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Protective effect of *Piper longum* Linn. on monosodium glutamate induced oxidative stress in rats

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Protective effect of ethanol extract of *Piper longum* Linn. against monosodium glutamate (MSG) induced toxicity was studied. Rats, orally administered with MSG at a dose of 8 mg/g body weight for 20 consecutive days, showed an increase in liver weight and rate of lipid peroxidation. Glutathione (GSH) in serum, liver and kidney showed decreased concentration. Significant increase was noticed in activities of serum alanine amino transferase (ALT) and aspartate amino transferase (AST), levels of serum triacylglycerol, total cholesterol and urea. Histopathological examination of liver and kidney showed central venous congestion, diffuse degeneration and necrosis of hepatocytes in para cortical and midzonal areas of liver and diffuse cortical tubular degeneration of kidney. Oral administration of ethanol extract of *P. longum* fruits at 300 mg/kg body weight along with MSG significantly reduced the levels of lipid peroxides in serum, liver and kidney, serum AST activity, serum levels of triacylglycerol and total cholesterol. Though, there was an increase in the level of GSH in tissues it was not significant. However, the treatment failed to reduce the levels of ALT and urea. Examination of tissue sections also exhibited normal histological architecture of both the organs. The present study revealed that administration of *P. longum* provided significant protection to liver and kidney from the oxidative stress of MSG, though the dose rate was not sufficient to provide a complete protection.

Keywords: Monosodium glutamate, *Piper longum*, Rats, Toxicity

Monosodium glutamate (MSG), a food flavour enhancer, is used all over the world especially in Chinese and Japanese foods[1]. However, its use has been questioned due to a number of reports describing the toxic effects in human beings[2]. MSG was found to be the cause of 'Chinese restaurant syndrome' characterized by headache, burning sensation along the back of the neck, chest tightness, nausea and sweating[1,2]. Additionally, MSG is known to elicit toxic effects such as, impairment in memory retension[3], damage in the hypothalamic neurons, alterations in mitochondrial lipid peroxidation and antioxidant status in different regions of brain[4] and induce hyperphagia leading to obesity[5]. The asthma provoking potential of MSG in sensitive individuals is also proved[6]. Alterations in the level of certain biochemical indices such as, carbohydrates, lipids and proteins in rats treated with MSG suggested that it induces a shift in the carbohydrate metabolism towards lipogenesis leading to hyperlipidemia[7]. Hyperglycemia and hyperlipidemia induced by MSG could result in lipid peroxidation of biomembranes and production of oxygen free radicals[8]. MSG induced oxidative stress in erythrocytes, liver, kidney, heart and brain of experimental animals has also been documented[9,10].

Piper longum, Indian long pepper in English, *Pippali* in Hindi, is an important medicinal plant, the fruits of which find use in Ayurvedic and Unani systems of medicine. The chemical constituents present in the ethanol extract include piperine, pipalartene, piperlongumine, volatile oils, starch, resins, gum, fatty oils and inorganic matter[11]. The fruits are used for the treatment of asthma, bronchitis, tumors, spleen disorders, inflammation, leprosy, insomnia and jaundice[12]. Reports are available regarding the enhancement of bioavailability of other drugs such as, phenytoin, pentobarbitone, nimesulide and oxytetracycline by the alkaloid, piperine[13]. Evaluation of permeability characteristics of piperine showed that it is a weak base, highly lipophilic in nature which is absorbed very fast across the

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intestinal barrier through transcellular pathway. Piperine forms apolar complex with drugs and solutes with which it is compounded. It modulates the membrane dynamics, which increases the absorptive area resulting in efficient permeability of drugs through membranes and enhances their bioavailability[14]. There are also evidences for the antiulcer, antidiarrhoeal, antimicrobial, immuno-modulatory, hepatoprotective and antioxidant effects of the plant[11,15 -19].

Present study elucidates the protective effect of ethanol extract of *P. longum* against MSG induced hepatic and renal toxicity in rats.

Materials and Methods

Drugs and chemicals—Monosodium L-glutamate, 5-5'Dithiobis 2-nitrobenzoic acid (DTNB), 1,1,3,3 Tetramethoxypropane (TMP), and Thio barbituric acid (TBA) were procured from Himedia Laboratories Pvt. Ltd, Mumbai. Sodium dodecyl sulphate (SDS) was from Sigma – Aldrich India, Bangalore. All other reagents and chemicals were analytical grade from Merck India Ltd, Mumbai.

Fruits of *P. longum* were identified and authenticated by Dr K T Prasanna Kumari, 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 aqueous solution of gum acacia (5%).

Monosodium glutamate was dissolved in deionised double distilled water to make aqueous solution (50%).

Animals—Male Wistar rats weighing 100-150 g were housed in appropriate cages in a well ventilated experimental animal room under 12:12 hr L:D cycle at 22° to 28°C with free access to standard rat pellet diet and drinking water. Animals were randomly divided into 3 groups, each comprising 6 animals and administered as per the treatment schedule for a period of 20 consecutive days. Experiments were conducted in accordance with the rules, regulations and approval of the Institutional Animal Ethics Committee.

