation of blood glucose level in mice. They reported that daily oral administration of piperine at a dose of 2.5 mg/kg for 15 days lowered the serum levels of both the thyroid hormones, T3 and T4 as well as glucose concentrations with a concomitant decrease in hepatic glucose-6-phosphatase activity.

Min *et al*. (2004) discovered that piperlonguminine from *Piper longum* fruits has an inhibitory effect on alpha- melanocyte stimulating hormone- induced melanogenesis in melanoma B16 cells.

Immunomodulatory and antitumor activities of methanolic extract of *Piper longum* and piperine were studied by Sunila and Kuttan (2004) in mice. Administration of *Piper longum* extract at a dose of 10 mg and piperine at 1.14 mg/animal was found to possess a stimulatory effect on haematopoeitic system and humoral immune system as evidenced by the increased total leukocyte count and circulating antibody titer. They also observed that administration of these drugs increased the bone marrow cellularity indicating its effect on stem cell proliferation and inhibited the growth of solid tumors induced by Dalton's lymphoma ascites cells and ascites tumor induced by Ehrlich ascites carcinoma cells.

Ali *et al*. (2007) reported that ethyl acetate, chloroform and methanolic extracts obtained from *Piper longum* roots, stem and leaves showed *in vitro* antibacterial activity against *Bacillus subtilis*, *Sarcina lutea*, *Escherichia coli*, *Pseudomonas aerogenosa*, *Salmonella typhi*, *Shigella boydii* and *Klebsiella* sp. Ethyl acetate extracts also exhibited a mild *in vitro* antifungal activity against *Aspergillus fumigatus*, *Aspergillus niger* and *Fusarium* sp.

Kanungo *et al*. (2007) compared the hypolipidemic activities of five different marketed herbal formulations along with a prepared formulation containing *Piper longum*, in triton induced hyperlipidemic rats. They observed significant decrease in the levels of cholesterol and triacylglycerol in all the treated animals. Treatment of rats with the herbal formulations after the administration of triton also resulted in an increase in the HDL level.

Pathak and Khandelwal (2007) studied the cytoprotective and immunomodulating properties of piperine on cadmium induced immuno-compromised murine spleenocytes. Piperine at 50 µg/ml dose rate restored the cell viability, suppressed the generation of reactive oxygen species and prevented the cadmium induced depletion of GSH. They also observed that piperine exhibited anti-apoptotic activity and mitigated the adverse effects of cadmium on cytokines.

Freire-de-Lima *et al*. (2008) investigated the toxic effects of piperine against *Trypanosoma cruzi*. They observed a strong decrease in the growth of epimastigotes when cultured in the presence of 10-25 µg/ml of piperine for 7 days. The inhibition of *Trypanosoma cruzi* replication by piperine was followed by the formation of bizarre morphologic forms as observed by scanning electron microscopy. Epimastigotes treated with piperine became round shaped, with swelling of the mitochondrion matrix, intense intracellular vacuolation and plasma membrane blebs.

Recently Han *et al*. (2008) synthesized a novel starch piperinic ester with anti-hyperlipidemia activity by coupling a carboxylic group of piperic acid and a hydroxyl group on the backbone of starch. Piperic acid was obtained by hydrolyzing piperine that was extracted from seeds of *Piper longum*.

Matsuda *et al*. (2008) found that piperine at a concentration of 19 μM inhibited the lipid droplet accumulation in mouse peritoneal macrophages incubated with liposomes. Piperine selectively inhibited the cholesteryl ester synthesis at 25 μM and slightly inhibited triacylglycerol synthesis at 200 μM in macrophages. They also observed that piperine inhibited the acyl-CoA-cholesterol acyl transferase activity in mouse macrophage and liver microsomal fractions.

2.2. MONOSODIUM GLUTAMATE (MSG)

2.2.1. Toxicity in humans

Schaumburg *et al.* (1969) had shown that MSG can produce undesirable effects in humans in the amounts used in the preparation of widely consumed foods such as soups. They observed that MSG causes burning sensation, facial pressure, chest pain and headache. These pharmacological effects obeyed a dose – effect relationship. They also observed considerable variation in oral threshold doses among individuals.

Ghadimi *et al.* (1971) presented evidences indicating that the ingestion of MSG at a dose rate of 150 mg/kg body weight causes transient increase in an acetyl choline-like substance which is responsible for symptoms of Chinese restaurant syndrome in humans including head ache, sweating, nausea, weakness, thirst, flushing of the face, sensation of burning or tightness, abdominal pain and lacrimation.

Allen *et al*. (1987) investigated the asthma provoking potential of MSG. They challenged 32 subjects - number of whom gave histories of severe asthma after Chinese restaurant meals - in a single blind placebo - controlled fashion with

increasing doses of MSG from 0.5 – 5 g. 13 subjects reacted to the challenge. This study established that MSG can provoke severe and life threatening asthma due to its peripheral neuroexcitatory effect such as stimulation of irritant receptors in the lung, leading to reflex bronchoconstriction.

2.2.2. Toxicity in experimental animals:

Creasey and Malawista (1971) observed that intraperitoneal injections of MSG at a dose rate of 300mg/kg body weight to adult male Swiss mice reduced the uptake of glucose into whole brain by up to 35.5%. They suggested that the interference with glucose uptake is the basis for the toxicity of MSG.

Intraperitoneal or intra gastric injection of MSG (4mg/g body weight) to infant rats invariably caused an increase in brain glutamine not brain glutamate. Administration of monosodium salts of L- glutamate, D- glutamate and L- aspartate gave similar symptoms. Therefore, the neurotoxicity of MSG characterized by convulsions is not due to ammonia but to amino acid anions. (Mushahwar and Koeppe, 1971).

Malik and Ahluwalia (1994) conducted studies on the effect of monosodium glutamate on various fractions of lipids and certain carbohydrate metabolic enzymes in liver and blood of adult male mice by administering MSG s.c. for 6 days at dose levels 2, 4 and 8 mg/g body weight. Dose levels above 4 mg/g body weight showed significant increase in the content of liver total lipids, phospholipids, triacylglycerol and free fatty acids 31 days after the last injection. Blood pyruvate, glucose and glucose-6-phosphate dehydrogenase activity in RBC and liver were also increased significantly whereas liver glycogen, blood lactate and LDH activity in serum and liver were reduced significantly in these groups. These findings suggested that MSG

administration shifted the carbohydrate metabolism towards lipogenesis leading to hyperlipidemia.

Subcutaneous administration of MSG at dose levels above 4 mg/g body weight induced a significant increase in the erythrocyte glucose content accompanied by oxidative stress in RBC. Lipid peroxidation and activities of glutathione reductase, glutathione peroxidase and glutathione-S- transferase were found to be significantly increased in RBC whereas non protein bound glutathione was significantly decreased (Ahluwalia *et al*., 1996).

Choudhary *et al.* (1996) reported that daily administration of MSG to adult male mice at dose levels of 4 mg and 8 mg/ g body weight s.c. for 6 days significantly increased lipid peroxidation in hepatic microsomes, 31 days after the last injection. They observed a highly significant increase in the levels of hepatic calcium and ascorbic acid. The GSH content was significantly decreased and the activities of glutathione reductase, glutathione peroxidase and glutathione -S- transferase were found to be significantly increased, suggestive of the oxidative stress induced by MSG at dose levels above 4 mg/g body weight.

Bopanna *et al*. (1998) studied the monosodium glutamate potentiated atherogenesis in rats receiving atherogenic diet and observed that MSG at the dose rates of 250 mg/kg and 1 g/kg body weight orally for 30 days caused a significant increase in the formation of malondialdehyde, hydroperoxides and conjugated dienes in liver, kidney, lungs, brain and heart. Levels of reduced glutathione, superoxide dismutase and catalase were lowered significantly in liver, heart and kidney. The increase in lipid peroxidation potential was predominantly observed on higher dose of MSG. They suggested that this might be due to high lipogenic activity of MSG itself and in supplementation with atherogenic diet.

In another study, Bopanna *et al*. (1999) evaluated the histological and ultra structural changes in liver, kidney, heart and brain produced by MSG in rats on atherogenic diet. They found that MSG at a dose rate of 1 g/kg body weight caused an increase in the activities of 5′ nucleotidase, and Na^{+}-K^{+}-ATPase in these organs. Membrane fluidization was also increased in all these tissues. On histological study, liver section showed foci of necrosis, ballooning degeneration, fatty change and hypertrophy of kupffer cells. In kidney section, glomeruli showed mesangial proliferation and matrix deposition. Hydropic degeneration of tubular epithelium, vacuolation of epithelial cells, oedema and tubulointerstitial infiltration with inflammatory cells were also noticed.

Macho *et al*. (2000) reported that early postnatal administration of MSG to rats induces obesity, hyperinsulinemia and hyperglycemia in adulthood suggesting the presence of insulin resistance. An increase of plasma insulin, glucose and leptin levels was found in 3 months old rats treated with MSG during postnatal period. Attenuation of insulin stimulatory effect on glucose transport was also observed in MSG treated rats. This was found to be due to lower insulin binding to plasma membranes of liver, skeletal muscles and adipocytes and lower content of GLUT4 protein in adipose tissue. MSG treated rats also showed a shift in glucose metabolism towards lipid synthesis in fat tissues.

Singh *et al*. (2003) examined the prolonged and delayed effects of glutamate excitotoxicity on mitochondrial lipid peroxidation and antioxidant parameters in cerebral hemispheres, cerebellum, brain stem and diencephalon of adult male Wistar rats. They found that administration of MSG at dose rate of 4 mg/g body weight i.p. for 6 consecutive days produced decrease in mitochondrial manganese superoxide dismutase, catalase and GSH. They also observed an increase in lipid peroxidation and glutathione peroxidase activity. This study revealed that mitochondrial function

impairment is an important mechanism of excitatory amino acid mediated neurotoxicity in chronic neurodegeneration.

Diniz *et al.* (2004) examined the effects of a hypercaloric diet on hepatic glucose metabolism of young weaned Wistar rats (21 days old) with MSG administration. At 45 days of treatment they observed that the rats which where given MSG alone and MSG along with a hypercaloric diet had developed hyperinsulinemia, hyperglycemia, decreased hexokinase and increased glucose-6 phosphatase in hepatic tissue. These animals had impaired oral glucose tolerance test, increased lipid hydroperoxides and decreased superoxide dismutase in hepatic tissue. These findings indicated that hypercaloric diet and MSG administration induced alterations in the metabolic rate of glucose utilization and decreased the antioxidant defenses.

Studies conducted by Hong and Liu (2004) revealed that incubation of PC 12 cells with 10 *mM* glutamate induced oxidative cytotoxicity (oxitosis) characterized by increased intracellular accumulation of reactive oxygen species (ROS), thiobarbituric acid reactive substances and decreased level of GSH. The antioxidant, scutellarin protected against this cytotoxicity, production of ROS and lipid peroxidation induced by glutamate.

Administration of MSG to newborn rats causes the destruction of the ventromedial hypothalamic and arcuate nuclei, leading to the development of obesity due to the lack of control between absorption and energy expenditure (Diemen *et al*., 2006).

According to Farombi and Onyema (2006) MSG administered intraperitoneally at a dose of 4 mg/g body weight markedly increased malondialdehyde formation in liver, kidney and brain of rats. They observed that

MSG elicited decrease in GSH level and a marked increase in the activities of glutathione-S- transferase, catalase and superoxide dismutase in these organs. There were marked increase in the activities of serum ALT, AST and GGT in MSG treated rats. They also observed that MSG at the above dose rate induced the formation of micronucleated polychromatic erythrocytes.

Onyema *et al*. (2006) reported that MSG administered at a dose of 0.6 mg/g body weight by gavage for 10 days induced oxidative stress and hepatotoxicity in rats. They observed a significant increase in lipid peroxidation, glutathione-S-transferase, superoxide dismutase and catalase activities in liver. A decrease in the liver GSH level was also noticed 24 hours after 10 days of administration. The activities of ALT, AST and GGT in serum were also found to be increased.

Ortiz *et al*. (2006) evaluated the toxic effects of MSG in liver and kidney after an intraperitoneal injection at a dose rate of 4 mg/g body weight in rats and observed increase in the activities of ALT and AST at 30 and 45 minutes after the injection. Also an increase in the lipid peroxidation products was observed in kidney at 15, 30 and 45 minutes while in liver it was found at 30 and 45 minutes. Degenerative changes including oedema, congestion, cloudy swelling and hydropic degeneration were also observed in these organs at 15, 30 and 45 min after the injection.

Parshad and Natt (2007) studied the food acceptance and toxic effects of feeding sodium selenite alone and in combination with MSG in rats. They observed that MSG stimulated food intake in a dose related manner. Food intake increased gradually from four percent level of MSG, but at 10 percent level marked increase occurred only in the first three meals and declined thereafter. It has been suggested that the higher level of MSG (10 percent) probably had detrimental effects that decreased the food consumption on the following days.

2.2.3. Protection against MSG toxicity in experimental animals

Bopanna *et al*. (1998) studied the protective effect of S-allyl cysteine sulphoxide (SACS) isolated from garlic in monosodium glutamate administered rats receiving atherogenic diet. They observed that SACS produced a significant antioxidant effect. Administration of SACS at a dose of 250 mg/kg body weight along with MSG at the same dose rate for 30 days significantly decreased the formation of malondialdehyde, hydroperoxides and conjugated dienes in liver, kidney, lungs and heart. It also increased the levels of GSH and the activities of superoxide dismutase and catalase in tissues.

In another study by Bopanna *et al*. (1999) the protective effect of SACS on histological and ultrastructural changes produced by MSG in liver, kidney, heart and brain of rats on atherogenic diet was evaluated. Rats on atherogenic diet with MSG at two different doses (250 mg/kg and 1 g/kg body weight, p.o.) received SACS at a dose of 250 mg/kg body weight, p.o. for 30 days. SACS significantly decreased the Na^+-K^+-ATPase and 5′ nucleotidase activities and increased the membrane fluidization pattern in liver, kidney and heart in rats treated with MSG at both doses. But there was no significant effect of SACS on architectural, ultrastructural and histological changes induced by MSG at higher dose on cell membrane and cell organelles of hepatic and renal tissues.

Farombi and Onyema (2006) studied the modulatory role of vitamin E, vitamin C and quercetin in MSG induced oxidative damage in liver, kidney and brain of rats. They also investigated the effect of these antioxidants on the genotoxicity of MSG in rat bone marrow micronuclei model. Vitamin C, vitamin E (both at 200 mg/kg body weight) and quercetin (at 10 mg/kg body weight) inhibited the induction of oxidative injury by MSG. Vitamin E was the most effective in reducing lipid

peroxidation in liver followed by vitamin C and then quercetin while vitamin C and quercetin showed greater ability to protect brain than vitamin E. The three antioxidants were effective in ameliorating the effects of MSG on GSH, glutathione-S- transferase, catalase, superoxide dismutase, ALT, AST and GGT. Co-treatment of rats with vitamin C and quercetin inhibited the induction of genotoxicity whereas vitamin E failed to protect against the formation of micronucleated polychromatic erythrocytes.

Onyema et al. (2006) studied the effect of vitamin E on MSG induced hepatotoxicity and oxidative stress in male Wistar rats by administering vitamin E at 0.2 mg/g body weight p.o. along with MSG at a dose of 0.6 mg/g body weight p.o. for 10 days. They observed that vitamin E reduced the MSG induced increase in liver weight by its action as a radical scavenger. Co- administration of vitamin E and MSG significantly reduced the lipid peroxidation and the activities of glutathione-S-transferase, superoxide dismutase and catalase and significantly increased the level of GSH in tissues. Vitamin E treatment also reduced the activities of the markers of hepatocellular damage, ALT, AST and GGT.

Ramanathan *et al*. (2007) evaluated the neuroprotective effect of chloroform: methanolic (80:20) extract of *Centella asciatica* on the course of free radical generation and excitotoxicity in rats treated with MSG at the dose rate of 2 mg/g i.p. for 7 days. The extract at 100 and 200 mg/kg dose rates attenuated the glutamate induced excitation and showed significant increase in the levels of the enzymes catalase and superoxide dismutase. They also observed a significant decrease in the lipid peroxide level in hippocampus and striatum regions of brain whereas the GSH level was not altered

Materials and Methods

3. MATERIALS AND METHODS

3.1. EXPERIMENTAL ANIMALS

The study was conducted in 56 adult male Wistar rats weighing 100-150g. The rats were purchased from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy. The animals were housed in appropriate cages in a well ventilated experimental animal room and were maintained under identical feeding and managemental practices. An acclimatization period of one week was allowed before the commencement of the experiment. The experiment was conducted for a period of 35 days.

3.2. DRUG AND PLANT MATERIAL

3.2.1. Monosodium glutamate (MSG)

MSG was purchased from HiMedia Laboratories Pvt. Ltd, Mumbai. It was dissolved in deionised double distilled water to make 50% aqueous solution and administered at a dose rate of 8 mg/g body weight p.o. for 20 days to induce toxicity.

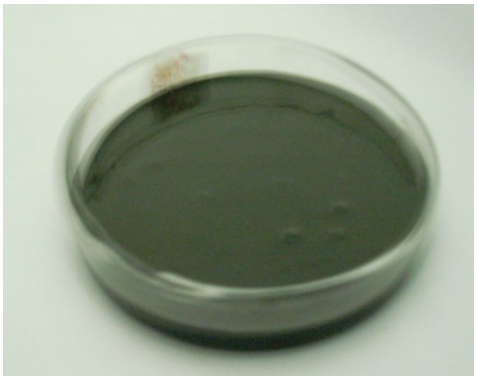
3.2.2. *Piper longum*

Dried ripe fruits of *Piper longum* were procured locally (Plate 1). They were washed thoroughly, dried under shade, coarsely powdered in a pulverizer and used for the preparation of alcoholic extract.

The alcoholic extract of *Piper longum* fruits was prepared using ethyl alcohol in a soxhlet apparatus. The extract was concentrated in a rotary vacuum evaporator

A. *Piper longum* (whole plant)

B. Dried ripe fruits of *Piper longum*



C. Ethanolic extract of *Piper longum* fruits

Plate 1. *Piper longum*

under reduced pressure and at a temperature of 50^{o}C. The concentrated extract was then dried by keeping at room temperature. On an average 100g of dried *Piper longum* fruits gave 20 g of dry extract. The extract was dissolved in 5 percent aqueous solution of gum acacia to obtain a final concentration of 5 g of dry extract per 100 ml and administered using an orogastric tube.

3.3. EXPERIMENTAL DESIGN

The animals were randomly divided into seven groups comprising 8 animals each. Curative and protective studies were carried out as detailed below:

Groups	Treatment
Treatment studies	
G0	Healthy Control, administered with 5 percent solution of gum acacia at the rate of 1.2 ml/100 g body weight (b.w.) p.o. daily for 14 days from 21st day onwards.
G1	Positive control, MSG alone at a dose rate of 8 mg/g b.w. p.o. for 20 consecutive days.
G2	Ethanolic extract of fruits of *Piper longum* at a dose rate of 300 mg/kg b.w. p.o. daily for 14 days after the induction of toxicity by administering MSG at the dose rate of 8 mg/g b.w. p.o. for 20 consecutive days.
G3	Ethanolic extract of fruits of *Piper longum* at a dose rate of 600 mg/kg b.w. p.o. daily for 14 days after the induction of toxicity by administering MSG at the dose rate of 8 mg/g b.w. p.o. for 20 consecutive days.

Protective studies	
G4	Healthy control, administered with 5 percent solution of gum acacia at the rate of 0.6 ml/100 g b.w. p.o. daily for 20 consecutive days.
G5	Positive control, MSG alone at a dose rate of 8 mg/g b.w. p.o. for 20 consecutive days.
G6	Ethanolic extract of fruits of *Piper longum* at a dose rate of 300 mg/kg b.w. p.o. daily along with MSG for 20 consecutive days.

Blood samples were collected from all animals of G0, G1, G2 and G3 on days 0, 21, 28 and 35 of experiment, while that of G4, G5 and G6 on days 0 and 21. Weight of the animals was also recorded on the above mentioned days. Animals in G4, G5 and G6 were euthanized on day 21 of experiment, while those of G0, G1, G2 and G3 were euthanized on 35th day of experiment.

3.4. COLLECTION OF BIOLOGICAL MATERIALS

3.4.1. Collection of blood and separation of serum

Blood samples were collected from the retroorbital plexus under mild ether anesthesia, using heparinised capillary tubes, into sterile microfuge tubes. The tubes were kept undisturbed for one hour at room temperature. Then they were transferred to 4°C and kept for 30 minutes for clot retraction, after which the tubes were thawed and centrifuged at 3000 rpm for 10 minutes at 15ºC to separate the serum.

3.4.2. Collection of internal organs and preparation of tissue homogenates

The animals were euthanized by cervical dislocation, dissected upon and collected the liver, spleen and kidney. The organs were washed in ice cold saline to

remove blood clots and kept in chilled saline. Weight of liver and spleen were recorded.

Liver and kidney tissue homogenates were prepared by weighing 1 g of each tissue and homogenized in suitable buffer (detailed under sections 3.5.3.2 and 3.5.3.3) for the estimation of lipid peroxides and reduced glutathione.

3.5. PARAMETERS ANALYSED

3.5.1. Estimation of serum Enzymes

Activity of serum enzymes were determined using commercial kits and directly measured in U/L using semiautomatic blood analyzer, Microlab – 200 (M/s E. Merck India Limited, Mumbai).

3.5.1.1. Alanine amino transferase (ALT) activity

Serum ALT activity was determined using Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Principle:

Photometric determination of serum ALT activity is based on reference method of International Federation of Clinical Chemistry (IFCC).

$$\text{L-Alanine} + \text{2-Oxoglutarate} \xleftrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xleftrightarrow{\text{LDH}} \text{L-Lactate} + \text{NAD}^+$$

The rate of NADH consumption is measured photometrically and is directly proportional to the ALT concentration in the sample.

Reagents:

Reagent 1:	TRIS, pH 7.5	100 mmol/L
	L-Alanine	500 mmol/L
	Lactate dehydrogenase(LDH)	≥ 1.2 kU/L
Reagent 2:	2-Oxoglutarate	15 mmol/L
	NADH	0.18 mmol/L

Procedure:

Reagent 1 and reagent 2 were mixed in the ratio of 4:1 to prepare the working reagent and proceeded as follows.

