

Effect of *Piper longum* Linn. in monosodium glutamate toxicity in rats

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ABSTRACT

Effect of ethanol extract of *Piper longum* (*P. longum*) Linn. in monosodium glutamate (MSG) induced toxicity was studied in rats. Oral administration of MSG at a dose of 8 mg/g body weight for 20 consecutive days showed an increase in body weight and liver weight. Oxidative stress was evidenced by significant increase in the level of lipid peroxides and decrease in reduced glutathione (GSH) content of serum, liver and kidney. There was significant increase in serum ALT and AST activities. Level of serum urea increased significantly whereas creatinine level did not altered. Significant increase was also observed in the levels of serum triacylglycerol and total cholesterol. Histopathological examination revealed degeneration and necrosis of hepatocytes, renal tubules and glomeruli of liver and kidney respectively. Rats treated with ethanol extract of *P. longum* fruits at 300 mg/kg body weight and 600 mg/kg body weight for 14 days significantly decreased body weight, serum triacylglycerol, lipid peroxides in serum, liver and kidney, serum ALT and AST activities and increased the content of GSH in serum, liver and kidney. However, 300 mg/kg body weight was found to be more effective in decreasing the level of triacylglycerol, serum ALT, lipid peroxides in serum, liver and kidney and increasing the content of GSH in serum and liver. Significant reduction in liver weight and serum cholesterol was observed only in rats treated with the extract at this dose. Serum urea level did not vary significantly between treated groups and positive control. Histopathological examination of liver and kidney showed normal histological architecture except for mild congestion. The present study revealed that ethanol extract of fruits of *P. longum* has antioxidant and hypolipidaemic properties and may be used for treating MSG toxicity.

Key words: GSH, Lipid peroxides, Monosodium glutamate, Oxidative stress, *Piper longum*

Monosodium glutamate (MSG) is a widely used food flavor enhancer. 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). Research on the toxic effect of MSG as a food additive increased greatly because of the association of this compound with Chinese restaurant syndrome (Schaumburg *et al.* 1969), neuroexcitotoxicity (Park *et al.* 2000, Singh *et al.* 2003) obesity (Nagata *et al.* 2006) and hyperlipidemia (Ahluvalia and Malik 1989). Chronic administration of MSG induces oxidative injury to vital organs by increasing lipid peroxidation, depletion of reduced glutathione (GSH) and alteration in antioxidant status resulting in ultimate degeneration of tissues (Singh *et al.* 2003, Onyema *et al.* 2006).

Piper longum (Indian long pepper/pippali) is an important medicinal plant, which is used 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 fruits are useful in asthma, bronchitis, tumors, spleen disorders, inflammation, leprosy, insomnia and jaundice and the roots are used as laxative and anthelmintic (Kirtikar and Basu 1975).

The chemical constituents present in fruits include piperine, pipalartene, piperlongumine, volatile oils, starch, resins, gum, fatty oils and inorganic matter (Jalalpure *et al.* 2003). Piperine is capable of enhancing bioavailability of other drugs such as, phenytoin, pentobarbitone, nimesulide and oxytetracycline (Singh *et al.* 2005). Piperine is highly lipophilic and is absorbed very fast across the intestinal barrier through transcellular pathway. It modulates the membrane dynamics, which increases the absorptive area resulting in efficient permeability of drugs through membranes and enhances their bioavailability (Khajuria *et al.* 1998). Reports are also available on the antiulcer, antidiarrhoeal, antimicrobial, immunomodulatory, hepatoprotective and antioxidant effects of the plant (Jalalpure *et al.* 2003, Agrawal *et al.* 2000, Bajad *et al.* 2001, Sunila and Kuttan 2004, Karthikeyan and Rani 2003, Ali *et*

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al. 2007). However, *in vivo* studies on the antioxidant and hypolipidaemic potentials of *P. longum* are limited. The present study was aimed to evaluate the antioxidant and hypolipidaemic effect of ethanol extract of fruits of *P. longum* in rats induced with MSG toxicity.

MATERIALS AND METHODS

Drugs and chemicals: Monosodium L-glutamate, 5–5' Dithiobis 2-nitrobenzoic acid (DTNB), 1,1,3,3 tetramethoxypropane (TMP), thio barbituric acid (TBA), sodium dodecyl sulphate (SDS) and all other reagents and chemicals were obtained commercially from reputed producers.

Fruits of *P. longum* were identified and authenticated by the Professor, Department of Plant breeding and Genetics, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur. Ethanol extract of *P. longum* fruits were prepared as follows: Dried ripe fruits, procured locally, were washed, dried under shade, coarsely powdered and extracted with ethanol in a soxhlet apparatus. The extract was concentrated in a rotary vacuum evaporator at 50°C under reduced pressure and dried at room temperature. The extract was reconstituted to a final concentration of 5% (w/v) using 5% aqueous solution of gum acacia. Monosodium glutamate was dissolved in deionised double distilled water to make aqueous solution (50%).

Animals and experimental design: Male Wistar rats weighing 100–150 g were housed in appropriate cages in a well ventilated experimental animal room under 12:12 h L : D cycle at 22 to 28°C with free access to standard rat pellet diet and drinking water. Animals were randomly divided into four groups, each comprising six animals and were treated, as per the schedule given below, for a period of 35 consecutive days. Experiments were conducted in accordance with the approval of Institutional Animal Ethics Committee.

Normal control group (G_N) 5% aqueous solution of gum acacia for 14 days from 21st day onwards.

Positive control group (G_P) MSG at a dose of 8 mg/g body weight for 20 consecutive days.

Treatment group 1 (G_{T1}) MSG at a dose of 8 mg/g body weight for 20 consecutive days followed by ethanol extract of fruits of *P. longum* at the dose of 300 mg/kg body weight for 14 days

Treatment group 2 (G_{T2}) MSG at a dose of 8 mg/g body weight for 20 consecutive days followed by ethanol extract of fruits of *P. longum* at the dose of 600 mg/kg body weight for 14 days

Standardization of dose and route of administration of MSG: Dose and route of administration of MSG were standardized through experimental trials with varying concentrations of MSG and different routes of administration. MSG was administered at 2, 4, 6 and 8 mg/g body weight both subcutaneously and orally, each route of administration comprised four groups of male Wistar rats, having six animals in each group.

Intra peritoneal injections were tried only at 4 mg/g dose, which resulted in about 80% mortality. Subcutaneous injections were given for six consecutive days (Ahluwalia *et al.* 1996). Hepatic damage was monitored by analyzing serum enzymes, alanine amino transferase (ALT) and aspartate amino transferase (AST) and serum lipid peroxide level on 20th and 30th days after injection. Significant alterations could not be detected in any of the above parameters as compared with pre administration values. Oral administrations were carried out continuously and serum samples were analyzed on 11th, 16th and 21st day. Among the 4 doses administered, significant increase in the level of ALT, AST and serum lipid peroxides was observed only in the group administered with MSG at 8 mg/g body weight for 20 consecutive days. Moreover, when the dose (8 mg/g) was continued beyond 20 days, about 50% animals died between 25 to 30 days due to severe diarrhoea and neurological problems. Thus, it was concluded from the experimental trials that oral administration of MSG at a dose of 8 mg/g body weight for 20 consecutive days could effectively induce toxicity in rats and therefore, the dose was fixed for this experiment.

Treatment with ethanol extract of *P. longum*: Two doses, 300 mg/kg body weight (Jalalpure *et al.* 2003) and a higher dose of 600 mg/kg body weight were selected and administered orally from day 21 onwards for 14 consecutive days.

Weight of animals was recorded on days 0, 21, 28 and 35. Blood samples were collected from the retro orbital plexus under mild ether anesthesia, using heparinised capillary tubes, into sterile microfuge tubes on the above days and centrifuged at $1000 \times g$ for 10 minutes at 15°C to separate serum. On day 35, the rats were euthanized, separated liver and kidney, washed in ice cold saline, dried on a filter paper and the weight of liver was noted.

Biochemical assay: Serum ALT, AST activities, serum triacylglycerol, total cholesterol, urea and creatinine were estimated using commercially available kits.

The extent of lipid peroxidation in serum was determined by the method of Yagi (1984). Lipid peroxides in liver and kidney tissue homogenates were determined by the method described by Ohkawa *et al.* (1979). Tissue homogenates (10% w/v) were prepared in 1.15% KCl solution using a glass homogenizer, centrifuged at $2750 \times g$ for 5 min. and supernatant was used for estimation of lipid peroxides.