Serum	100 µl
Working reagent	1000 µl
Mixed, after 1 minute, read the decrease in absorbance (ΔA) every minute for three minutes at 340nm.	

Calculation:

ALT activity in U/L = ΔA × 1746

3.5.1.2. Aspartate amino transferase (AST) activity

Serum AST activity was determined by Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Principle:

Photometric determination of AST is based on reference method of International Federation of Clinical Chemistry (IFCC).

L-Aspartate + 2-Oxoglutarate $\xleftrightarrow{\text{AST}}$ L-Glutamate + Oxaloacetate

Oxaloacetate + NADH + H^+ $\xleftrightarrow{\text{Malate dehydrogenase}}$ L-Malate + NAD^+

The rate of NADH consumption is measured photometrically and is directly proportional to the AST concentration in the sample.

Reagents:

Reagent 1:	TRIS, pH 7.8	80 mmol/L
	L-Aspartate	240 mmol/L
	Malate dehydrogenase(MDH)	≥ 420 U/L
	Lactate dehydrogenase(LDH)	≥ 600 U/L
Reagent 2:	2-Oxoglutarate	12 mmol/L
	NADH	0.18 mmol/L

Procedure:

Reagent 1 and reagent 2 were mixed in the ratio of 4:1 to prepare the working reagent and proceeded as follows.

Serum	100 µl
Working reagent	1000 µl
Mixed, after 1 minute, read the decrease in absorbance (ΔA) every minute for three minutes at 340 nm.	

Calculation:

AST activity in U/L = ΔA × 1746

3.5.2. Estimation serum metabolites

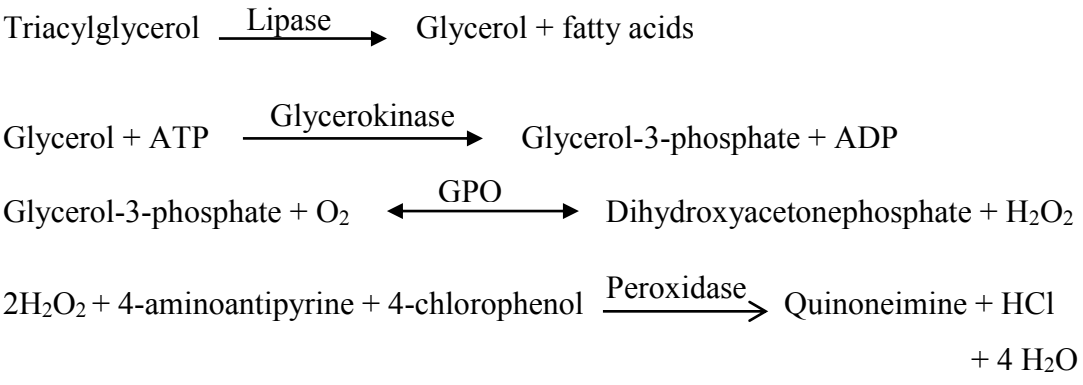
Serum metabolites were estimated using commercial kits. The concentrations of various metabolites were directly measured using semiautomatic blood analyzer, Microlab – 200 (M/s E. Merck India Limited, Mumbai).

3.5.2.1. Triacylglycerol

Concentration of serum triacylglycerol was estimated using Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Principle:

Photometric determination of triacylglycerol involves enzymatic splitting with lipoprotein lipase. Indicator is quinoneimine which is generated from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase.



Reagents:

Reagent 1:	Good's buffer, pH 7.2	50 mmol/L
	4-chlorophenol	4 mmol/L
	ATP	2 mmol/L

	Mg^{2+}	15 mmol/L
	Glycerokinase	≥0.4 kU/L
	Peroxidase	≥2 kU/L
	Lipoprotein lipase	≥2 kU/L
	4-aminoantipyrine	0.5 mmol/L
	Glycerol-3-phosphate oxidase (GPO)	≥1.5 kU/lL
Standard:	Triacylglycerol	200 mg/dl

Procedure:

	Blank	Sample/Standard
Sample/standard	-	10 µl
Distilled water	10 µl	-
Reagent 1	1000 µl	1000 µl
Mixed, incubated for 5 minutes at 37ºC. Absorbance was read against the blank at 546 nm within 60 minutes.		

Calculation:

$$\text{Triacylglycerol in mg/dl} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard.}$$

3.5.2.2. Cholesterol

Concentration of serum cholesterol was estimated by Cholesterol oxidase phenol amino antipyrine (CHOD-PAP) method using Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Principle:

Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyses the esters. In the subsequent oxidation by cholesterol oxidase, H_2O_2 is liberated. The colorimetric indicator is quinoneimine which is generated from 4-aminoantipyrine and phenol by H_2O_2 under the catalytic action of peroxidase.

Reagents:

Reagent 1:	PIPE's buffer, pH 7.5	99 mmol/L
	Salicylic alcohol	3.96 mmol/L
	Peroxidase	≥1000 U/L
	4-aminoantipyrine	0.5 mmol/L
	Cholesterol oxidase	≥100 U/L
	Cholesterol esterase	≥100 U/L
Standard:	Cholesterol	200 mg/dl

Procedure:

	Blank	Sample/Standard
Sample/standard	-	10 µl
Distilled water	10 µl	-
Reagent 1	1000 µl	1000 µl
Mixed, incubated for 5 minutes at 37ºC. Absorbance was read against the blank at 546 nm within 60 minutes.		

Calculation:

$$\text{Cholesterol in mg/dl} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

3.5.2.3. Total protein

Amount of total protein in serum was determined according to biuret method using Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Principle:

Serum proteins together with copper ions form a violet blue colour complex in alkaline solution. The absorbance of the colour is directly proportional to the concentration.

Reagents:

Reagent 1:	Sodium hydroxide	100 mmol/L
	Potassium sodium tartrate	16 mmol/L
Reagent 2:	Sodium hydroxide	100 mmol/L
	Potassium sodium tartrate	16 mmol/L
	Potassium iodide	15 mmol/L
	Copper sulphate	6 mmol/L
Standard:	Protein solution	5 g/dl.

Procedure:

Reagent 1 and reagent 2 were mixed in the ratio of 4:1 to prepare the working reagent and proceeded as follows.

	Blank	Sample/Standard
Sample/standard	-	20 µl
Distilled water	20 µl	-
Working Reagent	1000 µl	1000 µl
Mixed, incubated for 5 minutes at 37ºC. Absorbance was read against the blank at 546 nm within 60 minutes.		

Calculation:

$$\text{Total protein in g/dl} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard.}$$

3.5.2.4. Albumin

Amount of albumin in serum was determined according to bromocresol green method using Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Principle:

Serum albumin forms yellow-green to green-blue complex at a slightly acidic pH which is measured photometrically. The absorbance of the colour is directly proportional to the concentration.

Reagents:

Reagent 1:	Citrate buffer, pH 4.2	30 mmol/L
	Bromocresol green	0.26 mmol/L
Standard:	Albumin solution	5 g/dl

Procedure:

	Blank	Sample/Standard
Sample/standard	-	10 µl
Distilled water	10 µl	-
Reagent 1	1000 µl	1000 µl
Mixed, incubated for 10 minutes at 37°C. Absorbance was read against the blank at 546 nm within 60 minutes.		

Calculation:

$$\text{Albumin in g/dl} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

3.5.2.5. A: G ratio

Serum globulin content was determined by subtracting serum albumin level from total serum protein content and A: G ratio was calculated from the values of albumin and globulin obtained.

3.5.2.6. Bilirubin

Concentration of serum total bilirubin was estimated using Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Principle:

Total bilirubin in serum was determined using Jendrassik and Grof method by coupling with diazotized sulfanilic acid after addition of caffeine, sodium benzoate and sodium acetate. A blue azobilirubin is formed in alkaline Fehling solution II, which is measured photometrically.

Reagents:

Reagent 1:	Sulfanilic acid	29 mmol/L
	HCl	170 mmol/L
Reagent 2:	Sodium nitrite	29 mg/dl
Reagent 3:	Caffeine	130 mmol/L
	Sodium benzoate	156 mmol/L
	Sodium acetate	460 mmol/L
Reagent 4:	Sodium potassium tartrate	930 mmol/L
	Sodium hydroxide	1.9 mmol/L

Procedure:

Reagent 1 and reagent 2 were mixed in the ratio of 4:1 to prepare diazo solution and proceeded as follows.

	Sample blank	Sample
Diazo solution	-	100 µl
Reagent 1	100 µl	-
Reagent 3	500 µl	500 µl
Serum	100 µl	100 µl
Mixed and incubated for 30 minutes at 37ºC.		
Fehling solution II	500 µl	500 µl
Mixed, incubated for 15 minutes at 37ºC and read the absorbance against sample blank at 578 nm.		

Calculation:

Total bilirubin concentration in mg/dl = Absorbance of sample ×10.3.

3.5.2.7. Urea

Concentration of urea in serum was determined according to the urease glutamate dehydrogenase (GLDH) method using Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Principle:

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + 2HCO_3^-$$

$$\text{2-Oxoglutarate} + NH_4^+ + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + NAD^+ + H_2O$$

Reagents:

Reagent 1:	TRIS, pH 7.8	120 mmol/L
	2-Oxoglutarate	7 mmol/L
	ADP	0.6 mmol/L
	Urease	≥6 kU/L
	GLDH	≥1 kU/L
Reagent 2:	NADH	0.25 mmol/L
Standard:	Urea solution	50 mg/dl.

Procedure:

Reagent 1 and reagent 2 were mixed in the ratio of 4:1 to prepare the monoreagent and proceeded as follows.

Sample/standard	10 µl
Monoreagent	1000 µl
Mixed, incubated for 60 seconds at 25°C and absorbance (A1) was read. After exactly 60 seconds absorbance (A2) was read at 340 nm.	

Calculation:

$\Delta A = A1-A2$

$$\text{Urea in mg/dl} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times \text{Concentration of standard.}$$

3.5.2.8. Creatinine

Photometric determination of creatinine based on Jaffe kinetic method was done using Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Principle:

Creatinine forms a yellow-orange compound in alkaline solution with picric acid. At low concentration of picric acid precipitation of proteins does not take place. As a result of rapid reaction between creatinine and picric acid, the secondary reactions do not cause interference.

Reagents:

Reagent 1	NaOH	313 mmol/L
	Phosphate	12.5 mmol/L
Reagent 2	Picric acid	8.73 mmol/L
Standard	Creatinine	1 mg/dl

Procedure:

Reagent 1 and reagent 2 were mixed in the ratio of 1:1 to prepare the monoreagent and proceeded as follows.

Sample/standard	100 µl
Monoreagent	1000 µl
Mixed, incubated for 60 seconds at 25ºC and absorbance (A1) was read. After exactly 120 seconds absorbance (A2) was read at 340 nm.	

Calculation:

$\Delta A = A1-A2$

$$\text{Creatinine in mg/dl} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times \text{Concentration of standard.}$$

3.5.3. Assessment of oxidative damage

3.5.3.1. Estimation of lipid peroxides in serum

Level of lipid peroxides in serum was determined by the method of Yagi, (1984)

Principle:

Thiobarbituric acid (TBA) reacts with lipid peroxides and malondialdehyde to form a red coloured pigment that can be determined by colorimetry. 1,1,3,3

Tetramethoxypropane (TMP) was used as a standard since it can be converted to malondialdehyde quantitatively by reacting with TBA.

Reagents:

TBA, phosphotungstic acid and TMP were purchased from Himedia Laboratories Pvt. Ltd, Mumbai. All the other chemicals required were purchased from Merck India Limited, Mumbai.

N/12 H_2SO_4

10% Phosphotungstic acid solution

TBA reagent: 0.67% aqueous solution of TBA was prepared and mixed with an equal volume of glacial acetic acid

n-Butanol

Procedure:

1. To 200µl of serum 4ml of N/12 H_2SO_4 was added and the mixture was shaken gently. To this 0.5 ml of 10% phosphotungstic acid was added and mixed. After standing at room temperature for 5 minutes the mixture was centrifuged at 3000 rpm for 10 minutes.
2. The supernatant was discarded, and the sediment was mixed with 2 ml of N/12 H_2SO_4 and 0.3 ml of 10% phosphotungstic acid. The mixture was centrifuged at 3000 rpm for 10 minutes and discarded the supernatant.
3. The sediment was suspended in 4 ml of distilled water and added 1 ml of TBA reagent. The reaction mixture was kept at 95^oC in a water bath for 60 minutes.

4. After cooling with tap water, 5 ml of n-butanol was added and the mixture was shaken vigorously. Then centrifuged at 3000 rpm for 15 minutes and the absorbance of the n- butanol layer was measured at 532 nm.
5. A standard was prepared by taking 0.6nmol of 1,1,3,3 tetramethoxypropane and followed steps from 3 – 4.

Calculation:

The value was expressed in terms of malondialdehyde.

$$\text{Level of lipid peroxides (nmol/ml of serum)} = \frac{a}{A} \times \frac{0.6}{0.2}$$

Absorbance of Standard – A

Absorbance of sample - a

Volume of sample - 0.2 ml

3.5.3.2. Estimation of lipid peroxides in tissues

Level of lipid peroxides in tissue homogenate was determined by the method of Ohkawa *et al*. (1979).

Principle:

Thiobarbituric acid reacts with lipid peroxides and malondialdehyde to form a red coloured pigment that can be determined by colorimetry. TMP was used as a standard since it can be converted to malondialdehyde quantitatively by reacting with TBA.

Reagents:

TBA and TMP were purchased from Himedia Laboratories Pvt. Ltd, Mumbai. Sodium dodecyl sulphate (SDS) was procured from Sigma- Aldrich India, Bangalore; and all the other chemicals were purchased from Merck India Limited, Mumbai.

8.1% SDS
20% acetic acid solution, pH adjusted to 3.5 with NaOH
0.8% aqueous solution of TBA
1.15% KCl

Procedure:

Preparation of tissue homogenate:

Homogenates of liver and kidney were prepared in a ratio of 1g of wet tissue to 9 ml of 1.15% KCl solution (10% w/v) using a glass homogenizer. The tissue homogenate was centrifuged at 5000 rpm for 5 minutes and the supernatant was used for the estimation of lipid peroxides.

To 100 µl of the supernatant, added 200 µl of 8.1% SDS, 1.5 ml 20% acetic acid solution (pH 3.5) and 1.5 ml of 0.8% aqueous solution of TBA. The mixture was made up to 4 ml with distilled water, and heated in a water bath at 95^{o}C for 60 minutes. After cooling under tap water, 1 ml of distilled water and 5ml of n-butanol were added and shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, absorbance of the organic layer was taken at 532 nm.

Preparation of standard curve:

Standard curve was prepared using concentrations varying from 0.5 *nM* to 5 *nM* of TMP in deionised double distilled water by following the above procedure. A graph was plotted between optical density and concentration of the standards. The level of lipid peroxides were read directly from the standard curve, and expressed as nmol of malondialdehyde/g wet tissue.

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3.5.3.3. Estimation of reduced glutathione in serum and tissue homogenate

Level of reduced glutathione in serum and tissue homogenate was estimated by the method of Moron *et al*. (1979).

Principle:

Reduced glutathione is measured by its reaction with 5-5' dithiobis 2-nitrobenzoic acid (DTNB) to give a yellow coloured complex with an absorption maxima at 412 nm.

Reagents:

Disodium hydrogen phosphate, monosodium dihydrogen phosphate and DTNB were purchased from Himedia Laboratories Pvt. Ltd, Mumbai. Trichloro acetic acid (TCA) was procured from Qualigens Fine Chemicals, Glaxo Smith Kline Pharmaceuticals Ltd, Mumbai. Mono and disodium hydrogen phosphates were used for the preparation of phosphate buffer.

0.2 *M* phosphate buffer, pH 8
25% TCA
5% TCA

Procedure:

Preparation of tissue homogenate:

Homogenates of liver and kidney were prepared in a ratio of 1g of wet tissue to 9ml of 0.2 *M* phosphate buffer, pH 8 (10%w/v) using a glass homogenizer. The tissue homogenate was centrifuged at 5000 rpm for 5 minutes and the supernatant was used for the estimation of reduced glutathione.

Added 125 µl of 25% TCA to 500 µl of supernatant/ serum for the precipitation of proteins. The tubes were cooled on ice for 5 minutes and the mixture was further diluted with 575 µl of 5% TCA. Centrifuged the tubes for 5 minutes and 300 µl of resulting supernatant was taken for the estimation of reduced glutathione. The volume of aliquot was made up to 1 ml with 0.2 *M* phosphate buffer (pH, 8). Added 2ml of freshly prepared 0.6 mmol DTNB to the tubes and the intensity of yellow colour formed was read at 412 nm. The level of reduced glutathione was measured from the standard curve.

Preparation of standard curve:

Standard curve was prepared using concentrations varying from 1 µg to 10µg of reduced glutathione which was dissolved in 5% TCA. The volume of standard solution was made upto 1 ml with 0.2 *M* phosphate buffer (pH, 8). Added 2ml of freshly prepared 0.6 mmol DTNB to the tubes and the intensity of yellow colour formed was read at 412 nm. A graph was plotted between optical density and concentration of the standards. Knowing the optical density of the unknown samples, the corresponding concentration of GSH was read directly from the calibration curve and expressed as µg/ml in the case of serum and µg/g wet tissue in the case of liver and kidney.

3.5.4. Histopathological examination of liver and kidney

Representative samples of liver and kidney obtained from the dissected animals were fixed in 10% formalin. They were processed and paraffin embedded as described by Sheehan and Hrapchak, (1980). The sections were stained with haematoxyline and eosin as per the technique followed by Bancroft and Cook, (1984). The sections were examined in detail under light microscope.

3.6. STATISTICAL ANALYSIS OF THE DATA

Data obtained from the experiment were analysed and compared between different groups by analysis of variance (ANOVA) followed by Duncan's multiple range test. For period wise comparison of means paired t-test was used (Snedecor and Cochran, 1994).

Results

4. RESULTS

In the present study, effects of ethanolic extract of fruits of *Piper longum* were evaluated in rats after inducing toxicity with MSG and as a protective agent against the toxicity on co-administration with MSG. The results were analyzed and presented in tables and figures.

4.1. EFFECT OF ETHANOLIC EXTRACT OF FRUITS OF *Piper longum* AFTER INDUCING TOXICITY WITH MSG

The study was carried out in rats randomly divided into four groups,

- G0 – Healthy control
- G1 – Positive control (administered with MSG at the dose rate of 8 mg/g body weight, p.o. for 20 consecutive days).
- G2 – Treated with ethanolic extract of *Piper longum* at the dose rate of 300 mg/kg body weight, for 14 days after inducing toxicity.
- G3 – Treated with ethanolic extract of *Piper longum* at the dose rate of 600 mg/kg body weight, for 14 days after inducing toxicity.

During the treatment period, observations were made on days 7 (28^{th} day) and 14 (35^{th} day) of treatment.

4.1.1. Body weight

The values are presented in Table 1 and Fig. 1. On day 0 and day 21 no significant difference was observed in the body weight between the four groups. On day 28 though the body weight of G1 increased (180.00 ± 8.94 g) as compared to all the other groups, significant difference ($P<0.05$) was observed only between

Table 1. Effect of MSG and *Piper longum* on body weight (n = 6)

Groups	Body weight (g) (Mean ± SE)			
	0th day	21st day	28th day	35th day
G0	142.50 [a] ± 2.5	159.17 [a] ± 2.39	161.67[ab] ± 2.47	165.83 [a] ± 2.01
G1	135.00 [a] ± 5.00	172.50 [a] ± 9.29	180.00 [b] ± 8.94	190.00 [b] ± 8.17
G2	134.17 [a] ± 2.71	161.67 [a] ± 7.60	162.50[ab] ± 4.61	160.83 [a] ± 3.00
G3	145.00 [a] ± 5.48	150.83 [a] ± 6.38	152.50 [a] ± 6.80	155.83 [a] ± 7.90

Means bearing the same superscript in a column did not differ significantly (P<0.05).

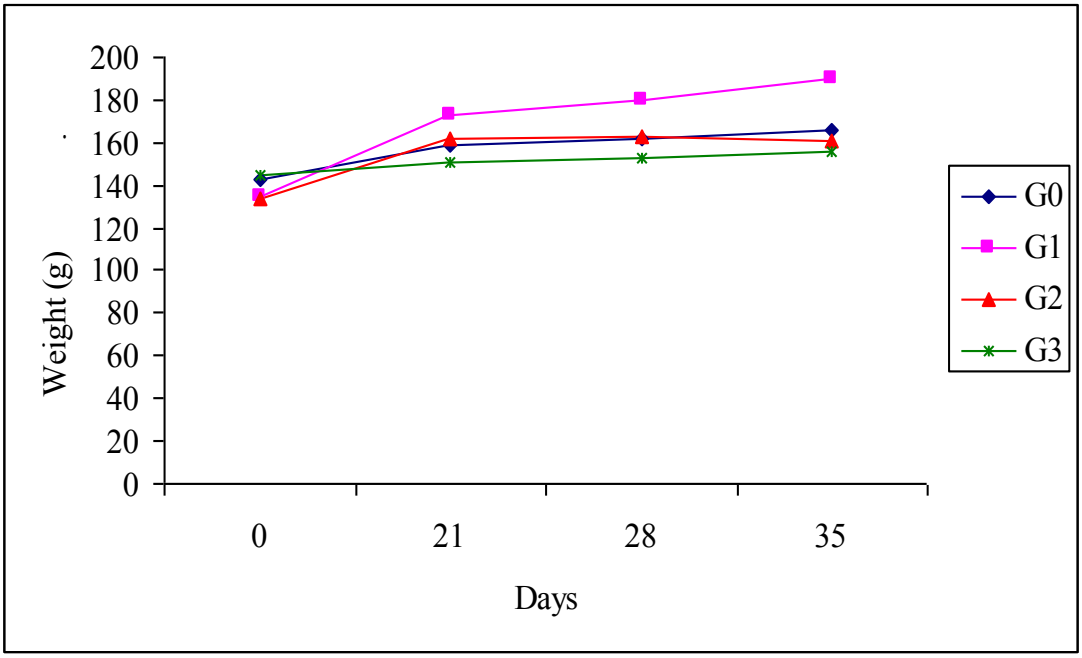

Fig. 1. Effect of MSG and *Piper longum* on body weight

G1 and G3 (152.50 ± 6.80 g) while on day 35 i.e. after 14 days treatment, the body weight significantly decreased ($P<0.05$) in G2 (160.83 ± 3.00 g) and G3 (155.83 ± 7.90 g) as compared to G1 (190.00 ± 8.17 g) and the values were comparable with that of G0 (165.83 ± 2.01 g).