Level of GSH in serum and tissue homogenates (10% w/v in 0.2 M phosphate buffer, pH 8) was determined by measuring the absorbance of yellow coloured complex formed by the reaction between GSH and DTNB, at 412 nm (Moron *et al.* 1979).

Statistical analysis: The data obtained were compared by analysis of variance (ANOVA) (Snedecor and Cochran 1989), followed by Duncan multiple range test to determine the level of significance (Steel and Torrie 1980). The value of $P < 0.05$ was considered statistically significant.

Histopathological examination: Liver and kidney sections were prepared by standard procedures and stained with haematoxylin and eosin (H and E). The sections were examined in detail under light microscope.

RESULTS AND DISCUSSION

Body weight and liver weight: There was no significant change in the body weight of animals under the groups G_p , G_{T1} and G_{T2} on day 21 while, a significant ($P < 0.05$) weight gain was noted in positive control (G_p) by day 35. Significant increase ($P < 0.05$) was also observed in liver weight of G_p (Table 1). Monosodium glutamate induced a gradual increase in body weight, which occurred during the period subsequent to MSG administration. Nagata *et al.* (2006) reported similar findings. Hypothalamic toxicity and release of hormones by MSG might have led to increased feed consumption and feed conversion rate, which resulted in increased body weight (Diniz *et al.* 2004). Significant increase in liver weight could be attributed to oxidative damage and resultant inflammation of liver tissues (Onyema *et al.* 2006). Treatment with

P. longum extract at both doses could prevent gain in body weight while 300 mg/kg body weight of the extract only could maintain the liver weight similar to that of normal control rats (Table 1). Antioxidant and neuroprotective effect of *P. longum* might be the reasons for preventing body weight gain. Ramanathan *et al.* (2007) reported neuroprotective effect of chloroform: methanol (80 : 20) extract of *Centella asiatica*, when administered to rats induced with MSG toxicity. They observed a significant reduction in free radical generation and excitotoxicity and an increase in the levels of the antioxidant enzymes, catalase and superoxide dismutase. Significant decrease was also observed in the level of lipid peroxides in hippocampus and striatum regions of brain. Reduction in liver weight of the treated group might be due to the antioxidant activity of *P. longum*, which prevented the oxidative stress and subsequent inflammation (Khajuria *et al.* 1997, Vijayakumar and Nalini 2006).

Lipid peroxides and GSH: Level of lipid peroxides increased significantly ($P < 0.05$) while a significant reduction ($P < 0.05$) was observed in the content of GSH in serum, liver and kidney of positive control rats (Tables 2, 3). This is a clear indication of oxidative stress. Similar observations have also been reported by earlier workers (Bopanna *et al.* 1998, Singh *et al.* 2003, Onyema *et al.* 2006). 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 decompose to yield a wide range of cytotoxic products. Glutathione functions as a direct free radical scavenger and is thought to

Table 1. Effect of *P. longum* on body weight and liver weight (g) of rats induced with MSG toxicity

Groups	Body weight				Liver weight
	Day 0	Day 21	Day 28	Day 35	
G_N	142.50±2.5 ^a	159.17±2.39 ^a	161.67±2.47 ^a	165.83±2.01 ^a	5.42±0.15 ^a
G_p	135.00±5.00 ^a	172.50±9.29 ^a	180.00±8.94 ^b	190.00±8.17 ^b	6.43±0.27 ^b
G_{T1}	134.17±2.71 ^a	161.67±7.60 ^a	162.50±4.61 ^a	160.83±3.00 ^a	5.66±0.17 ^a
G_{T2}	145.00±5.48 ^a	150.83±6.38 ^a	152.50±6.80 ^a	155.83±7.90 ^a	6.59±0.34 ^b

Values are mean±SE; a, b: columns bearing different alphabets differ significantly, ($P < 0.05$); G_N , Normal control group; G_p , positive control group; G_{T1} , group treated with 300 mg/kg body weight of the extract; G_{T2} , group treated with 600 mg/kg body weight of the extract.