4.1.2. Activity of serum enzymes

4.1.2.1. Alanine amino transferase activity

Serum ALT values presented in Table 2 and Fig. 2 show that on day 0 the level of the enzyme did not differ significantly between the groups. All the groups, G1, G2 and G3 showed a significant increase ($P<0.05$) in serum ALT activity on day 21 as compared to the healthy control (G0). Treatment with *Piper longum* extract for seven days resulted in significant decrease ($P<0.05$) in the level of serum ALT in groups G2 (71.50 ± 3.39 U/L) and G3 (76.83 ± 5.31 U/L) as compared to G1 (98.00 ± 3.97 U/L), but the levels were not near to that observed in G0 (45.50 ± 1.71 U/L). Fourteen days treatment showed a further significant decrease ($P<0.05$) in the enzyme level in G2 (55.67 ± 1.76 U/L) as compared to seven days treatment. It was also noticed that 14 days treatment caused a significant reduction ($P<0.05$) in the enzyme level in G2 as compared to G3 (72.83 ± 3.76 U/L) and positive control (101.67 ± 1.61 U/L) though the level did not reach near to that of G0 (45.67 ± 1.56 U/L). Even though, G3 also showed a decrease in the enzyme level after 14 days treatment, the value did not differ significantly from that observed after seven days treatment. The results indicate that 14 days treatment with 300 mg dose rate of *Piper longum* extract was more effective in decreasing the serum ALT activity as against 600 mg dose rate.

Table 2. Effect of MSG and *Piper longum* on serum ALT activity (n = 6).

Groups	ALT (U/L) (Mean ± SE)			
	0th day	21st day	28th day	35th day
G0	43.33[a] ± 1.36	44.33 [a] ± 1.69	45.50 [a] ± 1.71	[NS]45.67 [a] ± 1.56
G1	46.67 [a] ± 1.63	99.17 [b] ± 2.71	98.00 [c] ± 3.97	[NS]101.67 [d] ±1.61
G2	48.12 [a] ± 1.17	98.17 [b] ± 4.06	71.50 [b] ± 3.39	*55.67 [b] ± 1.76
G3	47.67 [a] ± 1.99	100.0 [b] ± 03.42	76.83 [b] ± 5.31	[NS] 72.83 [c] ± 3.76

Means bearing the same superscript in a column did not differ significantly (P<0.05).

Means bearing * indicates significant difference between day 35 and day 21 of G1 and day 35 and day 28 of other groups at P<0.05. [NS] indicates non- significance.

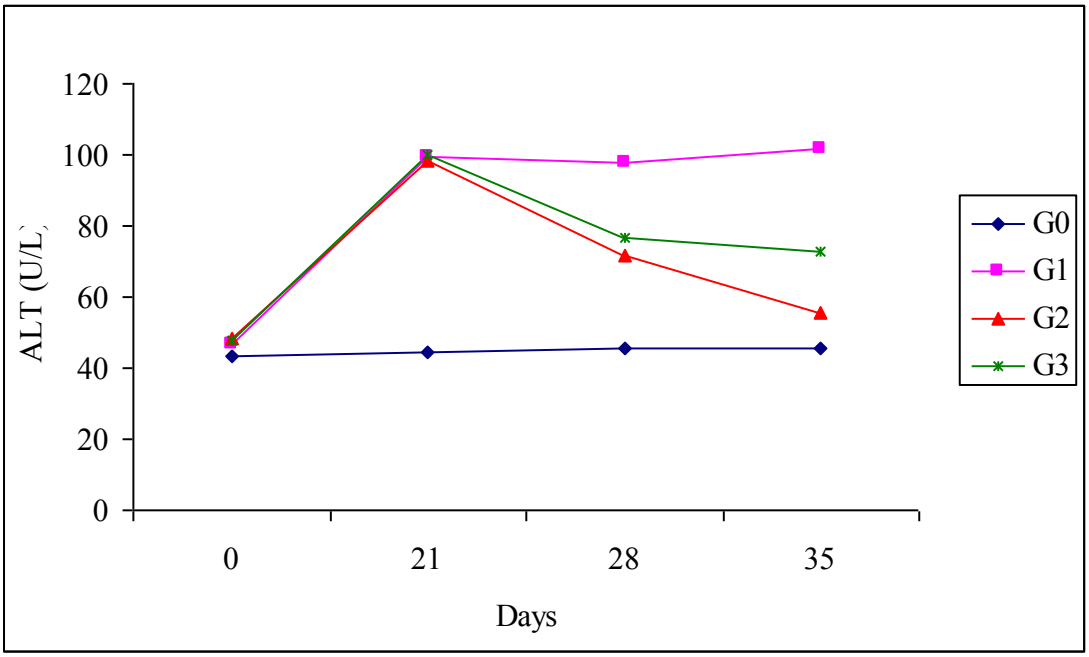

Fig. 2. Effect of MSG and *Piper longum* on serum ALT activity

4.1.2.2. Aspartate amino transferase activity

Serum AST activity (Table 3 and Fig. 3) did not show any significant difference between groups on day 0. Monosodium glutamate administration induced a significant increase ($P<0.05$) in the serum AST activity of all the groups, G1, G2 and G3, as compared to G0. Though, the level of enzyme decreased after 7 days treatment in both G2 and G3, a significant reduction ($P<0.05$) was observed only in G3 (230.50 ± 17.12 U/L) as compared to G1 (284.33 ± 4.07 U/L). After 14 days treatment, both the treated groups, G2 (234.67 ± 9.81 U/L) and G3 (235.50 ± 5.73 U/L) showed almost similar AST levels, which were significantly lower ($P<0.05$) as compared to the positive control, G1 (315.50 ± 10.88 U/L). It was also observed that a sudden decrease in the enzyme level occurred in G3 on day 28, which was maintained almost at the same level till day 35 while in G2, the decrease was gradual and reached a level similar to that in G3 on day 35. However, the enzyme activity in the treated groups did not reach a level as observed in the healthy control, G0 (194.17 ± 4.81 U/L).

4.1.3. Serum metabolites

4.1.3.1. Triacylglycerol

The values obtained are presented in Table 4 and Fig. 4. On day 0, the level of triacylglycerol did not differ significantly between the groups. On day 21, a significant increase ($P<0.05$) was observed in the serum triacylglycerol level in all the three groups, G1 (102.83 ± 5.97 mg/dl), G2 (112.17 ± 5.13 mg/dl) and G3 (103.17 ± 5.97 mg/dl) as compared to G0 (31.00 ± 2.17 mg/dl). On day 28, both the treated groups, G2 (46.50 ± 5.17 mg/dl) and G3 (51.83 ± 4.87 mg/dl), showed a significant reduction ($P<0.05$) in triacylglycerol level as compared to the positive control, G1 (95.50 ± 5.14 mg/dl). On day 35, though the level of triacylglycerol was further decreased in G2 (33.00 ± 3.51 mg/dl) and G3 (47.33 ± 5.89 mg/dl), a significant decrease ($P<0.05$) was observed only in G2.

Table 3. Effect of MSG and *Piper longum* on serum AST activity (n = 6).

Groups	AST (U/L) (Mean ± SE)			
	0th day	21st day	28th day	35th day
G0	191.67 a ± 3.89	192.50 a ± 4.06	193.67 a ± 3.81	NS194.17 a ±4.81
G1	197.00 a ± 2.54	317.33 b ± 17.29	284.33 c ± 4.07	NS315.50 c±10.88
G2	202.00a ± 2.85	309.50 b ± 6.83	278.17 c ± 11.02	*234.67 b ± 9.81
G3	201.83 a ± 2.39	324.33 b ± 11.82	230.50 b ± 17.12	NS235.50 b ± 5.73

Means bearing the same superscript in a column did not differ significantly (P<0.05).

Means bearing * indicates significant difference between day 35 and day 21 of G1 and day 35 and day 28 of other groups at P<0.05. NS indicates non- significance.

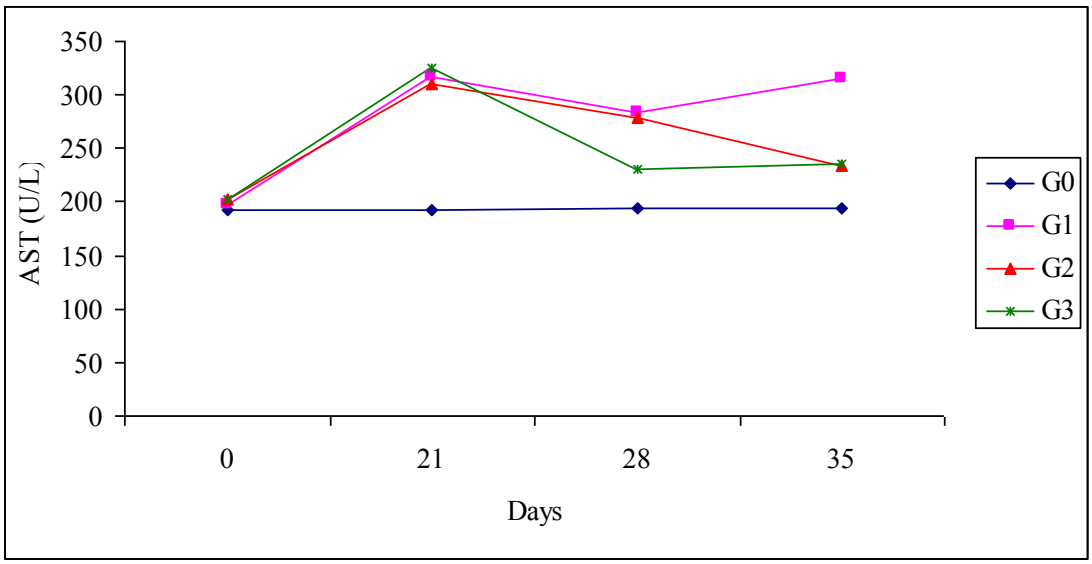

Fig. 3. Effect of MSG and *Piper longum* on serum AST activity

Table 4. Effect of MSG and *Piper longum* on serum triacylglycerol (n = 6)

Groups	Triacylglycerol (mg/dl) (Mean ± SE)			
	0th day	21st day	28th day	35th day
G0	29.33 [a] ± 0.67	31.00 [a] ± 2.17	29.17 [a] ± 1.64	[NS]30.83 [a] ±2.91
G1	30.17 [a] ± 2.44	102.83 [b] ± 5.97	95.50 [c] ± 5.14	*81.00 [c] ± 3.26
G2	34.00 [a] ± 1.88	112.17 [b] ± 5.13	46.50 [b] ± 5.17	*33.00 [a] ± 3.51
G3	33.50 [a] ± 2.49	103.17 [b] ±5.97	51.83 [b] ± 4.87	[NS] 47.33 [b] ± 5.89

Means bearing the same superscript in a column did not differ significantly (P<0.05).

Means bearing * indicates significant difference between day 35 and day 21 of G1 and day 35 and day 28 of other groups at P<0.05. [NS] indicates non- significance.

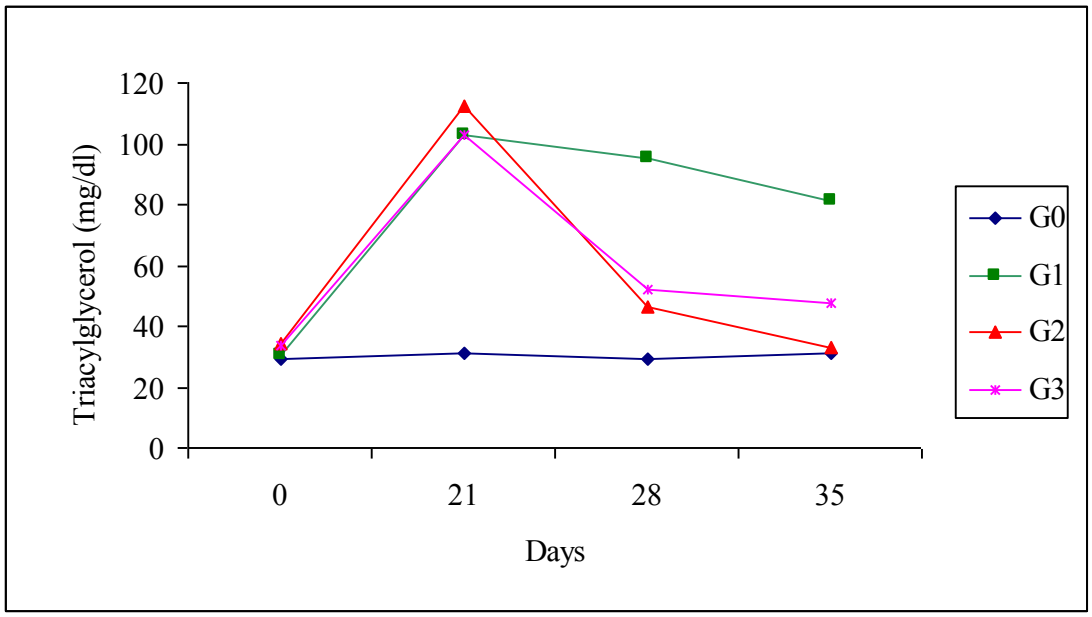

Fig. 4. Effect of MSG and *Piper longum* on serum triacylglycerol

Comparison between the treated groups indicate that treatment with *Piper longum* extract at 300 mg dose rate for 14 days was more effective in decreasing the serum triacylglycerol level and the level reached a value similar to that in the healthy control, G0 (30.83 ± 2.91 mg/dl). However, a significant reduction ($P<0.05$) in the triacylglycerol level was also noticed in the positive control, G1 on day 28 (95.50 ± 5.14 mg/dl) and day 35 (81.00 ± 3.26 mg/dl), but the values were significantly higher ($P<0.05$) as compared to the treated groups and healthy control.

4.1.3.2. Cholesterol

Serum total cholesterol values presented in Table 5 and Fig. 5 show that on day 0, the levels did not differ significantly between the groups. Twenty days of MSG administration induced significant increase ($P<0.05$) in the cholesterol levels of G1 (96.33 ± 5.73 mg/dl), G2 (91.33 ± 5.69 mg/dl) and G3 (90.33 ± 4.67 mg/dl) as compared to G0 (37.33 ± 2.14 mg/dl). On day 28, both the treated groups, G2 (59.33 ± 2.62 mg/dl) and G3 (60.83 ± 4.21 mg/dl) showed a significant decrease ($P<0.05$) in serum total cholesterol level as compared to G1 (77.50 ± 1.54 mg/dl) and almost similar values were observed in both the treated groups. After 14 days treatment (on day 35), a further significant decrease ($P<0.05$) was noticed in G2 (45.67 ± 2.84 mg/dl) as compared to the level on day 28 and that of the positive control (61.00 ± 1.77 mg/dl), but the level did not reach the value shown by healthy control, G0 (35.33 ± 2.95 mg/dl). On the other hand, in G3, the cholesterol level did not show any significant decrease (58.00 ± 4.73 mg/dl) and was similar to that observed on day 28. However, a significant reduction ($P<0.05$) in the cholesterol level was also observed in the positive control (G1) from 21st to 35th day and the level on 35th day was similar to that observed in G3. The results show that treatment with 300 mg dose rate was more effective as compared to 600 mg dose rate.

Table 5. Effect of MSG and *Piper longum* on serum total cholesterol (n = 6)

Groups	Total cholesterol (mg/dl) (Mean ± SE)			
	0th day	21st day	28th day	35th day
G0	38.50 [a] ± 1.89	37.33 [a] ± 2.14	36.67 [a] ± 2.25	[NS] 35.33 [a] ± 2.95
G1	37.50 [a] ± 2.46	96.33 [b] ± 5.73	77.50 [c] ± 1.54	*61.00 [c] ± 1.77
G2	38.83 [a] ± 1.64	91.33 [b] ± 5.69	59.33 [b] ± 2.62	*45.67 [b] ± 2.84
G3	39.50 [a] ± 1.71	90.33 [b] ± 4.67	60.83 [b] ± 4.21	[NS] 58.00 [c] ± 4.73

Means bearing the same superscript in a column did not differ significantly (P<0.05).

Means bearing * indicates significant difference between day 35 and day 21 of G1 and day 35 and day 28 of other groups at P<0.05. [NS] indicates non- significance.

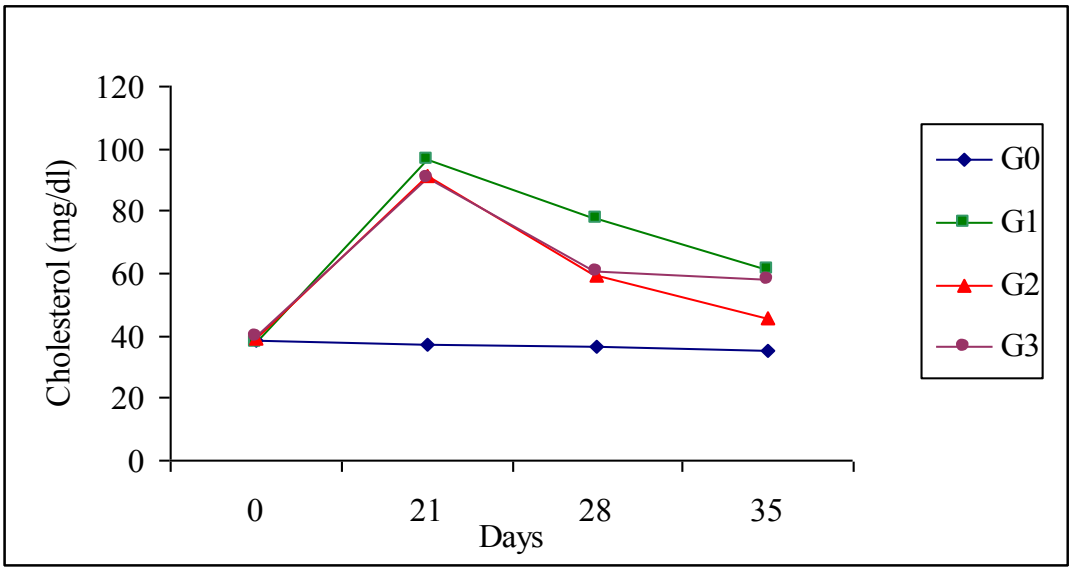

Fig. 5. Effect of MSG and *Piper longum* on serum total cholesterol

4.1.3.3. Total protein

Total protein values are presented in Table 6. The values did not differ significantly between the groups throughout the experimental period.

4.1.3.4. Albumin

Table 7 shows the level of albumin in serum. It was observed that serum albumin levels of all the groups were within the normal range throughout the experimental period and there was no significant difference between the groups.

4.1.3.5. A:G ratio

The results are presented in Table 8, which shows no significant difference in A:G ratio between the groups throughout the experimental period.

4.1.3.6. Bilirubin

Serum bilirubin concentrations are presented in Table 9. Analyses showed that the total bilirubin levels of all the groups were within the normal range throughout the experimental period and no significant difference was noticed between the groups.

4.1.3.7. Urea

The results obtained are presented in Table 10 and Fig. 6. On day 0, the serum urea levels did not differ significantly between the groups. All the MSG administered groups, G1 (62.17 ± 2.70 mg/dl), G2 (60.83 ± 2.50 mg/dl) and G3 (62.00 ± 2.73 mg/dl) showed a significant increase ($P<0.05$) in serum urea levels as compared to the healthy control, G0 (32.00 ± 2.21 mg/dl).

Table 6. Effect of MSG and *Piper longum* on serum total protein (n = 6)

Groups	Total protein (mg/dl) (Mean ± SE)			
	0th day	21st day	28th day	35th day
G0	7.17 [a] ± 0.31	7.50 [a] ± 0.43	7.33 [a] ± 0.33	7.00 [a] ± 0.26
G1	7.33 [a] ± 0.33	7.67 [a] ± 0.42	7.17 [a] ± 0.31	7.83 [a] ± 0.40
G2	7.50 [a] ± 0.22	7.33 [a] ± 0.49	7.00 [a] ± 0.26	6.67 [a] ± 0.21
G3	7.50 [a] ± 0.22	7.33 [a] ± 0.49	7.17 [a] ± 0.31	7.17 [a] ± 0.31

Means bearing the same superscript in a column did not differ significantly ($P<0.05$).

Table 7. Effect of MSG and *Piper longum* on serum albumin (n = 6)

Groups	Albumin (mg/dl) (Mean ± SE)			
	0th day	21st day	28th day	35th day
G0	3.63 [a] ± 0.12	3.60 [a] ± 0.11	3.62 [a] ± 0.11	3.58 [a] ± 0.10
G1	3.70 [a] ± 0.11	3.63 [a] ± 0.13	3.70 [a] ± 0.08	3.70 [a] ± 0.08
G2	3.62 [a] ± 0.11	3.55 [a] ± 0.08	3.58 [a] ± 0.11	3.67 [a] ± 0.09
G3	3.62 [a] ± 0.11	3.58 [a] ± 0.13	3.70 [a] ± 0.10	3.80 [a] ± 0.08

Means bearing the same superscript in a column did not differ significantly ($P<0.05$).

Table 8. Effect of MSG and *Piper longum* on serum A:G Ratio (n = 6)

Groups	A:G ratio (Mean ± SE)			
	0^{th} day	21^{st} day	28^{th} day	35^{th} day
G0	$1.09^{a} \pm 0.12$	$0.94^{a} \pm 0.05$	$1.09^{a} \pm 0.10$	$1.06^{a} \pm 0.04$
G1	$0.98^{a} \pm 0.03$	$0.92^{a} \pm 0.04$	$1.08^{a} \pm 0.05$	$0.96^{a} \pm 0.11$
G2	$0.96^{a} \pm 0.08$	$0.99^{a} \pm 0.09$	$1.07^{a} \pm 0.05$	$1.25^{a} \pm 0.09$
G3	$0.96^{a} \pm 0.08$	$0.94^{a} \pm 0.05$	$1.07^{a} \pm 0.05$	$1.16^{a} \pm 0.09$

Means bearing the same superscript in a column did not differ significantly ($P<0.05$).

Table 9. Effect of MSG and *Piper longum* on serum bilirubin (n = 6)

Groups	Bilirubin (mg/dl) (Mean ± SE)			
	0^{th} day	21^{st} day	28^{th} day	35^{th} day
G0	$0.73^{a} \pm 0.03$	$0.70^{a} \pm 0.03$	$0.63^{a} \pm 0.05$	$0.70^{a} \pm 0.06$
G1	$0.77^{a} \pm 0.04$	$0.60^{a} \pm 0.05$	$0.65^{a} \pm 0.06$	$0.60^{a} \pm 0.06$
G2	$0.73^{a} \pm 0.03$	$0.60^{a} \pm 0.04$	$0.67^{a} \pm 0.05$	$0.60^{a} \pm 0.04$
G3	$0.68^{a} \pm 0.02$	$0.65^{a} \pm 0.02$	$0.72^{a} \pm 0.04$	$0.70^{a} \pm 0.03$

Means bearing the same superscript in a column did not differ significantly ($P<0.05$).

Table 10. Effect of MSG and *Piper longum* on serum urea (n = 6)

Groups	Urea (mg/dl) (Mean ± SE)			
	0th day	21st day	28th day	35th day
G0	32.50[a] ± 1.89	32.00 [a] ± 2.21	31.00 [a] ± 1.63	[NS]33.50 [a] ± 0.89
G1	31.00[a] ± 1.63	62.17 [b] ± 2.70	51.33 [b] ± 1.05	*44.17 [b] ± 1.96
G2	34.50[a] ± 0.89	60.83 [b] ± 2.49	47.83 [b] ± 0.95	*40.83 [b] ± 1.49
G3	30.00[a] ± 2.98	62.00 [b] ± 2.73	48.50 [b] ± 2.23	*41.17 [b] ± 2.71

Means bearing the same superscript in a column did not differ significantly (P<0.05).

Means bearing * indicates significant difference between day 35 and day 28 at P<0.05. [NS] indicates non- significance.

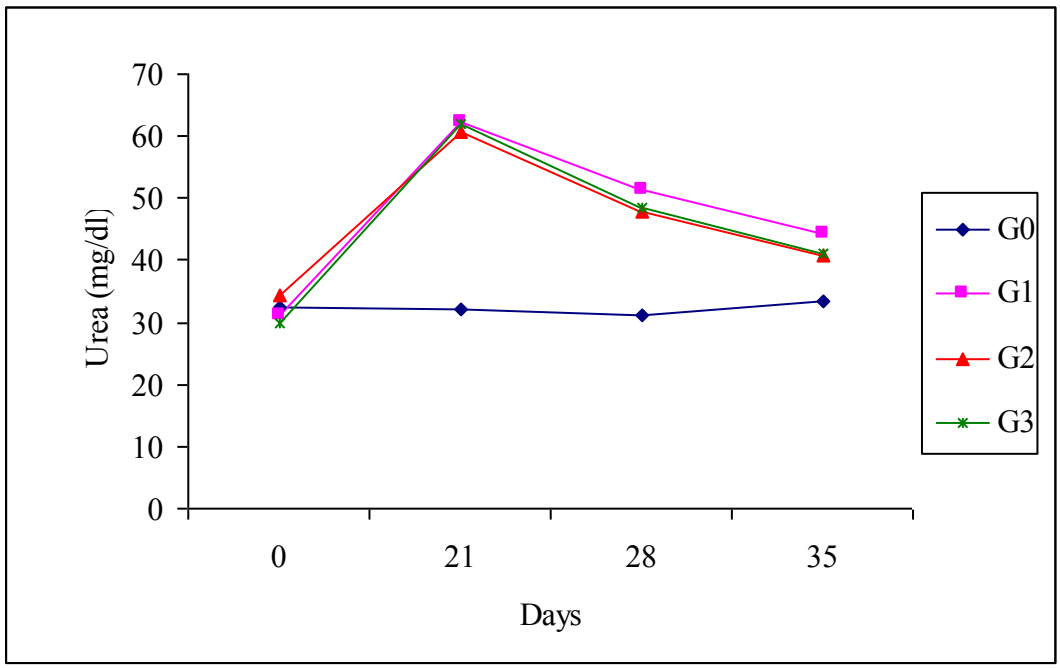

Fig. 6. Effect of MSG and *Piper longum* on serum urea

After 7 days treatment, serum urea level significantly decreased ($P<0.05$), in both the treated groups and in the positive control, from the level observed on day 21. A similar observation was noted in both the treated groups (G2 - 40.83 ± 1.49 mg/dl and G3 - 41.17 ± 2.71 mg/dl) and in the positive control (44.17 ± 1.96 mg/dl) even after 14 days treatment, but the levels were not similar to that of the healthy control (33.50 ± 0.89 mg/dl).

4.1.3.8. Creatinine

The values obtained are presented in Table 11. Serum creatinine levels of all the groups were within the normal range throughout the experimental period. No significant difference was noticed between the groups.

4.1.4. Weight of liver and spleen

The values are presented in Table 12, Fig. 7 and Fig. 8. Administration of MSG produced a significant increase ($P<0.05$) in the liver weight of G1 (6.43 ± 0.27 g) as compared to the healthy control, G0 (5.42 ± 0.15 g). Treatment with *Piper longum* extract at 300 mg dose rate (G2) showed a significant decrease ($P<0.05$) in liver weight (5.66 ± 0.17 g) whereas, treatment with 600 mg dose rate (6.59 ± 0.34 g) did not exhibit any effect in reducing the liver weight.

With regard to the weight of spleen, a significant increase ($P<0.05$) was observed in the positive control, G1 (0.44 ± 0.04 g) while in both the treated groups, G2 (0.29 ± 0.01g) and G3 (0.34 ± 0.02 g) the weight of spleen decreased significantly ($P<0.05$) and was similar to that of the healthy control, G0 (0.32 ± 0.02 g).

Table 11. Effect of MSG and *Piper longum* on serum creatinine

Groups	Creatinine (mg/dl)			
	0^{th} day	21^{st} day	28^{th} day	35^{th} day
G0	$0.52^{a} \pm 0.01$	$0.50^{a} \pm 0.02$	$0.52^{a} \pm 0.01$	$0.53^{a} \pm 0.04$
G1	$0.52^{a} \pm 0.01$	$0.67^{a} \pm 0.07$	$0.57^{a} \pm 0.03$	$0.58^{a} \pm 0.03$
G2	$0.50^{a} \pm 0.02$	$0.65^{a} \pm 0.04$	$0.57^{a} \pm 0.02$	$0.58^{a} \pm 0.04$
G3	$0.48^{a} \pm 0.04$	$0.65^{a} \pm 0.04$	$0.57^{a} \pm 0.04$	$0.50^{a} \pm 0.02$

Values are represented as Mean ± SE (n = 6)

Means bearing the same superscript in a column did not differ significantly ($P<0.05$).

Table 12. Effect of MSG and *Piper longum* on the weight of liver and spleen (n = 6)

Groups	Weight of liver (g) (Mean ± SE)	Weight of spleen (g) (Mean ± SE)
G0	5.42 [a] ± 0.15	0.32 [a] ± 0.02
G1	6.43 [b] ± 0.27	0.44 [b] ± 0.04
G2	5.66 [a] ± 0.17	0.29 [a] ± 0.01
G3	6.59 [b] ± 0.34	0.34 [a] ± 0.02

Means bearing the same superscript in a column did not differ significantly ($P<0.05$).

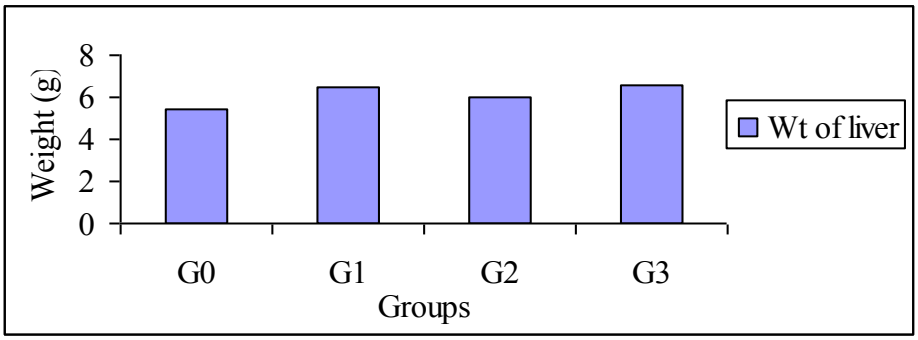

Fig. 7. Effect of MSG and *Piper longum* on the weight of liver

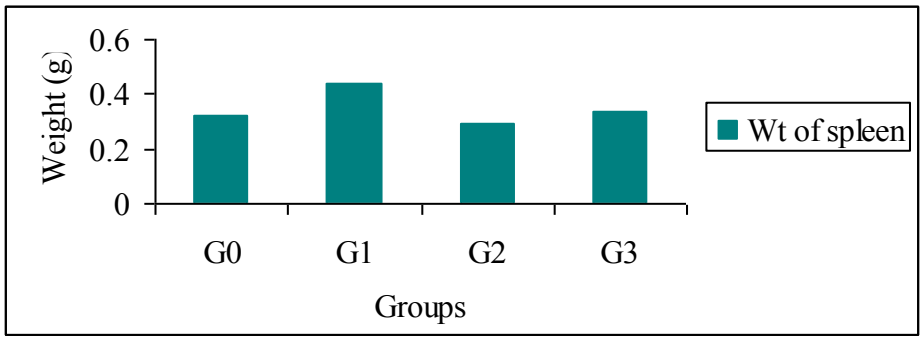

Fig. 8. Effect of MSG and *Piper longum* on the weight of spleen

4.1.5. Oxidative damage

4.1.5.1. Serum lipid peroxides

Serum lipid peroxide levels presented in Table 13 and Fig.9 show that on day 0, the level did not differ significantly between the groups. Monosodium glutamate administration induced a significant increase ($P<0.05$) in the serum lipid peroxide level of G1 (3.01 ± 0.06 nmol/ml), G2 (3.01 ± 0.06 nmol/ml) and G3 (2.89 ± 0.11 nmol/ml) as compared to G0 (1.97 ± 0.07 nmol/ml). After seven days treatment, both G2 (2.19 ± 0.11 nmol/ml) and G3 (2.58 ± 0.04 nmol/ml) showed a significant decrease ($P<0.05$) in the level of serum lipid peroxides as compared to the positive control, G1 (2.97 ± 0.06 nmol/ml). Among the two treated groups, a better effect was observed in G2, which was significantly lower ($P<0.05$) than that in G3. After 14 days treatment, G2 exhibited a further significant reduction ($P<0.05$) in serum lipid peroxide level (1.98 ± 0.05 nmol/ml) as compared to G1 (3.05 ± 0.09 nmol/ml) and G3 (2.54 ± 0.15 nmol/ml) and the level was in par with that in G0 (2.04 ± 0.06 nmol/ml), whereas G3 did not show any further decrease in the level of lipid peroxides after day 28. On comparison between the two treated groups, a better effect was observed in G2.

4.1.5.2. Serum reduced glutathione

Serum reduced glutathione levels presented in Table 14 and Fig.10 indicate no significant difference between the groups on day 0. Administration of MSG reduced the content of GSH nearly to half in all the groups, G1 (15.97 ± 0.82 µg/ml), G2 (15.15 ± 0.81 µg/ml) and G3 (15.00 ± 0.75 µg/ml) as compared to the healthy control, G0 (33.62 ± 1.11 µg/ml). Seven days treatment with *Piper longum* extract significantly increased ($P<0.05$) its level in G2 (23.38 ± 0.79 µg/ml) as compared to G1 (18.38 ± 0.45 µg/ml) while its level in G3 (18.65 ± 0.67 µg/ml) was similar to that in G1.

Table 13. Effect of MSG and *Piper longum* on the level of serum lipid peroxides (n = 6)

Groups	Serum lipid peroxides (nmol/ml) (Mean ± SE)			
	0^{th} day	21^{st} day	28^{th} day	35^{th} day
G0	$1.98^{a} \pm 0.08$	$1.97^{a} \pm 0.07$	$1.94^{a} \pm 0.09$	$2.04^{a} \pm 0.06$
G1	$1.98^{a} \pm 0.08$	$3.01^{b} \pm 0.06$	$2.97^{d} \pm 0.06$	$3.05^{c} \pm 0.09$
G2	$1.98^{a} \pm 0.06$	$3.01^{b} \pm 0.06$	$2.19^{b} \pm 0.11$	$1.98^{a} \pm 0.05$
G3	$1.99^{a} \pm 0.07$	$2.89^{b} \pm 0.11$	$2.58^{c} \pm 0.04$	$2.54^{b} \pm 0.15$

Means bearing the same superscript in a column did not differ significantly ($P<0.05$).

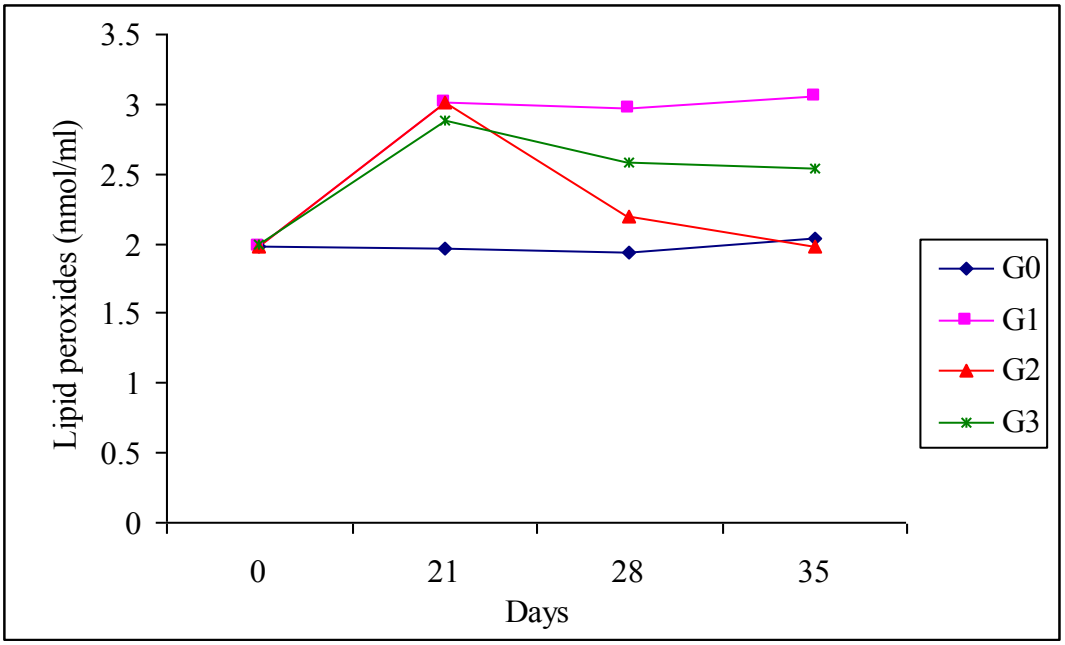

Fig. 9. Effect of MSG and *Piper longum* on the level of serum lipid peroxides

Table 14. Effect of MSG and *Piper longum* on the level of serum reduced glutathione (n = 6)

Groups	Serum reduced glutathione (µg/ml) (Mean ± SE)			
	0th day	21st day	28th day	35th day
G0	33.12 ^{a} ± 0.61	33.62 ^{b} ± 1.11	33.82 ^{c} ± 0.54	^{NS}34.38 ^{d} ± 1.11
G1	33.88 ^{a} ± 0.56	15.97 ^{a} ± 0.82	18.38 ^{a} ± 0.45	^{NS}18.67 ^{a} ± 0.28
G2	33.88 ^{a} ± 0.56	15.15 ^{a} ± 0.81	28.38 ^{b} ± 0.79	^{NS}29.68 ^{c} ± 0.63
G3	33.32 ^{a} ± 0.60	15.00 ^{a} ± 0.75	18.65 ^{a} ± 0.67	*24.30 ^{b} ± 0.85

Means bearing the same superscript in a column did not differ significantly ($P<0.05$).

Means bearing * indicates significant difference between day 35 and day 28 at $P<0.05$. ^{NS} indicates non- significance.

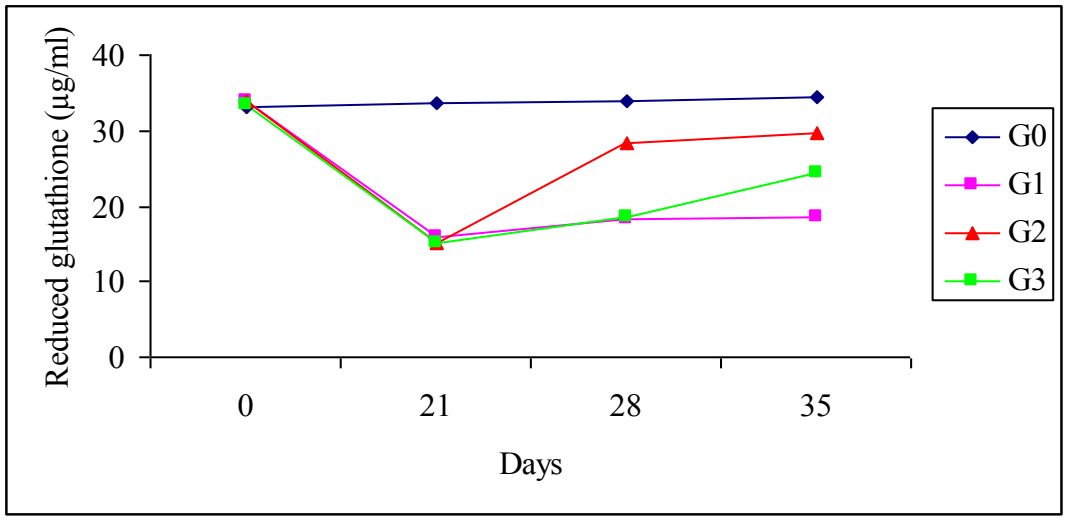

Fig. 10. Effect of MSG and *Piper longum* on the level of serum reduced glutathione

Fourteen days treatment did not cause any significant increase in the serum GSH level of G2 (29.68 ± 0.63 µg/ml) as compared to the value observed on day 28. But the increase was significant ($P<0.05$) as compared to G1. On the other hand, 14 days treatment showed a significant increase ($P<0.05$) in the level of GSH in G3 (24.30 ± 0.85 µg/ml) as compared to its level on 28th day as well as the level in positive control, G1 (18.67 ± 0.28 µg/ml). The results indicate that treatment with 300 mg dose rate exerts a better effect by rapidly increasing the reduced glutathione level as compared to 600 mg dose rate which showed a slow and gradual increase.

4.1.5.3. Liver and kidney lipid peroxides

The results are presented in Table 15 and Fig. 11. Administration of MSG resulted in a significant increase ($P< 0.05$) in the level of lipid peroxides in liver (534.88 ± 6.47 nmol/g) and kidney (585.72 ± 9.75 nmol/g) of G1 as compared the healthy control, G0 (liver- 323.00 ± 3.39 nmol/g; kidney- 340.92 ± 2.79 nmol/g). Both the treated groups, G2 (liver- 332.32 ± 7.07 nmol/g; kidney- 494.38 ± 6.49 nmol/g) and G3 (liver- 407.75 ± 12.76 nmol/g; kidney- 509.47 ± 16.72 nmol/g), showed a significant decrease ($P< 0.05$) in lipid peroxides in both liver and kidney as compared to the positive control, G1.

On comparison between the two treated groups, varying effect was observed in the two tissues. Liver lipid peroxide level was better controlled in G2, which was evident by observing a value significantly lower ($P< 0.05$) than that of G3 and near to that of the healthy control, G0. On the other hand, both G2 and G3 showed almost the same levels of kidney lipid peroxides. Thus, analyses of the results show that 300 mg dose rate is better to reduce lipid peroxides in both the tissues as compared to 600 mg dose rate.

Table 15. Effect of MSG and *Piper longum* on the level of lipid peroxides in the liver and kidney (n = 6)

Groups	Liver Lipid Peroxides(nmol/g) (Mean ± SE)	Lipid peroxides in kidney(nmol/g) (Mean ± SE)
G0	323.00 [a] ± 3.39	340.92 [a] ± 2.79
G1	534.88 [d] ± 6.47	585.72 [c] ± 9.75
G2	332.32 [b] ± 7.07	494.38 [b] ± 6.49
G3	407.75 [c] ± 12.76	509.47 [b] ± 16.72

Means bearing the same superscript in a column did not differ significantly ($P<0.05$).

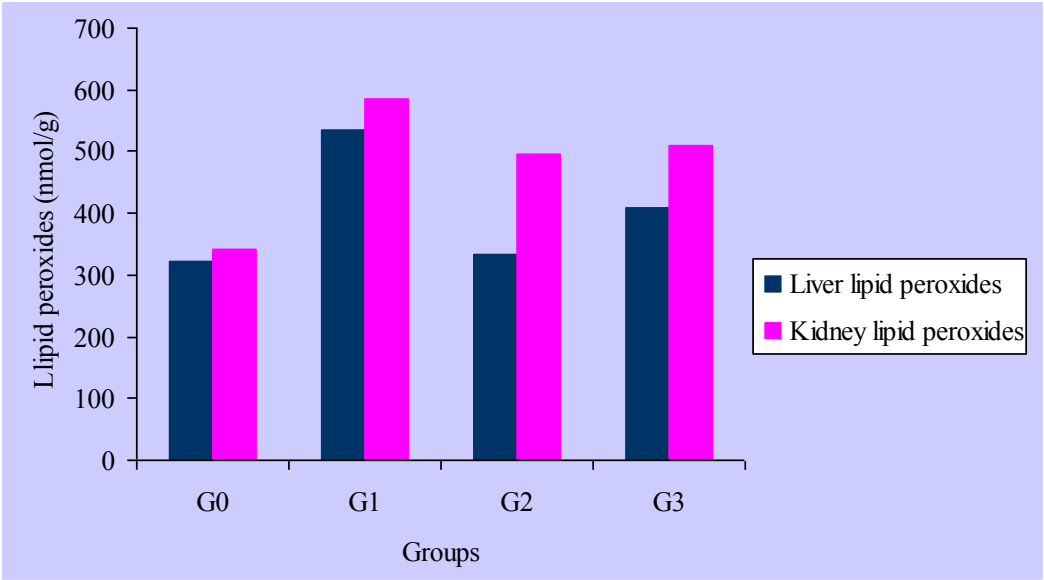

Fig. 11. Effect of MSG and *Piper longum* on the level of lipid peroxides in the liver and kidney

4.1.5.4. Liver and kidney reduced glutathione

Table 16 and Fig. 12 present the levels of reduced glutathione in liver and kidney. MSG administration produced a significant decrease ($P< 0.05$) in reduced glutathione level in the liver and kidney of G1 (liver- 414.28 ± 11.87 µg/g; kidney- 305.72 ± 7.19 µg /g) and it dropped nearly to half the level as compared to the healthy control, G0 (liver- 801.43 ± 33.45 µg/g; kidney- 593.37 ± 13.39 µg/g). Treatment with *Piper longum* extract showed a significant increase ($P< 0.05$) in its level in both the groups, G2 (liver- 567.65 ± 19.55µg/g; kidney- 403.78 ± 5.06µg/g) and G3 (liver- 503.90 ± 16.06 µg/g; kidney- 410.48 ± 18.34 µg/g) as compared to the positive control, G1. Though, there was no significant difference between the two treated groups, a better effect was observed in the liver of G2, which was treated with 300 mg dose rate. However, the level was significantly lower ($P< 0.05$) in both the groups as compared to the healthy control.