Table 2. Effect of *P. longum* on the level of lipid peroxides in serum (nM/ml), liver and kidney (nM/g) in rats induced with MSG toxicity

Groups	Lipid peroxides					
	Serum				Liver	kidney
	Day 0	Day 21	Day 28	Day 35		
G_N	1.98±0.08 ^a	1.97±0.07 ^a	1.94±0.09 ^a	2.04±0.06 ^a	323.00±3.39 ^a	340.92±2.79 ^a
G_p	1.98±0.08 ^a	3.01±0.06 ^b	2.97±0.06 ^d	3.05±0.09 ^c	534.88±6.47 ^d	585.72±9.75 ^c
G_{T1}	1.98±0.06 ^a	3.01±0.06 ^b	2.19±0.11 ^b	1.98±0.05 ^a	332.32±7.07 ^b	494.38±6.49 ^b
G_{T2}	1.99±0.07 ^a	2.89±0.11 ^b	2.58±0.04 ^c	2.54±0.15 ^b	407.75±12.76 ^c	509.47±16.72 ^b

Values are mean±SE; a, b, c, Columns bearing different alphabets differ significantly, ($P < 0.05$); G_N , Normal control group, G_p , positive control group; G_{T1} , group treated with 300 mg/kg body weight of the extract, G_{T2} , group treated with 600 mg/kg body weight of the extract.

Table 3. Effect of *P. longum* on the level of reduced glutathione (GSH) in serum ($\mu\text{g/ml}$), liver and kidney ($\mu\text{g/g}$) in rats induced with MSG toxicity

Groups	Reduced glutathione					
	Serum				Liver	kidney
	Day 0	Day 21	Day 28	Day 35		
GN	33.12 $\pm 0.61^a$	33.62 $\pm 1.11^b$	33.82 $\pm 0.54^c$	34.38 $\pm 1.11^d$	801.43 $\pm 33.45^c$	593.37 $\pm 13.39^c$
GP	33.88 $\pm 0.56^a$	15.97 $\pm 0.8^a$	18.38 $\pm 0.45^a$	18.67 $\pm 0.28^a$	414.28 $\pm 11.87^a$	305.72 $\pm 7.19^a$
GT1	33.88 $\pm 0.56^a$	15.15 $\pm 0.81^a$	28.38 $\pm 0.79^b$	29.68 $\pm 0.63^c$	567.65 $\pm 19.55^b$	403.78 $\pm 5.06^b$
GT2	33.32 $\pm 0.60^a$	15.00 $\pm 0.75^a$	18.65 $\pm 0.67^a$	24.30 $\pm 0.85^b$	503.90 $\pm 16.06^b$	410.48 $\pm 18.34^b$

Values are mean $\pm$ SE; a, b, c, d, Columns bearing different alphabets differ significantly, ($P < 0.05$); G_N, normal control group, G_p, positive control group, G_{T1}, group treated with 300 mg/kg body weight of the extract, G_{T2}, group treated with 600 mg/kg body weight of the extract

Table 4. Effect of *P. longum* on the activities of serum alanine amino transferase (ALT) and aspartate amino transferase (AST) (IU/L) in rats induced with MSG toxicity

Groups	Serum ALT activity				Serum AST activity			
	Day 0	Day 21	Day 28	Day 35	Day 0	Day 21	Day 28	Day 35
G _N	43.33 $\pm$ 1.36 ^a	44.33 $\pm$ 1.69 ^a	45.50 $\pm$ 1.71 ^a	45.67 $\pm$ 1.56 ^a	191.67 $\pm$ 3.89 ^a	192.50 $\pm$ 4.06 ^a	193.67 $\pm$ 3.81 ^a	194.17 $\pm$ 4.81 ^a
G _p	46.67 $\pm$ 1.63 ^a	99.17 $\pm$ 2.71 ^b	98.00 $\pm$ 3.97 ^c	101.67 $\pm$ 1.61 ^d	197.00 $\pm$ 2.54 ^a	317.33 $\pm$ 17.29 ^b	284.33 $\pm$ 4.07 ^c	315.50 $\pm$ 10.88 ^c
G _{T1}	48.12 $\pm$ 1.17 ^a	98.17 $\pm$ 4.06 ^b	71.50 $\pm$ 3.39 ^b	55.67 $\pm$ 1.76 ^b	202.00 $\pm$ 2.85 ^a	309.50 $\pm$ 6.83 ^b	278.17 $\pm$ 11.02 ^c	234.67 $\pm$ 9.81 ^b
G _{T2}	47.67 $\pm$ 1.99 ^a	100.0 $\pm$ 03.42 ^b	76.83 $\pm$ 5.31 ^b	72.83 $\pm$ 3.76 ^c	201.83 $\pm$ 2.39 ^a	324.33 $\pm$ 11.82 ^b	230.50 $\pm$ 17.12 ^b	235.50 $\pm$ 5.73 ^b