4.1.6. Histopathology of liver and kidney

Representative samples of liver and kidney of all groups were subjected to histopathological examination.

Microscopic examination of the liver (Fig. 13) and kidney (Fig. 14) of control group (G0) revealed normal histological architecture.

In the MSG treated group (G1) mild changes could be seen in both the kidney (Fig. 15) and liver (Fig. 16). Kidney lesions were characterised by diffuse cortical tubular degeneration, occasional necrosis and shrinkage of glomeruli. In the liver, piece meal type hepatocyte necrosis could be observed in the para cortical and midzonal areas.

In G2, treated with 300mg dose rate, the hepatocytes remained apparently normal (Fig. 17). In the kidney, the lesions were repaired by this dose rate except for mild tubular degeneration (Fig. 18).

In G3, treated with 600mg dose rate, repair process was effected in both the liver and the kidney. In liver, the hepatocytes remained apparently normal (Fig. 19) and in kidney, most of the tubules appeared to have been lined by multilayered hyperchromatic tubular cells. Crowding of epithelial cells could be observed in some of the tubules which at some regions appeared to be organised along the tubules (Fig. 20).

Table 16. Effect of MSG and *Piper longum* on the level of reduced glutathione in the liver and kidney (n = 6)

Groups	Reduced glutathione in liver (µg/g) Mean ± SE	Reduced glutathione in kidney (µg/g) Mean ± SE
G0	801.43 ^{c} ± 33.45	593.37 ^{c} ± 13.39
G1	414.28 ^{a} ± 11.87	305.72 ^{a} ± 7.19
G2	567.65 ^{b} ± 19.55	403.78 ^{b} ± 5.06
G3	503.90 ^{b} ± 16.06	410.48 ^{b} ± 18.34

Means bearing the same superscript in a column did not differ significantly ($P<0.05$).

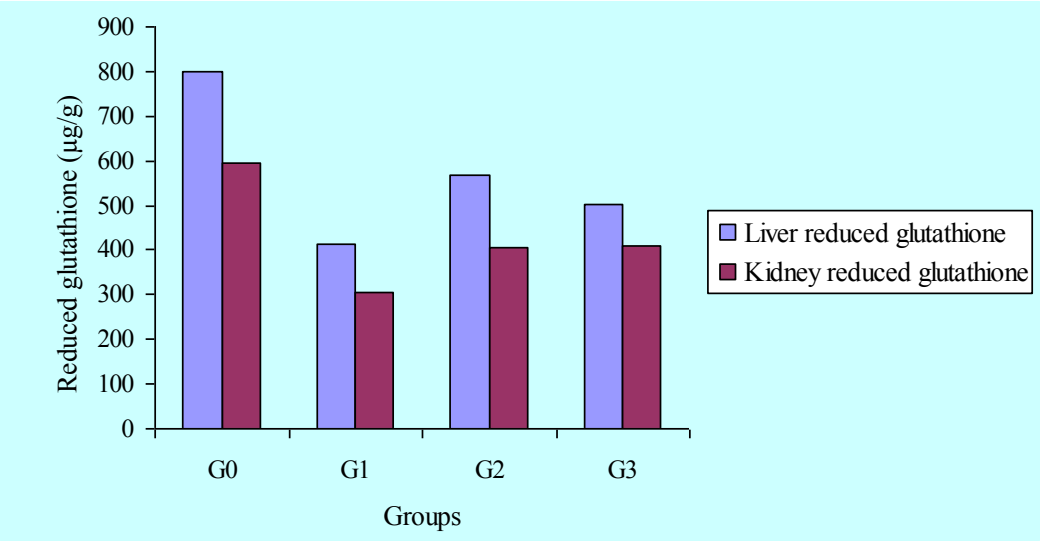

Fig. 12. Effect of MSG and *Piper longum* on the level of reduced glutathione in the liver and kidney

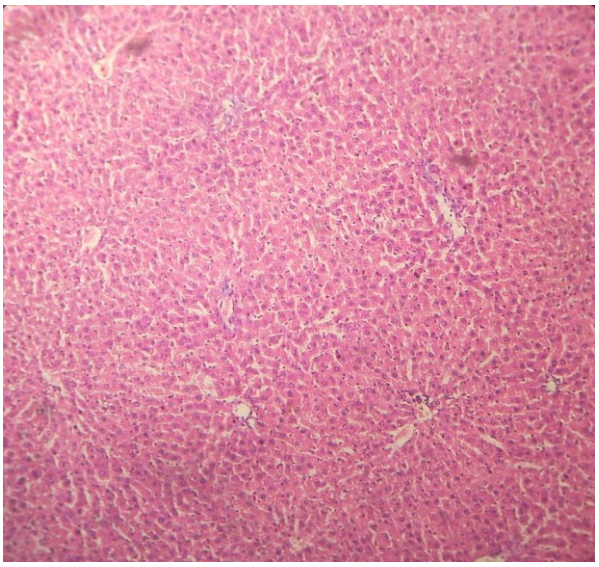

Fig. 13. Normal control group- liver

(H&E × 100)

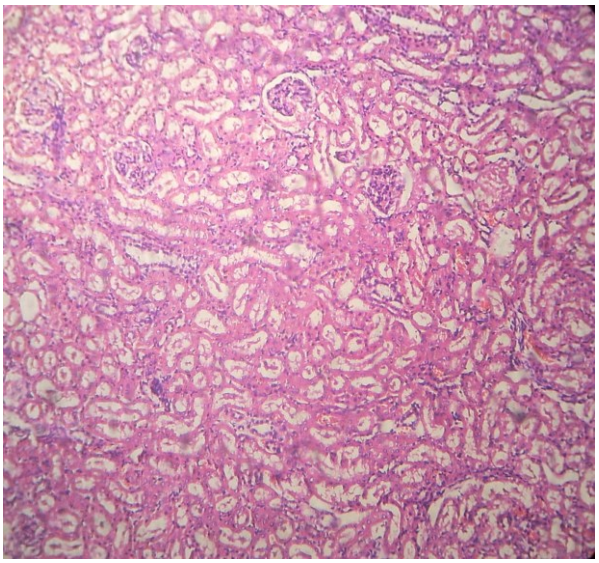

Fig. 1b. Normal control group- kidney

(H&E × 100)

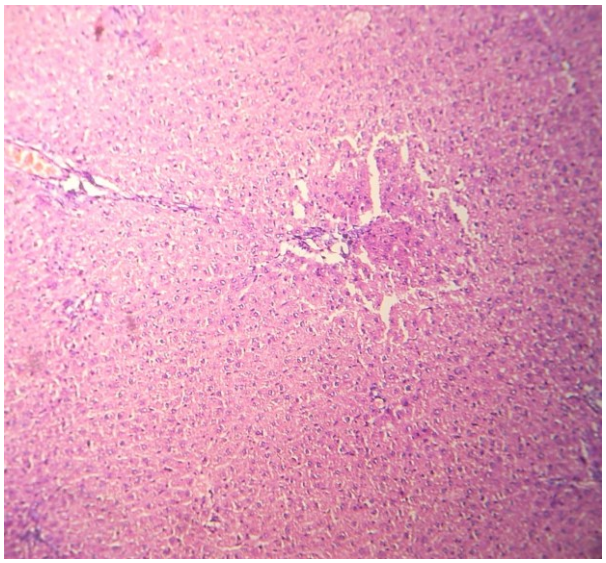

Fig. 15. Positive control group- liver
(H&E × 100)
Diffuse necrosis of hepatocytes

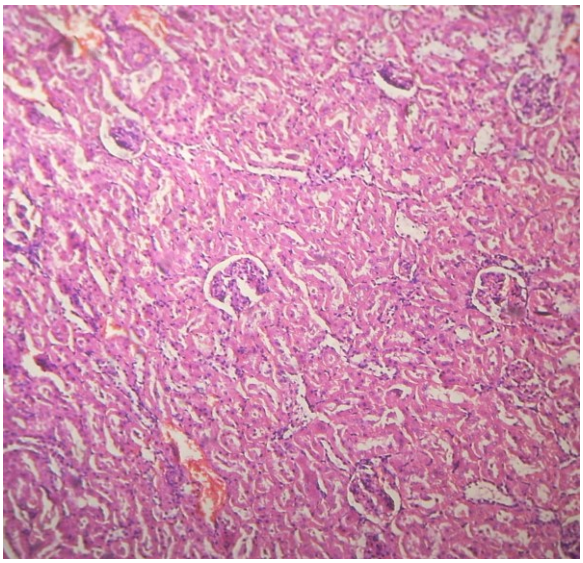

Fig. 16. Positive control group-kidney
(H&E × 100)
Degeneration of renal tubules, occasional necrosis and shrinkage of glomeruli

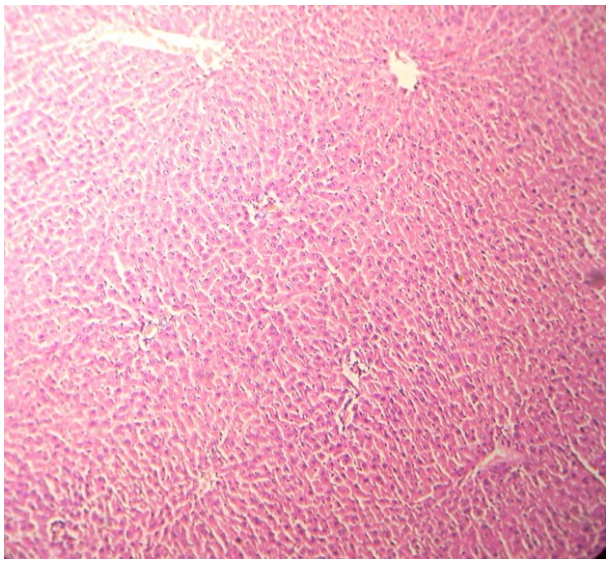

Fig. 17. Treatment group (300 mg/kg)- liver (H&E × 100)
Normal architecture of hepatocytes

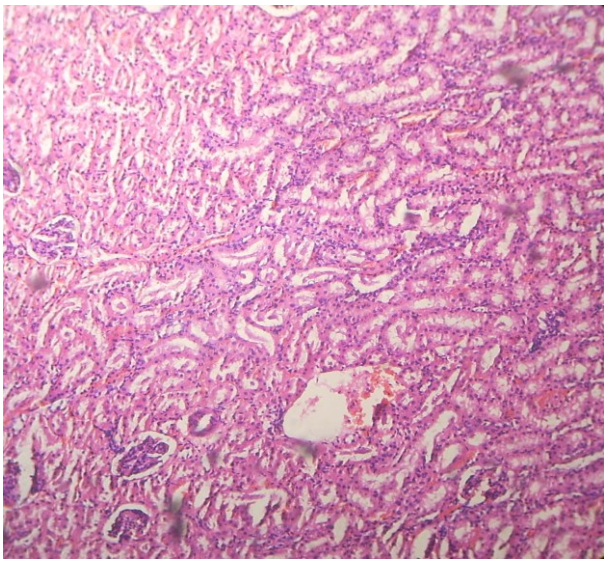

Fig. 18. Treatment group (300 mg/kg)- kidney (H&E × 100)
Normal architecture except for mild tubular degeneration

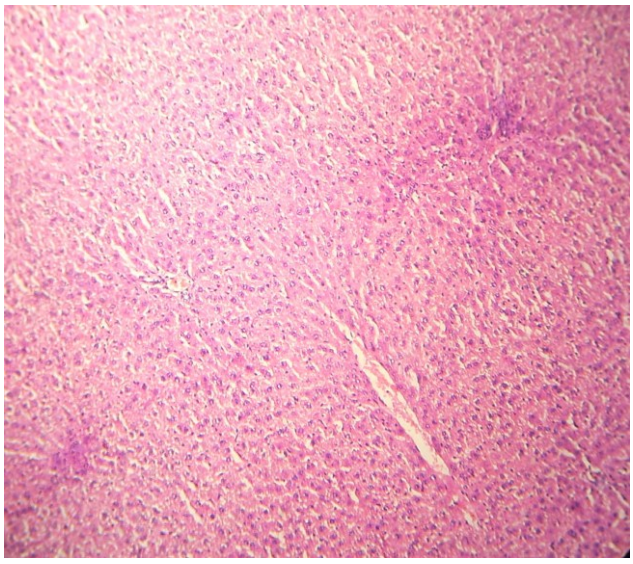

Fig. 19. Treatment group (600 mg/kg)- liver (H&E × 100)
Normal architecture of hepatocytes

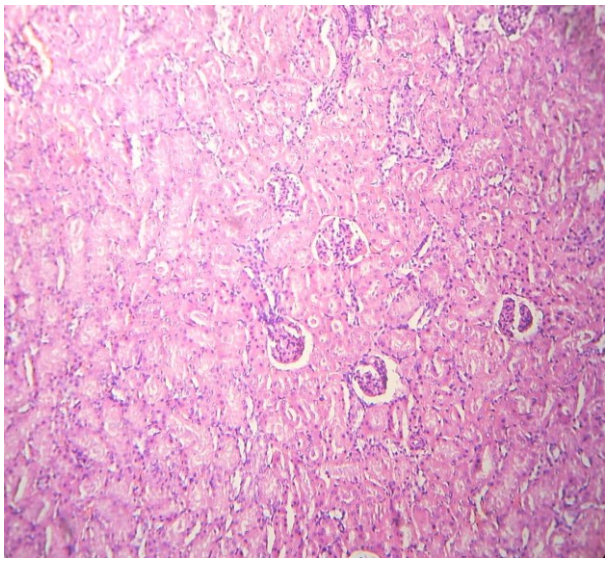

Fig. 20. Treatment group (600 mg/kg)- kidney (H&E × 100)
Crowding of hyperchromatic tubular epithelial cells.

4.2. PROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF FRUITS OF *Piper longum* ON CO-ADMINISTRATION WITH MSG

The study was carried out in three groups of rats as detailed below:

G4 – Healthy control

G5 – Positive control (administered with MSG at the dose rate of 8 mg/g body weight, for 20 consecutive days)

G6 – Co-administration of *Piper longum* extract at 300 mg/kg dose rate along with MSG

4.2.1. Body weight

The results obtained are presented in Table 17. There was no significant difference in the body weight of different groups throughout the experimental period.

4.2.2. Activity of serum enzymes

4.2.2.1. Alanine amino transferase activity

Table 18 and Fig. 21 present the serum ALT activity. The enzyme activities did not differ significantly between the groups on day 0. On day 21, the enzyme activity increased significantly ($P< 0.05$) in G5 (95.67 ± 2.11 U/L) and G6 (100.5 ± 6.97 U/L) as compared to the healthy control, G4 (44.38 ± 1.79 U/L). No significant difference was observed between G5 and G6. It appears that co-administration of *Piper longum* at 300 mg dose rate could not offer any protection against the rising serum ALT activity.

Table 17. Effect of co-administration of MSG and *Piper longum* on body weight (n = 6)

Groups	Body Weight (g) Mean ± SE	
	0^{th} day	21^{st} day
G4	143.20 [a] ± 2.52	158.47 [a] ± 2.39
G5	150.00[a] ± 9.66	156.67[a] ± 11.67
G6	145.00 [a] ± 4.28	157.50 [a] ± 6.68

Means bearing the same superscript in a column did not differ significantly ($P<0.05$).

Table 18. Effect of co-administration of MSG and *Piper longum* on serum ALT activity (n = 6)

Groups	ALT (U/L) (Mean ± SE)	
	0^{th} day	21^{st} day
G4	$43.43^{a} \pm 1.32$	$44.38^{a} \pm 1.79$
G5	$46.67^{a} \pm 1.63$	$95.67^{b} \pm 2.11$
G6	$48.50^{a} \pm 0.72$	$100.50^{b} \pm 6.97$

Means bearing the same superscript in a column did not differ significantly ($P<0.05$).

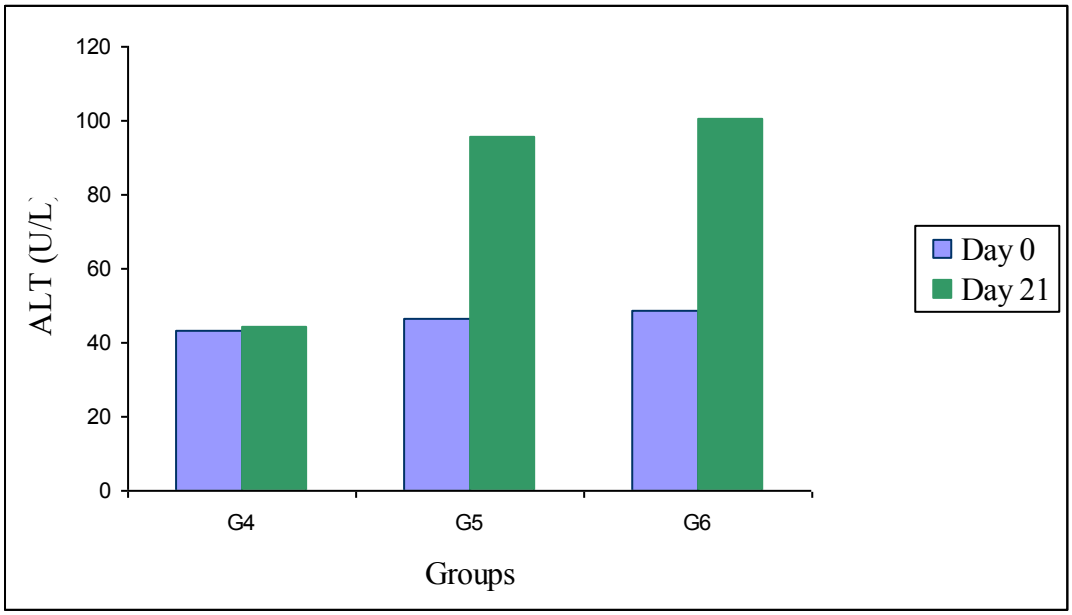

Fig. 21. Effect of co-administration of MSG and *Piper longum* on serum ALT activity

4.2.2.2. Aspartate amino transferase activity

On day 0, the AST activity did not differ significantly between the groups. On day 21, both G5 (309.50 ± 6.83 U/L) and G6 (218.33 ± 4.82 U/L) showed significant increase ($P< 0.05$) in the enzyme activity as compared to the healthy control, G4 (193.56 ± 3.06U/L). Comparison between G5 and G6 exhibited a significant decrease ($P< 0.05$) in the enzyme activity in G6, though the level did not reach a value similar to that of the healthy control (Table 19 and Fig. 22).

4.2.3. Serum metabolites

4.2.3.1 Triacylglycerol

Serum triacylglycerol values are presented in Table 20 and Fig. 23. The values did not differ significantly between the groups on day 0. MSG administration resulted in a significant increase ($P< 0.05$) in the triacylglycerol value of G5 (105.67 ± 6.43 mg/dl) as compared to the healthy control, G4 (31.64 ± 2.12 mg/dl). Co-administration of MSG and *Piper longum* extract at 300 mg dose rate significantly reduced ($P< 0.05$) the serum triacylglycerol level (54.83 ± 4.19 mg/dl) as against the positive control (G5) though, the level was significantly higher ($P< 0.05$) as compared to the healthy control (G4).

4.2.3.2. Cholesterol

Serum total cholesterol levels did not show any significant difference between the groups on day 0. The level increased significantly ($P< 0.05$) in both G5 (82.83 ± 4.40 mg/dl) and G6 (64.67 ± 6.81 mg/dl) as compared to G4 (33.06 ± 2.52 mg/dl) on day 21. Between G5 and G6, a significantly lower ($P< 0.05$) level was observed in G6, showing a protective effect of *Piper longum* extract in preventing hypercholesterolemia (Table 21 and Fig. 24).

Table 19. Effect of co-administration of MSG and *Piper longum* on serum AST activity (n = 6)

Groups	AST (U/L) (Mean ± SE)	
	0^{th} day	21^{st} day
G4	192.47 [a] ± 3.86	193.56 [a] ± 3.06
G5	197.33 [a] ± 2.74	309.50 [c] ± 6.83
G6	206.83 [a] ±1.60	218.33 [b] ± 4.82

Means bearing the same superscript in a column did not differ significantly (P<0.05).

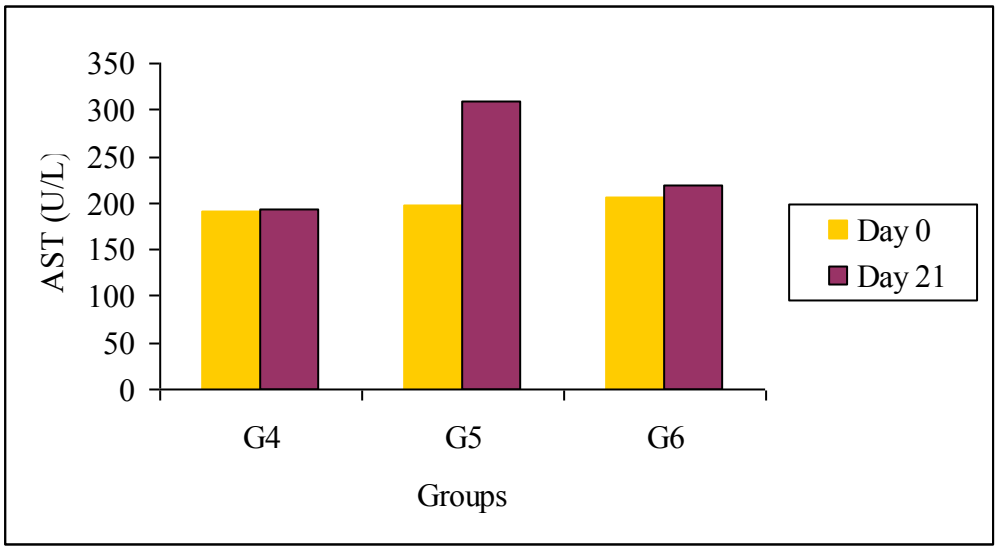

Fig. 22. Effect of co-administration of MSG and *Piper longum* on serum AST activity

Table 20. Effect of co-administration of MSG and *Piper longum* on serum triacylglycerol (n = 6)

Groups	Triacylglycerol (mg/dl) (Mean ± SE)	
	0^{th} day	21^{st} day
G4	30.14 [a] ± 1.67	31.64 [a] ± 2.12
G5	36.33 [a] ± 2.09	105.67 [c] ± 6.43
G6	28.83 [a] ± 1.74	54.83 [b] ± 4.19

Means bearing the same superscript in a column did not differ significantly (P<0.05).

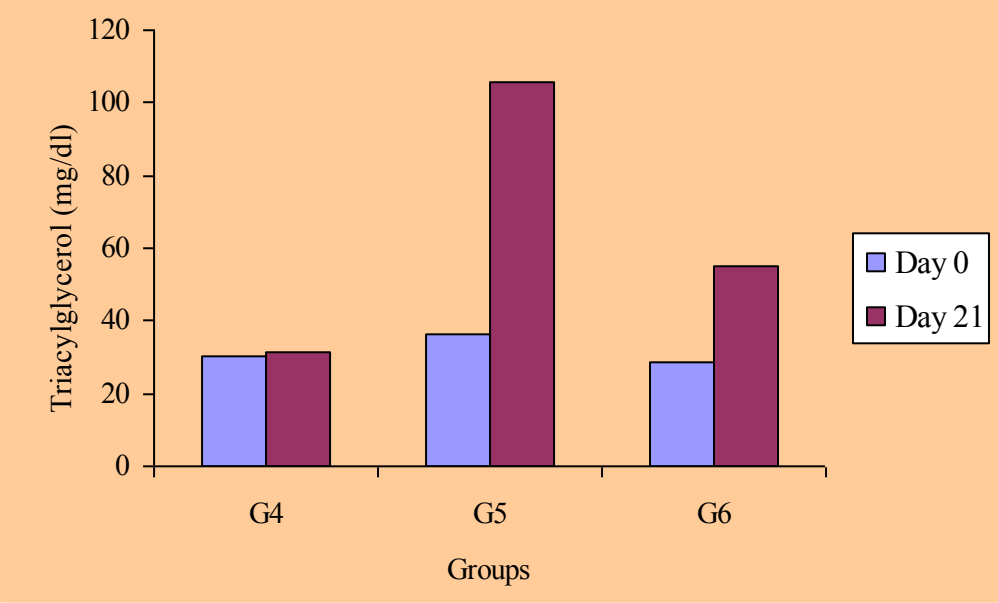

Fig. 23. Effect of co-administration of MSG and *Piper longum* on serum triacylglycerol

Table 21. Effect of co-administration of MSG and *Piper longum* on serum total cholesterol (n = 6)

Groups	Total cholesterol (mg/dl) (Mean ± SE)	
	0^{th} day	21^{st} day
G4	32.50 [a] ± 1.05	33.06 [a] ± 2.52
G5	39.50 [a] ± 1.54	82.83 [c] ± 4.40
G6	33.50 [a] ± 1.65	64.67 [b] ± 6.81

Means bearing the same superscript in a column did not differ significantly (P<0.05).

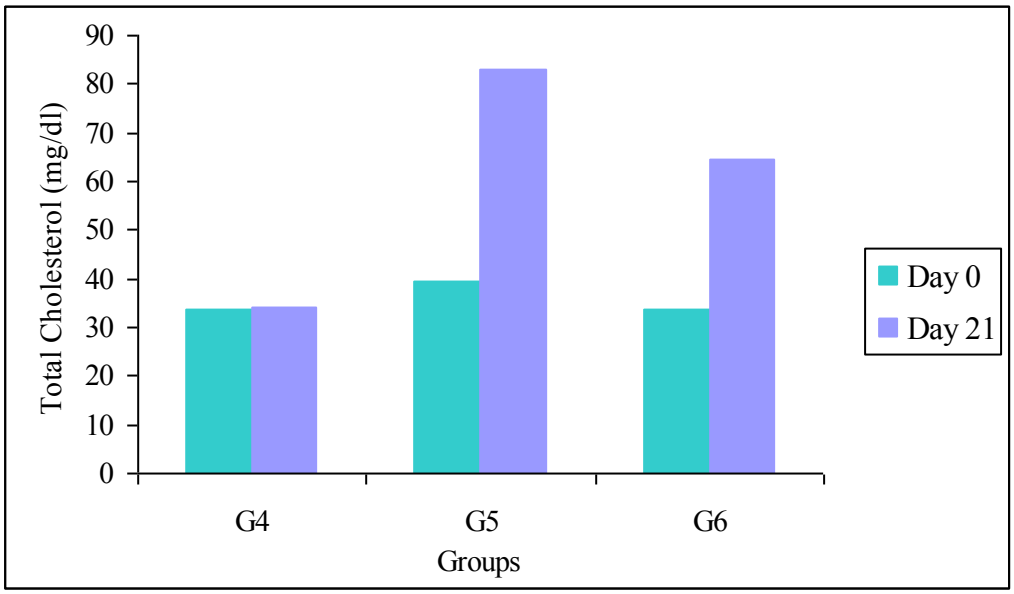

Fig. 24. Effect of co-administration of MSG and *Piper longum* on serum total cholesterol

4.2.3.3. Serum total protein, albumin and A:G ratio

Serum total protein, albumin and A:G ratio presented in Table 22 did not differ significantly between the groups either on day 0 or day 21 of the experimental period.

4.2.3.4. Serum bilirubin and creatinine

Serum total bilirubin and creatinine levels of all the groups were within the normal range and did not show any significant difference between the groups either on day 0 or day 21 of the experimental period (Table 23).

4.2.3.5. Urea

The results show no significant difference in urea levels between the groups on day 0. On day 21, significant increase ($P< 0.05$) was noticed in the urea levels of both G5 (56.00 ± 2.61 mg/dl) and G6 (52.00 ± 2.19 mg/dl) as compared to the healthy control, G4 (31.97 ± 2.30mg/dl). There was no significant difference between the groups, G5 and G6, which indicate that co-administration did not help in decreasing the urea level (Table 24 and Fig. 25).

4.2.4. Weight of Liver and spleen

The results obtained are presented in Table 25, Fig. 26 and Fig. 27. A significant increase ($P< 0.05$) in the weight of liver (6.96 ± 0.54 g) and spleen (0.41 ± 0.02 g) was observed in positive control (G5) than the healthy control (liver- 5.62 ± 0.15 g; spleen- 0.31 ± 0.02 g). In G6 the weight of liver (5.94 ± 0.58 g) and spleen (0.35 ± 0.01 g) were similar to that of the healthy control.

Table 22. Effect of co-administration of MSG and *Piper longum* on serum total protein, albumin and A:G ratio (n = 6)

Groups	0^{th} day	21^{st} day
	Total protein (mg/dl)	
G4	6.91 [a] ± 0.27	7.06 [a] ± 0.44
G5	6.83 [a] ± 0.17	6.83 [a] ± 0.40
G6	7.50 [a] ± 0.22	6.17 [a] ± 0.31
Albumin (mg/dl)		
G4	3.63 [a] ± 0.08	3.61 [a] ± 0.07
G5	3.62 [a] ± 0.07	3.47 [a] ± 0.10
G6	3.64 [a] ± 0.11	3.57 [a] ± 0.12
A:G ratio		
G4	1.07 [a] ± 0.08	1.08 [a] ± 0.06
G5	1.17 [a] ± 0.08	1.02 [a] ± 0.05
G6	0.96 [a] ± 0.09	1.24 [a] ± 0.11

Means bearing the same superscript in a column did not differ significantly ($P<0.05$).

Table 23. Effect of co-administration of MSG and *Piper longum* on serum bilirubin and creatinine (n = 6)

Groups	0th day	21st day
	Bilirubin (mg/dl)	
G4	0.68 [a] ± 0.03	0.73 [a] ± 0.03
G5	0.63 [a] ± 0.03	0.70 [a] ± 0.04
G6	0.65 [a] ± 0.04	0.67 [a] ± 0.04
Creatinine (mg/dl)		
G4	0.52[a] ± 0.02	0.53 [a] ± 0.03
G5	0.48 [a] ± 0.01	0.65 [a] ± 0.04
G6	0.50 [a] ± 0.00	0.50 [a] ± 0.02

Means bearing the same superscript in a column did not differ significantly (P<0.05).

Table 24. Effect of co-administration of MSG and *Piper longum* on serum urea (n = 6)

Groups	Urea (mg/dl) Mean ± SE	
	0^{th} day	21^{st} day
G4	32.70 [a] ± 1.87	31.97 [a] ± 2.30
G5	32.17 [a] ± 1.51	56.00 [b] ± 2.61
G6	31.83 [a] ± 2.57	52.00 [b] ± 2.19

Means bearing the same superscript in a column did not differ significantly (P<0.05).

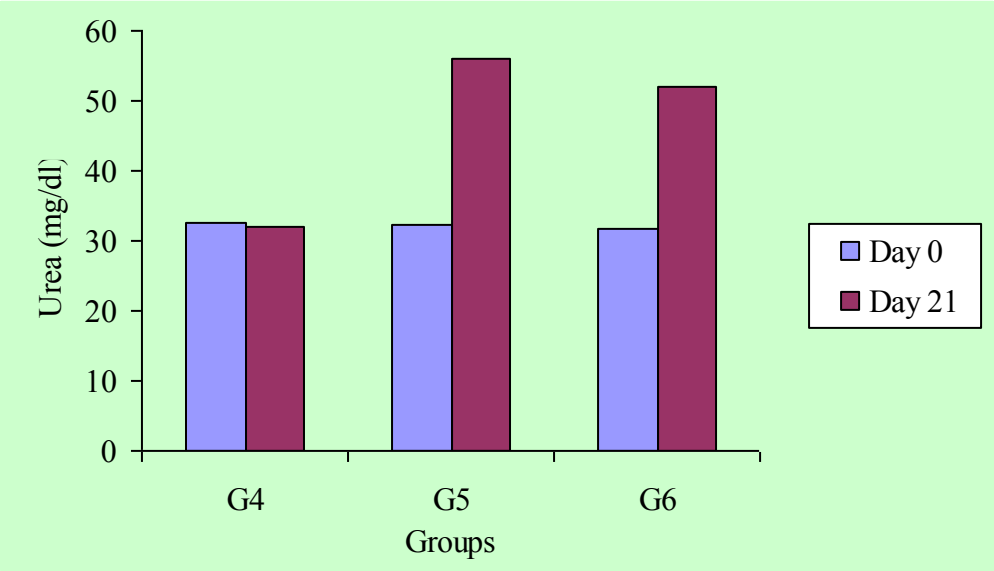

Fig. 25. Effect of co-administration of MSG and *Piper longum* on serum urea

Table 25. Effect of co-administration of MSG and *Piper longum* on the weight of liver and spleen (n = 6)

Groups	Weight of liver (g) Mean ± SE	Weight of spleen (g) Mean ± SE
G4	5.62 [a] ± 0.15	0.31 [a] ± 0.02
G5	6.96 [b] ± 0.54	0.41 [b] ± 0.02
G6	5.94 [a] ± 0.58	0.35 [a] ± 0.01

Means bearing the same superscript in a column did not differ significantly (P<0.05).

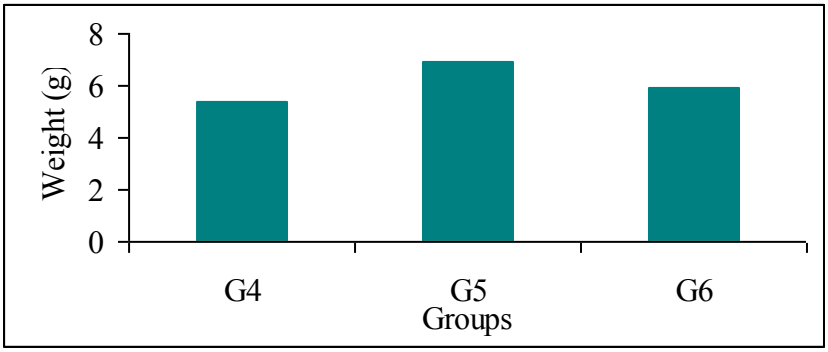

Fig. 26. Effect of co-administration of MSG and *Piper longum* on the weight of liver

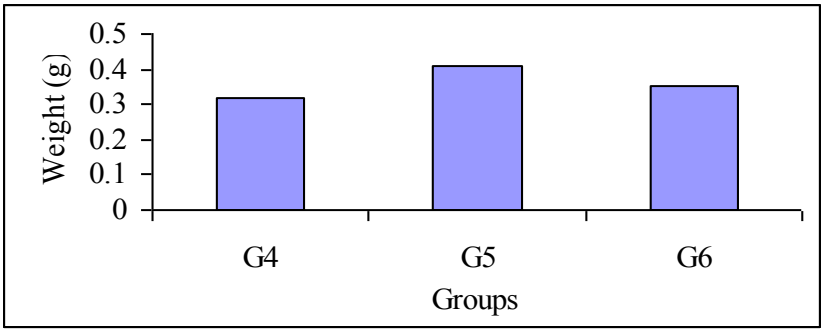

Fig. 27. Effect of co-administration of MSG and *Piper longum* on the weight of spleen

The results reveal that ethanolic extract of *Piper longum* extended a protective effect in maintaining the weight of the vital organs, liver and spleen when administered along with MSG.

4.2.5. Oxidative damage

4.2.5.1. Serum lipid peroxides

Level of serum lipid peroxides did not show any significant difference between the groups on day 0. On day 21, a significant increase ($P< 0.05$) was observed in the level of serum lipid peroxides in G5 (3.11 ± 0.07 nmol/ml) while the levels did not differ significantly between G4 (1.97 ± 0.08 nmol/ml) and G6 (1.88 ± 0.03 nmol/ml). The results indicate that ethanolic extract of *Piper longum* is capable of protecting against the oxidative damage caused by MSG (Table 26 and Fig. 28).

4.2.5.2. Serum reduced glutathione

There was no significant difference in the serum reduced glutathione levels between the groups on day 0. Administration of MSG significantly decreased ($P< 0.05$) the level of reduced glutathione in G5 (18.27 ± 0.59 μg/ml) as compared to the healthy control, G4 (34.32 ± 1.01 μg/ml). Co-administration of *Piper longum* extract at 300 mg dose rate along with MSG (G6) did not produce any significant change in its level (18.27 ± 0.51 μg/ml) as compared to the MSG control (Table 27 and Fig. 29).

Table 26. Effect of co-administration of MSG and *Piper longum* on the level of serum lipid peroxides (n = 6)

Groups	Serum lipid peroxides (nmol/ml) Mean ± SE	
	0^{th} day	21^{st} day
G4	1.96 [a] ± 0.06	1.97 [a] ± 0.08
G5	2.13 [a] ± 0.06	3.11 [b] ± 0.07
G6	1.84 [a] ± 0.08	1.88 [a] ± 0.03

Means bearing the same superscript in a column did not differ significantly (P<0.05).

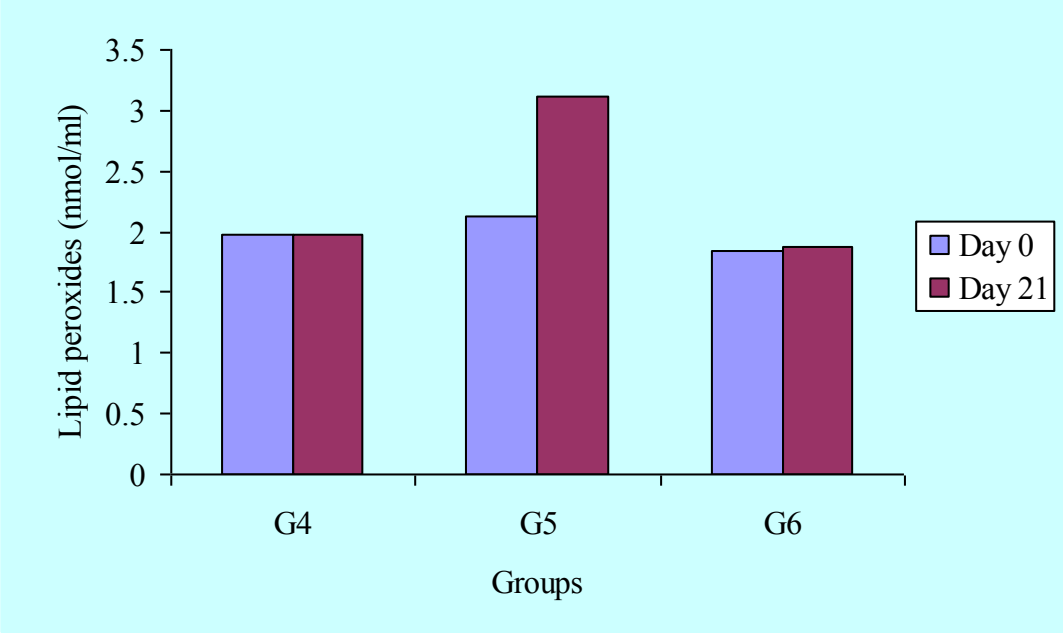

Fig. 28. Effect of co-administration of MSG and *Piper longum* on the level of serum lipid peroxides

Table 27. Effect of co-administration of MSG and *Piper longum* on the level of serum reduced glutathione (n = 6)

Groups	Serum reduced glutathione (µg/ml) Mean ± SE	
	0^{th} day	21^{st} day
G4	$32.82^{a} \pm 0.62$	$34.32^{b} \pm 1.01$
G5	$33.90^{a} \pm 0.51$	$18.27^{a} \pm 0.59$
G6	$35.05^{a} \pm 0.84$	$18.27^{a} \pm 0.51$

Means bearing the same superscript in a column did not differ significantly ($P<0.05$).

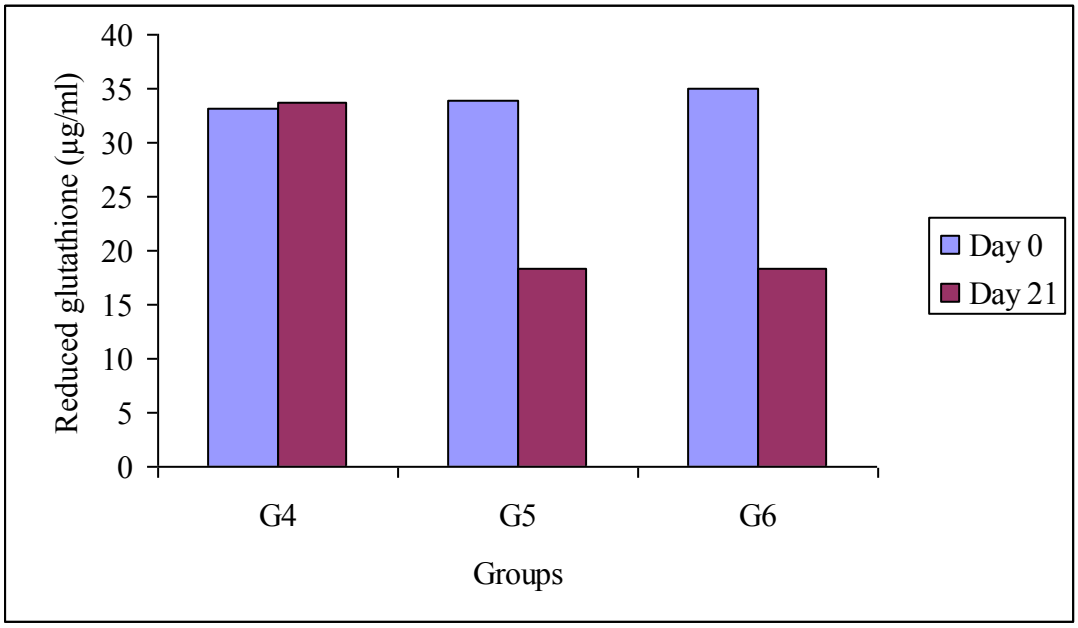

Fig. 29. Effect of co-administration of MSG and *Piper longum* on the level of serum reduced glutathione

4.2.5.3. Liver and kidney lipid peroxides

The results obtained are presented in Table 28 and Fig. 30. Lipid peroxide level increased significantly ($P< 0.05$) in both liver and kidney of G5 (liver- 523.57 ± 14.19 nmol/g; kidney- 643.18 ± 20.34 nmol/g) and G6 (liver- 472.73 ± 15.21 nmol/g; kidney- 446.33 ± 15.53 nmol/g) as compared to the healthy control, G4 (liver- 326.50 ± 3.59 nmol/g; kidney- 337.96 ± 2.76 nmol/g). Comparison between G5 and G6 showed a significant decrease ($P< 0.05$) in G6 and between the two tissues a better effect was observed in kidney.

4.2.5.4. Liver and kidney reduced glutathione

Reduced glutathione content significantly decreased ($P< 0.05$) in both liver and kidney of G5 (liver- 338.10 ± 49.79 μg/g; kidney- 354.30 ± 21.94 μg/g) and G6 (liver- 417.40 ± 8.85 μg/g; kidney- 372.38 ± 14.33 μg/g) as compared to the healthy control (liver- 804.60 ± 23.48 μg/g; kidney- 589.37 ± 14.37 μg/g). So also, the values obtained in both the tissues did not differ significantly between G5 and G6 (Table 29 and Fig. 31).