Values are mean $\pm$ SE; a, b, c, d, Columns bearing different alphabets differ significantly, ($P < 0.05$); G_N, normal control group, G_p, positive control group, G_{T1}, group treated with 300 mg/kg body weight of the extract, G_{T2}, group treated with 600 mg/kg body weight of the extract.

stabilize membrane structure by the removal of products of lipid peroxidation.

Treatment with both doses of *P. longum* extract significantly decreased ($P < 0.05$) the level of lipid peroxides and increased the content of GSH in serum, liver and kidney compared to positive control. However, the levels of lipid peroxides and GSH were significantly different from those of normal control rats except for serum lipid peroxides in G_{T1} on day 35 (Tables 2, 3). Comparison between the two treated groups revealed a significantly lower ($P < 0.05$) level of serum and liver lipid peroxides and higher level ($P < 0.05$) of serum GSH in rats treated with 300 mg/kg body weight of the extract. These results correlate with the antioxidant effect of *P. longum* (Karthikeyan and Rani 2003). It has been shown that piperine protected cells against oxidative damage by elevating the GSH content and maintaining high pool of GSH either by decreasing its catabolism or increasing the transport or synthesis (Khajuria *et al.* 1997, Vijayakumar and Nalini 2006). The antioxidant activity of *Piper longum* could be attributed to the high GSH pool, which helped to eliminate reactive intermediates and quenched the products of lipid peroxide (Khajuria *et al.* 1998, Wakade *et al.* 2008).

Serum enzymes: Serum ALT and AST activities increased significantly ($P < 0.05$) on MSG administration (Table 4). Cytotoxic effect of MSG on hepatocytes and other tissues might have resulted in increased permeability and thereby elevated the activity of these enzymes in serum (Onyema *et al.* 2006, Ortiz *et al.* 2006). Treatment with both doses of *P. longum* extract significantly decreased ($P < 0.05$) activity of these enzymes in serum. When the two treated groups were compared, G_{T1} showed significantly lower level of ALT activity than G_{T2} on day 35 (Table 4). Significant reduction in the level of the enzymes in the treated groups suggests the ability of the extract to augment tissue repair mechanisms. Jalalpure *et al.* (2003) reported similar observations in rats induced with CCl₄ toxicity when treated with *P. longum* extract.

Serum lipids: Administration of MSG also caused hyperlipidemia with a significant increase ($P < 0.05$) in the levels of serum triacylglycerol and cholesterol (Table 5), possibly due to increased levels of plasma insulin, glucose and leptin, which caused a shift in glucose metabolism towards lipogenesis (Malik and Ahluwalia, 1994, Macho *et al.* 2000, Diniz *et al.* 2004). Treatment with both doses of

Table 5. Effect of *P. longum* on the activities of serum triacylglycerol and total Cholesterol (mg/dl) in rats induced with MSG toxicity

Groups	Triacylglycerol				Total cholesterol			
	Day 0	Day 21	Day 28	Day 35	Day 0	Day 21	Day 28	Day 35
G _N	29.33±0.67 ^a	31.00±2.17 ^a	29.17±1.64 ^a	30.83±2.91 ^a	38.50±1.89 ^a	37.33±2.14 ^a	36.67±2.25 ^a	35.33±2.95 ^a
G _p	30.17±2.44 ^a	102.83±5.97 ^b	95.50±5.14 ^c	81.00±3.26 ^c	37.50±2.46 ^a	96.33±5.73 ^b	77.50±1.54 ^c	61.00±0.77 ^c
G _{T1}	34.00±1.88 ^a	112.17±5.13 ^b	46.50±5.17 ^b	33.00±3.51 ^a	38.83±1.64 ^a	91.33±5.69 ^b	59.33±2.62 ^b	45.67±2.84 ^b
G _{T2}	33.50±2.49 ^a	103.17±5.97 ^b	51.83±4.87 ^b	47.33±5.89 ^b	39.50±1.71 ^a	90.33±4.67 ^b	60.83±4.21 ^b	58.00±4.73 ^c

Values are mean±SE; a, b, c: Columns bearing different alphabets differ significantly, (P< 0.05); G_N, Normal control group, G_p, positive control group, G_{T1}, group treated with 300 mg/kg body weight of the extract, G_{T2}, group treated with 600 mg/kg body weight of the extract.