4.2.7. Histopathology of liver and kidney

Representative samples of liver and kidney of all groups were subjected to histopathological examination.

Microscopic examination of the liver and kidney of the healthy control group revealed normal histological architecture.

In the positive control (G5) liver showed central venous congestion, centrilobular and midzonal diffuse necrosis of hepatocytes characterised by both

cytoplasmic granularity and nuclear changes (Fig. 32), whereas degeneration and necrosis of the tubules was observed in the kidney (Fig. 33).

In the group G6, which received 300 mg dose rate of ethanolic extract of *Piper longum* along with MSG, liver (Fig. 34) and kidney (Fig. 35) showed a normal architecture except for the mild renal tubular degeneration.

Table 28. Effect of co-administration of MSG and *Piper longum* on the level of lipid peroxides in the liver and kidney (n = 6)

Groups	Lipid peroxides in liver (nmol/g) Mean ± SE	Kidney Lipid Peroxides (nmol/g) Mean ± SE
G4	326.50 [a] ± 3.59	337.96 [a] ± 2.76
G5	523.57 [c] ± 14.19	643.18 [c] ± 20.34
G6	472.73 [b] ± 15.21	446.33 [b] ± 15.53

Means bearing the same superscript in a column did not differ significantly (P<0.05).

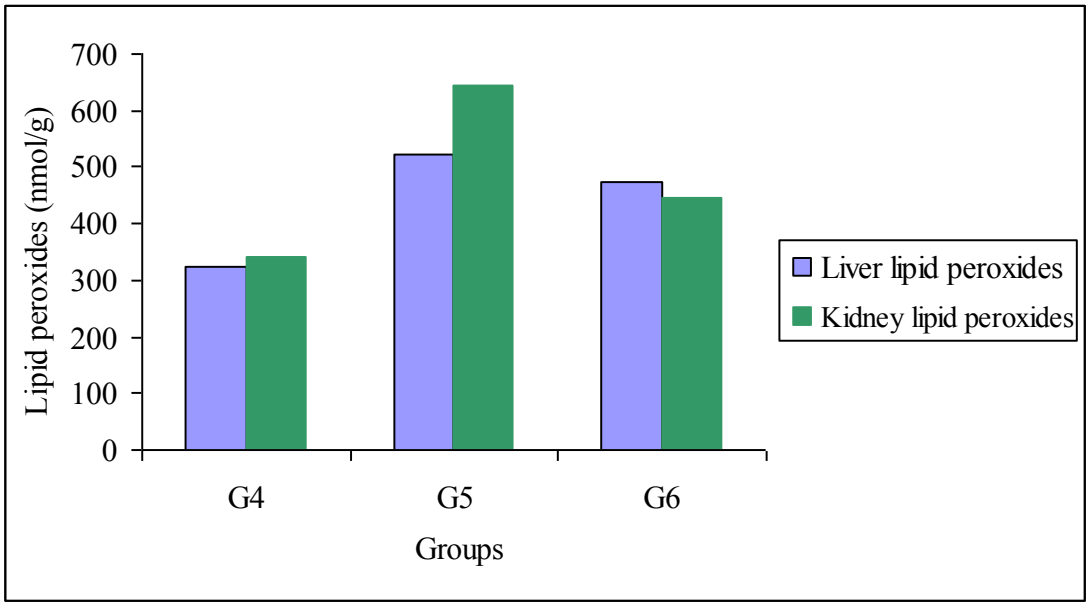

Fig. 30. Effect of co-administration of MSG and *Piper longum* on the level of lipid peroxides in the liver and kidney

Table 29. Effect of co administration of MSG and *Piper longum* on the level of reduced glutathione in the liver and kidney (n = 6)

Groups	Reduced glutathione in liver (µg/g) Mean ± SE	Reduced glutathione in kidney (µg/g) Mean ± SE
G4	804.60 [b] ± 23.48	589.37 [b] ± 14.37
G5	338.10 [a] ± 49.79	354.30 [a] ± 21.94
G6	417.40 [a] ± 8.85	372.38 [a] ± 14.33

Means bearing the same superscript in a column did not differ significantly (P<0.05).

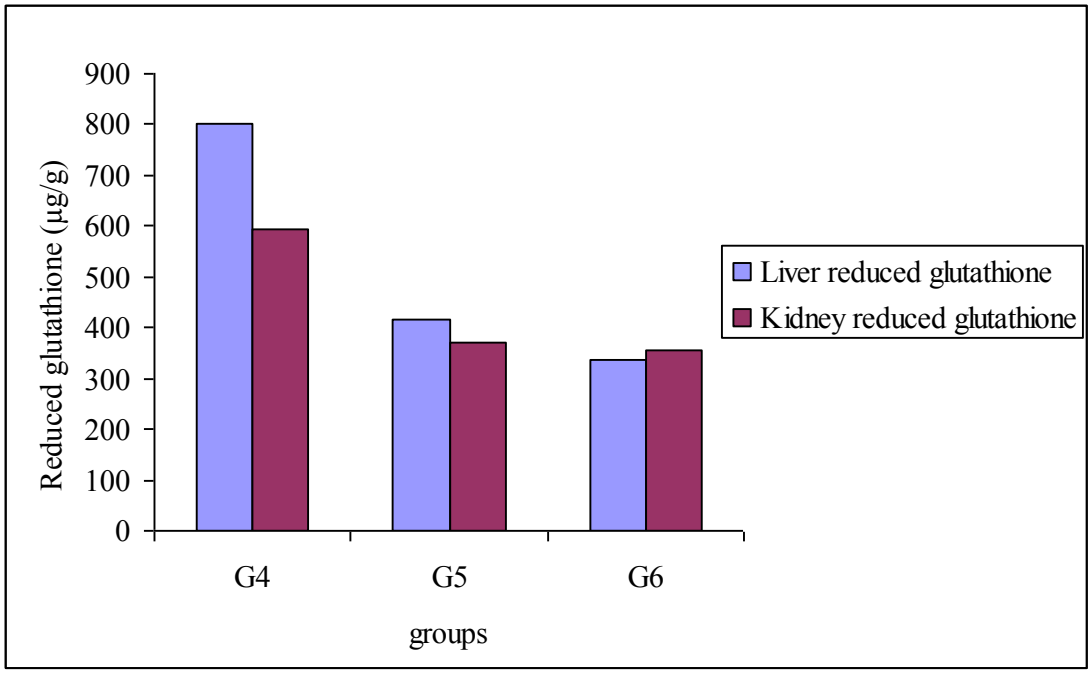

Fig. 31. Effect of co administration of MSG and *Piper longum* on the level of reduced glutathione in the liver and kidney

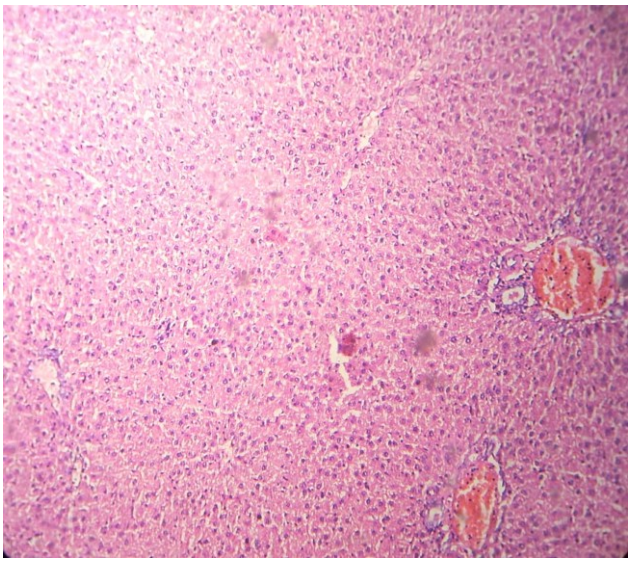

Fig. 32. Protective study- positive control, liver (H&E × 100)

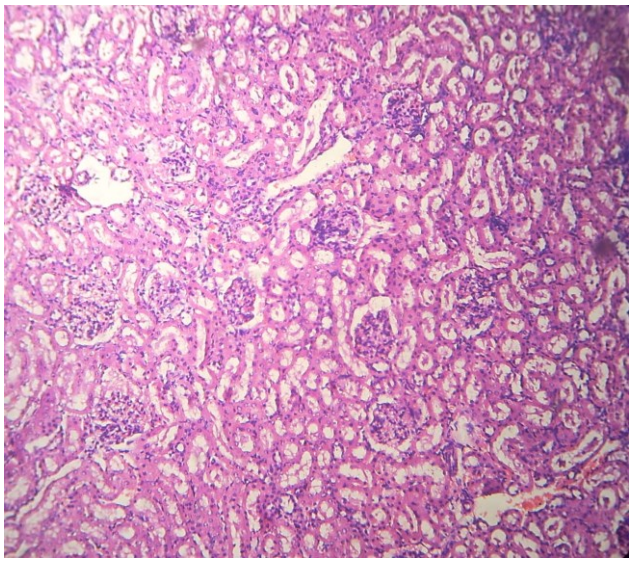

Fig. 33. Protective study- positive control, kidney (H&E × 100)

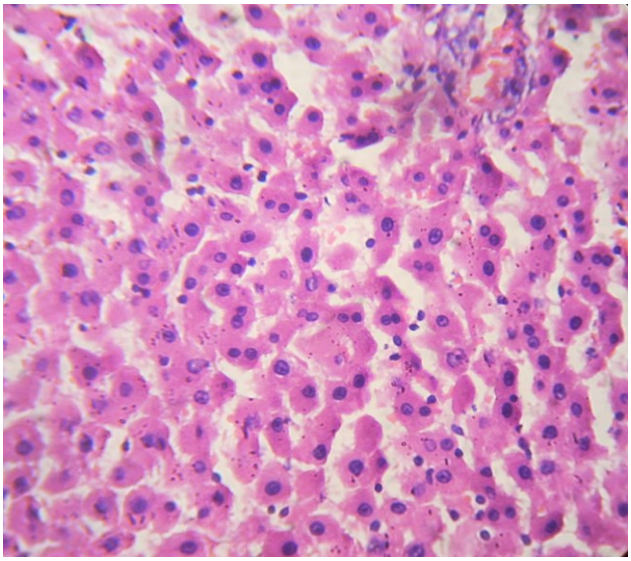

Fig. 34. Protective study- co-administered group, liver (H&E × 400)

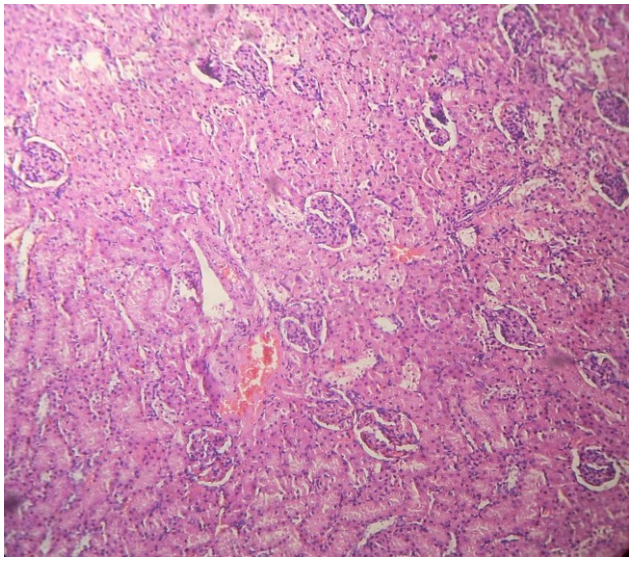

Fig. 35. Protective study- co-administered group, kidney (H&E × 100)

Discussion

5. DISCUSSION

The present study was undertaken to assess the effect of *Piper longum* against the toxicity produced by prolonged oral administration of MSG in rats. Monosodium glutamate is a widely used food flavor enhancer which causes oxidative injury to vital organs as evidenced by the induction of lipid peroxidation, depletion of reduced glutathione and alteration in the antioxidant status resulting in ultimate degeneration of the tissues (Bopanna *et al.*, 1998 and Ortiz *et al.*, 2006).

Public, academic and government interest to explore the time tested remedies from the traditional herbal medicine is growing exponentially in recent times due to the increased incidence of adverse drug reactions and economic burden of modern system of medicine. *Piper longum* is an important medicinal plant which is known to possess immunomodulatory, hepatoprotective and antioxidant effects

In this study, assays were carried out to evaluate the effect of ethanolic extract of *Piper longum* fruits after inducing toxicity with MSG (treatment study) as well as its protective effect on co-administration with MSG (protective study) in rats. The oxidative stress and subsequent damage to the tissues of liver and kidney were considered.

Adult male Wistar rats were used as experimental animals which were divided into seven groups. In the treatment study, group G0 served as the normal control. The group G1 was kept as positive control by administering MSG at a dose rate of 8 mg/g body weight p.o. for 20 consecutive days. G2 and G3 were the two treatment groups which received ethanolic extract of *Piper longum* fruits at 300 mg/kg and 600 mg/kg dose rates respectively for 14 days after inducing toxicity with MSG. Animals of all the four groups were euthanised on day 35 of experiment.

The protective study was carried out in three groups, G4 (healthy control), G5 (positive control, treated as described for G1) and G6 (received *Piper longum* extract at 300 mg/kg dose rate along with MSG). Animals of the groups G4, G5 and G6 were euthanized on day 21 of experiment.

5.1. PHYSIOLOGICAL PARAMETERS

5.1.1. Body weight

There was no change in the body weight of animals in all the MSG treated groups (G1, G2 and G3) immediately after the course of its administration whereas a significant weight gain was noted in the positive control after 14 days. This indicates that MSG induces a gradual increase in the body weight. Similar findings were also reported by earlier workers. Nagata *et al*. (2006) demonstrated that subcutaneous administration of MSG at the dose rate of 2 mg/g body weight for four days to newborn mice caused an increase in the body weight and body mass index at 7-29 week of age. Diniz *et al*. (2004) reported that subcutaneous injection with MSG at the dose rate of 4 mg/g body weight on post natal days 4 and 12 to rats resulted in increase in the body weight at 66 days post partum. They opined that MSG induced hypothalamic toxicity which led to increased food consumption, energy intake and feed conversion rate resulting in body weight gain. Parshad and Natt (2007) also reported that the presence of MSG in the rat feed pellets caused dose related stimulation of food intake. At a concentration of 4 percent of MSG, the amount of food intake increased gradually but at 10 percent MSG concentration, marked increase occurred in the first three meals and declined thereafter.

Treatment with *Piper longum* extract at both the dose levels could prevent the excess body weight gain.

Protective study also showed a similar effect and confirmed the observations in the treatment study. There was no increase in the body weight of G5 and G6 on day 21.

5.1.2. Weight of liver

Administration of MSG resulted in a significant increase in the liver weight of G1. This finding is in accordance with the observations of Onyema *et al*. (2006). They observed that MSG administered to adult rats at a dose rate of 0.6 mg/g body weight p.o. for 10 consecutive days produced a significant increase in liver weight. Nagata *et al*. (2006) also reported an increase in liver weight of mice at 29 and 54 weeks of age as a result of neonatal MSG treatment. The increase in the liver weight of animals could be attributed to oxidative damage and resultant inflammation of liver tissues. Treatment with *Piper longum* extract at 300 mg/kg dose rate was effective in decreasing the liver weight and the weight of liver in this group was similar to that observed in normal control while, 600 mg/kg dose rate failed to reduce the liver weight.

In the protective study also the positive control showed a significant increase in the liver weight while, the group fed with MSG and *Piper longum* extract simultaneously, exhibited a protective effect against the induction of MSG toxicity by preventing an increase in liver weight. These findings suggest the possible anti inflammatory action of *Piper longum*. Reduction in the liver weight may also be due to the antioxidant activity exerted by *Piper longum* which contributed to the prevention of oxidative stress and subsequent inflammation. Khajuria *et al*. (1997) and Vijayakumar and Nalini (2006) also have demonstrated that piperine – the principal alkaloid of *Piper longum* – has a potential antioxidant activity.

5.1.3. Weight of spleen

Monosodium glutamate administration resulted in an increase in the weight of spleen in G1. This might be due to an increased rate of destruction of erythrocytes. Monosodium glutamate induces oxidative stress in the erythrocytes by increasing lipid peroxidation (Ahluwalia *et al*., 1996). Oxidative injury makes the cells more fragile and such cells will be removed from the circulation by the sinusoidal phagocytic cells of spleen. This might have led to the hypertrophy of spleenocytes resulting in an increased spleen weight. Treatment with *Piper longum* at both the dose rates proved to be effective in reducing the spleen weight.

The results of the protective study also support the above findings, where the MSG administered group showed an increase in the spleen weight and co-administration of *Piper longum* and MSG could offer protection against the toxic effect of MSG. These findings correlate with the earlier reports of Vijayakumar and Nalini (2006). They observed that piperine markedly protected the erythrocytes from oxidative stress by improving antioxidant status and normalized the osmotic fragility of the cells.

5.2. BIOCHEMICAL PARAMETERS

5.2.1. Lipid peroxidation

Lipid peroxidation is the oxidative deterioration of poly unsaturated lipids by reactive oxygen species and transition metal ions. It is a molecular mechanism of cell injury leading to the generation of peroxides and lipid hydro peroxides which can decompose to yield a wide range of cytotoxic products, most of which are aldehydes such as, malondialdehyde (MDA) and 4- hydroxynonenal (Karthikeyan and Rani, 2003). Accumulation of these products in turn cause lipid peroxidation and cause

serious damage to the cell membrane and leakage of intracellular enzymes resulting in loss of cell function and cell death.

Administration of MSG caused an increase in endogenous lipid peroxides in serum, liver and kidney, which is in agreement with the findings of earlier workers. Onyema *et al*. (2006) observed elevated levels of lipid peroxides in the liver of adult rats treated with MSG at a dose rate of 0.6 mg/ g body weight p.o. for 10 days. Choudhary *et al*. (1996) reported that daily administration of MSG to adult male mice s.c. for 6 days at dose levels of 4 and 8 mg/g body weight significantly increased lipid peroxidation in the hepatic microsomes, 31 days after the last injection. Diniz *et al*. (2004) also demonstrated increased lipid peroxide concentration in the serum and liver tissue of rats after s.c. administration of MSG at a dose rate of 4 mg/g body weight. According to Ramanathan *et al*. (2007) administration of MSG at the dose rate of 2 mg/g body weight i.p. for one week to rats depleted the glutathione and increased the lipid peroxides in hippocampus and striatum of brain.

Oral administration of MSG at a dose level of 1 g/ kg body weight along with atherogenic diet for 30 days caused significant increase in the formation of MDA, hydro peroxides and conjugated dienes in the liver, kidney, lungs and heart of rats (Bopanna *et al*., 1998). According to Ortiz *et al*. (2006) single i.p. injection of MSG at a dose rate of 4 mg/g body weight resulted in an increase in lipid peroxidation of liver and kidney of rats at 30 and 45 minutes after injection. Furthermore, the reports of Singh *et al*. (2003) and Ahluwalia *et al*. (1996) also support the results confirming the MSG induced oxidative damage in tissues.

Treatment with *Piper longum* extract at both the dose levels was proved to be effective in decreasing the lipid peroxidation induced by MSG. However, 300 mg/kg dose level was found to be more effective and the level was found to be near to that

of normal control group. With regard to the lipid peroxides in kidney tissues, both the dose rates were equally effective.

Protective study also showed a similar effect and confirmed the observations in the treatment study. In G5, administration of MSG resulted in a significant increase in the level of lipid peroxides in serum, liver and kidney. Co-administration of MSG and *Piper longum* was capable of protecting against the increase in lipid peroxides.

The results of the present study correlate with the findings of Karthikeyan and Rani (2003), who demonstrated the *in vitro* anti oxidant effect of *Piper longum* against free radicals. Khajuria *et al*. (1998) showed that piperine inhibited the carcinogen induced accumulation of thiobarbituric acid reacting substances in rat intestinal lumen. Vijayakumar and Nalini (2006) also reported that supplementation of piperine simultaneously with high fat diet effectively reduced the plasma and erythrocyte lipid peroxidation. Significant hepatoprotective effect of piperine by decreasing lipid peroxidation against tert-butyl hydroperoxide and carbon tetrachloride hepatotoxicity was also demonstrated (Koul and Kapil, 1993). Wakade *et al*. (2008) also reported that pretreatment with methanolic extract of *Piper longum* fruits at 250 mg/kg and 500 mg/kg body weight by gavage for 21 days prevented the increase in the level of lipid peroxides in heart, induced by adriamycin.

However, the reports of Khajuria *et al*. (1997) state that piperine failed to decrease the lipid peroxide level in rat intestinal lumen model treated with hydrogen peroxide and cumene hydroperoxide.

5.2.2. Reduced glutathione

Reduced glutathione level reflects the antioxidant status of the tissues. Administration of MSG greatly reduced the level of reduced glutathione (GSH) in the serum, liver and kidney, which is in agreement with the findings of earlier workers (Bopanna *et al*., 1998, Farombi and Onyema, 2006, Onyema *et al*., 2006, Ramanathan *et al*., 2007, Ahluwalia *et al*., 1996, Choudhary *et al*., 1996 and Singh *et al*., 2003). They too reported that MSG induced the depletion of GSH in liver, kidney, heart, lungs, brain, erythrocytes, hepatic microsomes and mitochondria of brain in rats and mice. Decreased GSH level could not neutralize the increased level of reactive oxygen species (ROS). Thus it could be suggested that oxidative stress plays a prominent role in MSG induced organ toxicity due to the depletion of GSH.

The treatment with both the dose levels of *Piper longum* extract proved to be effective in replenishing the decreased level of GSH resulted from MSG toxicity. The dose rate of 300 mg/kg increased the serum GSH rapidly by 7 days whereas; 600 mg/kg dose rate produced a slow increase which became significant only after 14 days of treatment. On comparison between the effects of the two dose levels in tissues, though there was no significant difference, the lower dose rate showed a tendency for better effect in liver.