Table 6. Effect of *P. longum* on the activities of serum urea and creatinine (mg/dl) in rats induced with MSG toxicity

Groups	Urea				Creatinine			
	Day 0	Day 21	Day 28	Day 35	Day 0	Day 21	Day 28	Day 35
G _N	32.50±1.89 ^a	32.00±2.21 ^a	31.00±1.63 ^a	33.50±0.89 ^a	0.52±0.01 ^a	0.50±0.02 ^a	0.52±0.01 ^a	0.53±0.04 ^a
G _p	31.00±1.63 ^a	62.17±2.70 ^b	51.33±1.05 ^b	44.17±1.96 ^b	0.52±0.01 ^a	0.67±0.07 ^a	0.57±0.03 ^a	0.58±0.03 ^a
G _{T1}	34.50±0.89 ^a	60.83±2.49 ^b	47.83±0.95 ^b	40.83±1.49 ^b	0.50±0.02 ^a	0.65±0.04 ^a	0.57±0.02 ^a	0.58±0.04 ^a
G _{T2}	30.00±2.98 ^a	62.00±2.73 ^b	48.50±2.23 ^b	41.17±2.71 ^b	0.48±0.04 ^a	0.65±0.04 ^a	0.57±0.04 ^a	0.50±0.02 ^a

Values are mean±SE; a, b: Columns bearing different alphabets differ significantly, (P< 0.05); G_N, Normal control group, G_p, positive control group, G_{T1}, group treated with 300 mg/kg body weight of the extract, G_{T2}, group treated with 600 mg/kg body weight of the extract.

the extract significantly decreased (P<0.05) the level of serum triacylglycerol while significant reduction in cholesterol level was observed only in G_{T1} compared with G_p. Comparison between the treated groups revealed that both serum triacylglycerol and cholesterol levels were significantly lower (P<0.05) in G_{T1} than G_{T2} and serum triacylglycerol level in G_{T1} was similar to that of G_N. However, a gradual decrease was observed in the levels of both serum triacylglycerol and cholesterol in positive control too by day 35 but the levels were significantly higher than that of normal control and treated groups (Table 5). Lipid lowering effect of piperine has been reported by Vijayakumar and Nalini (2006). They observed that, piperine supplementation lowered the plasma cholesterol and cholesterol/phospholipids ratio in rats fed with high fat diet.

Serum NPN substances: Though, MSG administration increased serum urea to a significant level it decreased significantly by day 35 and did not exhibit any significant difference between the treated groups and positive control. Serum creatinine level did not show any variation between G_N, G_p and treated groups (Table 6). Elevation in serum urea level might be due to increased intake of the amino acid, glutamate in the form of MSG. 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 treated and positive control groups, which is suggestive of an accelerated rate of amino acid catabolism.

Histopathology: Histopathological examination of liver (Figs 1 a, b) revealed diffuse degeneration and necrosis of

hepatocytes in the para cortical and midzonal areas while that of kidney (Figs 2 a, b) showed diffuse cortical tubular degeneration, occasional necrosis and shrinkage of glomeruli of positive control rats. Liver sections of both the treated groups (G_{T1} and G_{T2}) showed apparently normal histological architecture (Figs 1. c, d). Kidney sections of G_{T1} showed a normal histological architecture except for mild tubular degeneration (Fig. 2c) while tubular regeneration was quite evident in the kidney of G_{T2} (Fig. 2d) where, 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 organized along the tubules. Histopathological observations of this study correlate with the biochemical findings. It appears that peroxidative damage of kidneys induced by MSG was not so severe to cause derangement in the filtration mechanisms to elevate the levels of non protein nitrogenous compounds in serum.

It is evident from the present study that ethanol extract of *P. longum* is capable of alleviating MSG induced oxidative stress, hyperlipidaemia and tissue damage in rats. Among the doses tried, 300 mg/kg body weight was found to be more effective in decreasing levels of serum triacylglycerol, cholesterol and all the parameters associated with inflammation and tissue damage such as, liver weight, serum ALT, lipid peroxides in serum, liver and kidney and increasing the content of GSH in serum and liver. This is in agreement with the findings of Mittal and Gupta (2000). They observed that lower doses of piperine, the principal alkaloid in *P. longum*, showed increased scavenging effect of free radicals while higher doses activated fenton reaction and

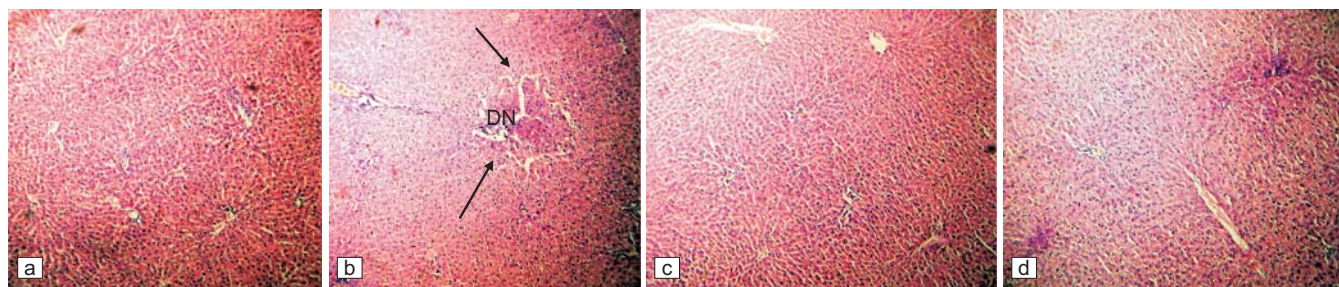


Fig. 1. **a-d.** Section of liver. **a.** Normal control group (G_N); **b.** Positive control group (G_P) showing diffuse necrosis (DN) of hepatocytes; **c.** Treated with 300 mg/kg body weight of ethanol extract of *P. longum* (G_{T1}) showing normal architecture of hepatocytes; **d.** Treated with 600 mg/kg body weight of ethanol extract of *P. longum* (G_{T2}) showing normal architecture of hepatocytes. H&E $\times$ 100.

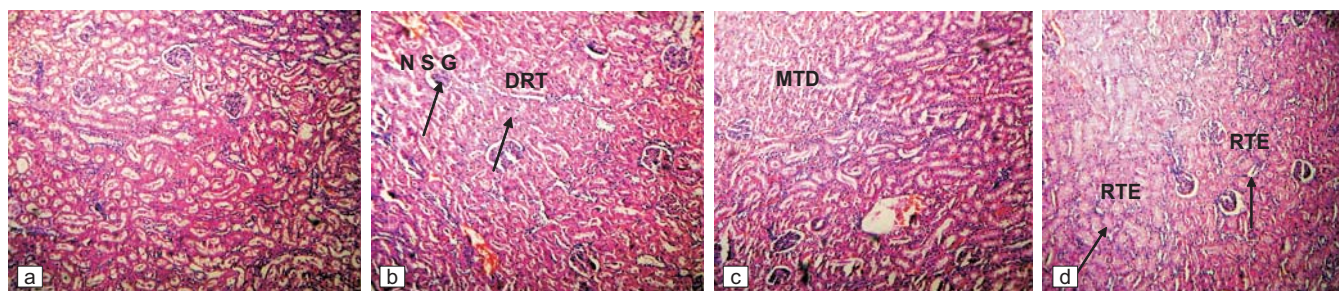


Fig. 2. **a-d.** Section of kidney. **a.** Normal control group (G_N); **b.** Positive control group (G_P) showing degeneration of renal tubules (DRT), occasional necrosis and shrinkage of glomeruli (NSG); **c.** Treated with 300 mg/kg body weight of ethanol extract of *P. longum* (G_{T1}) showing normal architecture except for mild tubular degeneration (MTD); **d.** Treated with 600 mg/kg body weight of ethanol extract of *P. longum* (G_{T2}) showing regeneration of tubular epithelium (RTE). H&E $\times$ 100.

increased generation of hydroxyl radicals.

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