It has been shown that piperine affected protection against oxidative damage by elevating the GSH content in cells and maintaining high pool of GSH either by decreasing its catabolism or increasing the transport or synthesis (Khajuria *et al*.,1997 and Vijayakumar and Nalini, 2006). The antioxidant activity of *Piper longum* could be attributed to this GSH pool which helps in elimination of reactive intermediates, quenching lipid peroxide products and maintaining the thiol redox (Khajuria *et al*., 1998). The reports of Wakade *et al*. (2008) also support the efficacy of *Piper longum* to protect against the oxidative damage by elevating the GSH content of cells.

In the protective study, the positive control showed similar decrease in the GSH content of serum, liver and kidney as that of G1. However, on contrary to the observations in the treatment study, co- administration of MSG and *Piper longum* failed to produce any significant increase in the GSH levels of serum, liver or kidney. It may be due to the rapid utilization of GSH for elimination of ROS and quenching the products of lipid peroxidation. The reduced level of lipid peroxidation observed in this group also supports this conclusion. Similar effect was also observed on co-administration of chloroform: methanolic (80:20) extract of *Centella asciatica* and MSG (Ramanathan *et al*., 2007). They observed that administration of *Centella asciatica* extract at dose rates of 100 and 200 mg/kg body weight orally along with MSG (at a dose rate of 2 mg/g body weight i.p.) failed to alter the decreased GSH level in the brain of rats.

5.2.3. Serum enzymes

Alanine amino transferase is employed as a marker of hepatocellular damage in a variety of species. It is a cytoplasmic enzyme and therefore is, readily released by mild hepatocellular compromise. Aspartate amino transferase is a cytoplasmic and mitochondrial enzyme which is widely distributed in tissues. The enzyme is released from the hepatocytes and myocytes by mild degenerative changes with increased membrane permeability (Evans, 1988).

The activity of serum ALT and AST was found to be increased on MSG administration. This too, is in agreement with the findings of earlier workers (Onyema *et al*., 2006 and Farombi and Onyema, 2006). They noted that administration of MSG at a dose rate of 0.6 mg/g body weight p.o. for 10 days induced hepatotoxicity in rats and resulted in a significant increase in serum ALT, AST and GGT activities. Ortiz *et al*. (2006) also reported that MSG at a dose rate of

4 mg/g body weight administered by i.p. injection to rats produced a highly significant increase in the serum ALT and AST activities from 15 minutes post injection. It has been suggested that MSG produced a cytotoxic effect on hepatocytes by inducing lipid peroxidation of cell membrane resulting in increased permeability and cell damage. This might have caused the release of enzymes thereby increasing their levels in serum.

Although treatment with *Piper longum* extract at both the dose rates was found to be effective in reducing the activity of these enzymes in serum, a better effect was observed in G2 after 14 days treatment. This could be attributed to the comparatively greater antioxidant effect of 300 mg/kg dose rate in reducing the lipid peroxidation of hepatocytes.

In the protective study, the positive control group showed a significant increase in the activity of serum ALT and AST. Since ALT is more specific to liver, its increase in serum reflect hepatocellular damage while, increased serum AST level reflects the damage to a variety of tissues including liver and muscle tissues (Evans, 1988). Administration of *Piper longum* extract along with MSG significantly decreased the serum AST activity, but no change was observed in serum ALT activity as compared to the positive control. The decreased serum AST activity may be due to the overall protective effect of *Piper longum* to hepatocytes and myocytes, where the myocytes are far greater in number as compared to hepatocytes. Wakade *et al*. (2008) also reported that pretreatment with methanolic extract of *Piper longum* fruits at 250 mg/kg and 500 mg/kg body weight by gavage for 21 days protected against adriamycin induced cardiotoxicity and decreased the serum AST level. From the observation on ALT activity it appears that *Piper longum* extract could not offer any substantial protection against the damage of hepatocytes caused by MSG on co-administration. The higher level of lipid peroxides and lower level of GSH in the co-

administered group (G6) as compared to the treatment study group (G2) also support the above view.

Earlier studies have proved that ethanolic extract of *Piper longum* at a dose rate of 300 mg/kg body weight p.o. produced significant hepatoprotective activity against carbon tetrachloride (CCl_4) induced liver damage in rats as evidenced by its potential to decrease the activity of ALT and AST in serum (Jalalpure et al., 2003). Koul and Kapil (1993) also observed that piperine exerted a significant protection against tert-butyl hydro peroxide and CCl_4 hepatotoxicity in rats by decreasing enzymatic leakage of ALT.

5.2.4. Serum metabolites

Assay of serum metabolites can be used as biochemical tools to assess the overall metabolic status of the animal with special reference to the functioning of vital organs such as liver and kidney.

5.2.4.1. Triacylglycerol

Oral administration of MSG to adult rats produced hyperlipidemia with an increase in the serum triacylglycerol level. This correlates with the reports of Diniz *et al*. (2004) stating that MSG induces a shift in the glucose metabolism towards lipogenesis in rats. Malik and Ahluwalia (1994) also showed that s.c. administration of MSG at dose levels above 4 mg/g body weight for 6 days produced a highly significant increase in the content of total lipids, phospholipids, triacylglycerol and free fatty acids of liver in mice, 31 days after the last injection.

According to Macho *et al*. (2000) postnatal MSG administration caused an increase in plasma insulin, glucose and leptin levels in 3 months old rats. Attenuation

of the insulin stimulatory effect on glucose transport was also observed in these rats. Despite the lower basal and insulin stimulated glucose uptake, the incorporation of glucose into lipids was significantly higher in MSG treated rats suggesting a shift in the glucose metabolism towards lipogenesis.

Nagata *et al*. (2006) have also reported an increase in the triacylglycerol level in the blood of mice which received neonatal MSG treatment at the dose rate of 2 mg/g body weight s.c. for four days.

Treatment with *Piper longum* extract at both the dose rates exhibited hypolipidaemic effect as evidenced by significant reduction in the serum triacylglycerol level by 7 days. On comparison between the two dose levels, 300 mg/kg dose rate was found to be more effective and it decreased the serum triacylglycerol level to normal by 14 days treatment. Though there was a gradual decrease in the serum triacylglycerol level of the positive control from day 21 to day 35 of experiment, its level was significantly higher than the other groups and not comparable with that of the normal control or the treated groups.

In the protective study also MSG administration resulted in an increase in the serum triacylglycerol level. Co-administration of MSG and *Piper longum* extract at 300 mg/kg dose rate exhibited a lower level of triacylglycerol, showing the protective effect of *Piper longum* against MSG toxicity. Hypolipidaemic effect of polyherbal preparations containing *Piper longum* has also been reported by Kanungo *et al*. (2007). They observed lowered levels of serum triacylglycerol and total cholesterol in triton induced hyperlipidaemic rats.

5.2.4.2. Total cholesterol

Monosodium glutamate administration also resulted in an increase in the serum total cholesterol level. Similar findings were also reported by Nagata *et al*. (2006). They noticed that MSG induced hypercholesterolemia in mice and confirmed the earlier reports suggesting that MSG produces a shift in the glucose metabolism towards lipid synthesis in the liver and adipose tissues (Ahluwalia *et al*., 1994 and Macho *et al*., 2000).

Piper longum extract at both the dose levels was found to be effective in treating MSG induced hypercholesterolemia and a better effect was observed with 300 mg/kg dose rate. Significant reduction in the serum cholesterol levels was noticed by 7 days treatment. The level further decreased significantly only in the group treated with 300 mg/kg dose rate. Though the positive control also showed a gradual decrease in the serum cholesterol level, it was significantly higher from that of the treated groups.

Hypocholesterolemic effect of *Piper longum* was also observed in the protective study. Co-administration of MSG and *Piper longum* extract significantly reduced the cholesterol level, showing a protective effect against MSG toxicity. This hypocholesterolemic effect of *Piper longum* is in agreement with the observations of Vijayakumar and Nalini (2006), who observed that piperine supplementation lowered the plasma cholesterol and cholesterol/phospholipids ratio in high fat diet fed rats.

5.2.4.3. Total protein, albumin A:G ratio and bilirubin

Estimation of total protein, albumin, A:G ratio and bilirubin concentrations in serum helps to assess the functioning of liver to some extent. Plasma protein has albumin and globulin as its major components. A low albumin to globulin ratio can raise suspicions of liver function.

Total protein, albumin, A:G ratio and bilirubin levels did not differ significantly between groups during the experimental period in both treatment and protective studies. This indicates that MSG induced liver damage was not so severe to alter the serum levels of these biochemical parameters. Histopathological examination of liver also supports this finding. It has been reported that a marked reduction in hepatic functional mass, usually to less than 20 percent of normal, is necessary to induce hypoalbuminemia due to decreased synthesis (Evans, 1988).

5.2.4.4. Serum urea and creatinine

The measurement of non protein nitrogenous metabolites such as urea and creatinine in serum is used to assess kidney function. Excess of urea and creatinine in blood (azotemia) is the terminal clinical expression of kidney failure and results from the failure of the kidneys to maintain adequate excretory, regulatory and endocrine functions. Urea is the major nitrogen containing metabolic product of protein catabolism accounting for more than 75 percent of the non protein nitrogen eventually excreted. During protein catabolism, amino acid nitrogen is converted to urea in the liver by the action of the urea cycle enzymes (Newman, 2001). The rate of urea formation depends upon the rate of protein (i.e., amino acid) catabolism (Finco, 1997).

Though, there was a significant increase in the serum urea level no change was observed in the creatinine level on MSG administration. It might be due to increased intake of the amino acid – glutamate in the form of MSG. It has been suggested that an increase in blood urea nitrogen may reflect an accelerated rate of protein catabolism rather than decreased urinary excretion of urea (Finco, 1997). According to Newman (2001) high urea nitrogen to creatinine ratio with normal creatinine levels may occur with hypercatabolic states, pre renal uremia or high protein intake. In the present study, it is also noted that the increased serum urea level decreased significantly in the treated as well as in the positive control groups which suggests an accelerated rate of amino acid catabolism. Histopathological examination of the kidneys also shows that peroxidative damage to the kidneys caused by MSG was not so severe to cause derangement in the filtration mechanism to elevate the levels of non protein nitrogenous compounds in serum.

Protective study also showed a similar effect and confirmed the observations in the treatment study. On comparison with the normal control (G4), both the positive control (G5) and the treated group showed (G6) a significant increase in serum urea level while no variation was noticed in the level of creatinine.

5.3. HISTOPATHOLOGICAL EXAMINATION OF LIVER AND KIDNEY

Monosodium glutamate causes uncontrolled peroxidation of biomembranes with a resultant loss in membrane integrity due to influx of calcium from the extra cellular space. It produces hypoxia by up regulating the membrane bound enzymes, like Na^+ K^+ ATPase and 5' nucleotidase through increase in membrane permeability. Furthermore, MSG down regulates membrane fluidization whereby initiating autocatalytic reactions to cause chain of damage resulting in cytological alterations characteristic of coagulative necrosis (Bopanna *et al*., 1999).

In the present study, oral administration of MSG along with normal diet produced only mild histological changes in the liver and kidney. In G1, kidney lesions were characterized by diffuse cortical tubular degeneration, occasional necrosis and shrinkage of glomeruli. In the liver, piece meal type hepatocyte necrosis could be observed in the para cortical and midzonal areas.

It has been reported that oral administration of MSG at a dose of 1 mg/g body weight along with atherogenic diet produced foci of necrosis, ballooning degeneration, fatty change and hypertrophy of kupffer cells in liver. In kidney section, glomeruli showed mesangial proliferation. Hydropic degeneration of tubular epithelium, vacuolation of epithelial cells, edema, patchy tubular necrosis and tubulointerstitial infiltration with inflammatory cells were also noted (Bopanna *et al*., 1999). Ortiz *et al*. (2006) demonstrated degenerative changes including oedema, congestion, cloudy swelling, hydropic degeneration and necrosis in the liver and kidney of rats at 15, 30 and 45 minutes after i.p. injection of MSG at 4 mg/g dose rate.

Treatment with *Piper longum* extract at both the dose rates normalized the histological architecture of the liver and kidney. These findings correlate with the

decreased concentration of lipid peroxides and increased level of reduced glutathione in the tissues of these groups produced by *Piper longum* treatment.

In the positive control group of the protective study, liver showed central venous congestion, centrilobular and midzonal diffuse necrosis of hepatocytes characterized by both cytoplasmic granularity and nuclear changes whereas occasional degeneration of the tubules was observed in the kidney. These observations support the MSG induced oxidative damage in the liver and kidney. In the group administered with *Piper longum* extract along with MSG, the histological architecture of liver and kidney was normalized except for the mild renal tubular degeneration.

This observation correlates with the findings of Jalalpure *et al*. (2003). They noted that administration of ethanolic extract of *Piper longum* fruits at the dose rate of 300 mg/kg body weight orally produced significant signs of amelioration of CCl_4 induced liver injury as evident from the presence of normal hepatic cords, absence of necrosis and less degree of infiltration as compared to the positive control.

From the findings of the treatment study it could be concluded that ethanolic extract of *Piper longum* fruits at the dose rates of 300 mg/kg and 600 mg/kg dose rates found to reduce the oxidative stress, tissue damage and metabolic derangements caused by the toxic effect of MSG. On comparison between the two dose rates, the 300 mg/kg dose level was more effective to treat MSG toxicity. Protective study shows that although the ethanolic extract of *Piper longum* fruits at 300 mg/kg dose rate could offer significant protection against the induction of toxicity by MSG, it appears that the dose rate is insufficient to provide a complete protection against the oxidative injury.

Summary

6. SUMMARY

The present study was undertaken to assess the effect of ethanolic extract of fruits of *Piper longum* in monosodium glutamate toxicity in rats. The effect of *Piper longum* extract was studied after inducing toxicity with MSG (treatment study) as well as on co-administration with MSG (protective study). The oxidative stress and subsequent damage to liver and kidney were assessed.

Experiments were carried out in 56 adult male Wistar rats, which were randomly divided into seven groups. In the treatment study, group G0 served as a healthy control, G1, positive control, G2 and G3, the two treatment groups. G1, G2 and G3 were administered with MSG at a dose rate of 8 mg/g body weight p.o. for 20 consecutive days followed by administration of ethanolic extract of *Piper longum* to G2 and G3 at 300 mg/kg and 600 mg/kg dose rates respectively for 14 days. Weights of all the animals were recorded and blood samples were collected on days 0, 21, 28 and 35 of experiment. All the animals were euthanized on day 35 of experiment.

The protective study was carried out in the remaining groups, where G4 served as normal control, G5, positive control, and G6 was administered with *Piper longum* extract at 300 mg/kg dose rate along with MSG for 20 consecutive days. Blood collection and weight recording of these animals were carried out on days 0 and 21 of experiment and then the animals were euthanized.

Biochemical parameters viz; activities of serum ALT, AST, concentration of serum triacylglycerol, total cholesterol, bilirubin, total protein, albumin, A:G ratio, urea, creatinine and the levels of serum and tissue (liver and kidney) lipid peroxides and GSH were analyzed. From the euthanized animals liver and spleen were separated and weighed. Representative samples of liver and kidney tissues were subjected to histopathological examination.

In the treatment study, gradual but significant increase in the body weight, weight of liver and spleen was observed in the positive control, while in both the treated groups a significant reduction in the body weight and weight of spleen was observed, which was near to that of the normal control group. Whereas the liver weight was decreased only in the group treated with *Piper longum* extract at 300 mg/kg dose rate.

Prolonged oral administration of MSG induced oxidative injury to the vital organs such as liver and kidney as evidenced by the increased level of lipid peroxides, decreased level of GSH and increased activities of the enzymes ALT and AST. Treatment with both the dose rates exerted a significant antioxidant effect and the 300 mg/kg dose rate was found to be more effective. Significant decrease in serum lipid peroxides and increase in GSH were observed in the treated groups. Similar effect was also observed in liver and kidney tissues. Activity of the serum enzymes, ALT and AST, also decreased significantly in the treated groups.

Administration of MSG also caused an increase in the serum triacylglycerol, cholesterol and urea levels. Increased levels of triacylglycerol and cholesterol may possibly due to a shift in glucose metabolism towards lipid synthesis. Administration of the amino acid glutamate in the form of MSG might be the reason for the rise in serum urea level. *Piper longum* extract at both the dose levels ameliorated the MSG induced hyperlipidemia. Here too, 300 mg/kg dose rate was found to be more effective than 600 mg/kg dose rate. The levels of total protein, albumin, A:G ratio, bilirubin and creatinine did not show any significant difference between groups during the experimental period and remained within the normal range. Histopathological examination of the liver and kidney of positive control animals revealed piece meal type hepatocyte necrosis in the para cortical and midzonal areas of liver and diffuse cortical tubular degeneration, occasional necrosis and shrinkage

of glomeruli of the kidney. Treatment with *Piper longum* extract at both the dose rates normalized the histological architecture of both the liver and the kidney.

Protective study also showed a similar effect and supported the observations of the treatment study. In the treatment study, positive control showed a significant increase in body weight only on day 35 and the weight gain occurred during the period from day 21 to 35. But, increase in body weight was not observed in the protective study, may be because the total duration of the study was only 20 days. Positive control showed a significant increase in the weight of liver, spleen, level of lipid peroxides in serum, liver, kidney and activities of serum ALT and AST. Moreover, there was a significant reduction in the level of GSH in serum, liver and kidney tissues. Hyperlipidaemic effect of MSG was also evidenced by the observation of increased levels of serum triacylglycerol and cholesterol.

Co-administration of *Piper longum* extract and MSG produced a protective effect by preventing an abnormal increase in the weight of vital organs and the level of lipid peroxides in serum, liver and kidney. But this treatment failed to produce any increase in the level of GSH. Among the serum enzymes, ALT level was similar to that in the positive control while, AST level significantly decreased. Administration of *Piper longum* extract along with MSG prevented the metabolic derangements caused by MSG and reduced the level of serum triacylglycerol and total cholesterol, whereas there was no change in the serum urea level as compared to the positive control. Other biochemical parameters such as the levels of total protein, albumin, A:G ratio, bilirubin and creatinine did not show any significant difference between groups during the experimental period as observed in the treatment study.

Histopathological examination of the tissues of positive control showed mild changes such as, central venous congestion, centrilobular and midzonal diffuse necrosis of hepatocytes in liver and occasional degeneration of the tubules in the

kidney. Administration of *Piper longum* extract at 300 mg dose rate along with MSG normalized the histological architecture of liver and kidney except for the occasional shrinkage of glomeruli in kidney.

Based on the findings of the treatment and protective studies on the toxic effect of MSG in rats it could be summed up as follows:

1. Though both dose rates of ethanolic extract of *Piper longum* fruits were effective in fighting the toxic effects of MSG, 300 mg/kg b.w dose rate showed a better effect in alleviating the oxidative injury and metabolic derangement.
2. Although, ethanolic extract of *Piper longum* fruits at 300 mg/kg b.w dose rate could offer significant protection against the induction of toxicity by MSG, it appears that the dose rate is insufficient to provide a complete protection against the oxidative injury.

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Abstract

EFFECT OF *Piper longum* Linn. (PIPPALI) IN MONOSODIUM GLUTAMATE TOXICITY IN RATS

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ABSTRACT

The present study was conducted to evaluate the effect of ethanolic extract of fruits of *Piper longum* in monosodium glutamate toxicity in rats. Treatment as well as protective effects of the plant extract against MSG toxicity were studied.

The experiments were carried out in adult male Wistar rats, which were divided into seven groups. The treatment study was conducted in four groups viz; G0- normal control, G1-positive control, G2 and G3- two treatment groups. G1, G2 and G3 were administered with MSG at a dose rate of 8 mg/g body weight p.o. for 20 days followed by treatment of G2 and G3 with *Piper longum* extract at dose rates of 300 mg/kg and 600 mg/kg b.w p.o. respectively for 14 days.. Blood collection and weight recording of these animals were carried out on days 0, 21, 28 and 35 of experiment and then the animals were euthanized. The remaining three groups viz; G4, G5 and G6 were subjected to protective study where G4 served as normal control, G5, positive control and G6 was administered with *Piper longum* extract at 300 mg/kg dose rate along with MSG for 20 days. Blood samples were collected and the animals were weighed on days 0 and 21 of experiment followed by euthanasia.

The oxidative stress and subsequent damage to liver and kidney were assessed by measuring the biochemical parameters viz; activities of serum ALT, AST, concentration of serum triacylglycerol, total cholesterol, bilirubin, total protein, albumin, A:G ratio, urea, creatinine and the levels of serum and tissue (liver and kidney) lipid peroxides and GSH. From the euthanized animals liver and spleen were separated and weighed. Representative samples of liver and kidney tissues were subjected to histopathological examination.

Administration of MSG induced a significant increase in the body weight, weight of liver and spleen. The increase in body weight was gradual and became significant only on day 35. Oxidative injury to the tissues of liver and kidney was

evident from the increased level of lipid peroxides, decreased level of GSH and increased activities of serum ALT and AST. There was also an increase in the levels of serum triacylglycerol, cholesterol and urea. Histopathological examination of the liver and kidney of positive control animals revealed necrosis of hepatocytes in the para cortical and midzonal areas of liver and diffuse cortical tubular degeneration, occasional necrosis and shrinkage of glomeruli of the kidney. However, the hepatic and nephro toxicities caused by MSG were not so severe to alter the levels of total protein, albumin, A:G ratio, bilirubin and creatinine in serum.

Piper longum extract at both the dose levels proved to be effective in treating the toxicity induced by MSG and helped to bring back the body weight and weight of spleen near to that of the control, significantly reduced the lipid peroxides and increased the GSH levels in serum, liver and kidney. Treated groups also showed a significant reduction in serum ALT and AST activity, ameliorated the MSG induced hyperlipidemia and normalized the histological architecture of both the liver and kidney. Among the two dose rates, only the 300 mg/kg dose rate was effective in maintaining the liver weight near to that of the control and this dose rate was found to be more effective in alleviating the toxicity caused by MSG.

Co-administration of *Piper longum* extract and MSG prevented the abnormal increase in the weight of vital organs, level of lipid peroxides in serum, liver and kidney, while no increase was observed in the level of GSH. Significant decrease was also observed in the levels of serum AST, triacylglycrol and total cholesterol, whereas no change was observed in the level of ALT and urea. Although, ethanolic extract of *Piper longum* fruits at 300 mg/kg dose rate could offer significant protection against the induction of toxicity by MSG, it appears that the dose rate is insufficient to provide a complete protection against the oxidative injury.