Normal control group (G_N) - 5% aqueous solution of gum acacia

Positive control group (G_P) - MSG at a dose of 8 mg/g body weight

Treated group (G_T) - Ethanol extract of fruits of *P. longum* at a dose of 300 mg/kg body weight along with MSG (8 mg/g body weight)

Standardization of dose and route of administration—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 mg/g, 4 mg/g, 6 mg/g and 8 mg/g body weight both subcutaneously and orally, each route of administration comprised of 4 groups of male Wistar rats, having 6 animals in each group.

Injections (ip) were tried only at 4 mg/g dose, which resulted in about 80% mortality. Subcutaneous injections were given for 6 consecutive days[9]. Hepatic damage was monitored by analyzing serum enzymes alanine amino transferase (ALT) and aspartate amino transferase (AST) and serum lipid peroxide level on 20^{th} and 30^{th} 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 11^{th}, 16^{th} and 21^{st} day. Among the four 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, experimental trials revealed 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 study in rats at two doses of ethanol extract of *P. longum*, 300 mg/kg and 600 mg/kg body

weight, showed that 300 mg/kg dose was more effective in treating MSG induced toxicity compared to 600 mg/kg (unpublished data). Therefore, 300 mg/kg dose was selected to evaluate its efficacy in protecting the animals from the toxic effects of MSG, when the two were administered together.

Blood samples were collected on days 0 and 21 of experiment and centrifuged at 1000 × g for 10 min. at 15°C to separate serum. On day 21, the rats were euthanized, separated liver and kidney, which were washed in ice cold saline, dried on filter paper and the weight of liver was noted.

Biochemical analysis—Serum ALT, AST acivities, serum triacylglycerol, total cholesterol, urea and creatinine were estimated using Ecoline Kits (M/s E. Merck India, Ltd, Mumbai).

The extent of lipid peroxidation in serum was determined by the method of Yagi[20]. Serum lipids were isolated by precipitating them along with proteins using phosphotungstic acid-sulphuric acid system. Lipid peroxides and malondialdehyde in the sediment reacted with TBA to form a red coloured pigment. The absorbance was measured at 532 nm. TMP was used as a standard since, it can be converted to malondialdehyde quantitatively by reacting with TBA. The level of lipid peroxides in liver and kidney tissue homogenates was determined by the method of Ohkawa *et al*[21]. Tissue homogenates (10% w/v) were prepared in 1.15% KCl solution using a glass homogenizer, centrifuged at 2750 × g for 5 min. and the supernatant was used for the 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[22].

Statistical analysis—The data obtained were compared by analysis of variance (ANOVA) followed by Duncan multiple range test to determine the level of significance. The value of $P < 0.05$ was considered statistically significant[23].

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

Administration of MSG in rats caused a significant increase in liver weight. Level of endogenous lipid peroxides elevated significantly while that of GSH showed significant decrease in serum as well as tissues of liver and kidney (Table 1). Similar observations have also been reported by earlier workers[4,24,25]. Increase in liver weight could be attributed to oxidative damage and resultant inflammation of liver tissues[26]. Reactive oxygen species (ROS) generated by the toxic effect of MSG might have caused lipid peroxidation and GSH depletion, which are indicators of tissue damage.

Administration of *P. longum* extract along with MSG could prevent excess liver weight gain, which might be due to the antioxidant activity of *P. longum*,

Table 1—Effect of co-administration of MSG and *P. longum* on liver weight, levels of lipid peroxides and reduced glutathione in the serum, liver and kidney
[Values are Mean ± SE of 6 animals]

		Lipid peroxides				Reduced glutathione			
		Serum (nmol/ml)		Liver (nmol/g)	Kidney (nmol/g)	Serum (µg/ml)		Liver (µg/g)	Kidney (µg/g)
Groups	Liver weight	Day 0	Day 21	Day 21	Day 21	Day 0	Day 21	Day 21	Day 21
G_N	$5.62^a \pm 0.15$	$1.96^a \pm 0.06$	$1.97^a \pm 0.08$	$326.50^a \pm 3.59$	$337.96^a \pm 0.76$	$32.82^a \pm 0.62$	$34.32^b \pm 1.01$	$804.60^b \pm 23.48$	$589.37^b \pm 14.37$
G_P	$6.96^b \pm 0.54$	$2.13^a \pm 0.06$	$3.11^b \pm 0.07$	$523.57^c \pm 14.19$	$643.18^c \pm 20.34$	$33.90^a \pm 0.51$	$18.27^a \pm 0.59$	$338.10^a \pm 49.79$	$354.30^a \pm 21.94$
G_T	$5.94^a \pm 0.58$	$1.84^a \pm 0.08$	$1.88^a \pm 0.03$	$472.73^b \pm 15.21$	$446.33^b \pm 15.53$	$35.05^a \pm 0.84$	$18.27^a \pm 0.51$	$417.40^a \pm 8.85$	$372.38^a \pm 14.33$

Level of significance was determined column wise between G_N, G_P and G_T
Values not bearing a common superscript letter (a, b and c) differ significantly
G_N - Normal control group, G_P - Positive control group, G_T - Treated group

which protected the liver tissues from oxidative stress and subsequent inflammation to a certain extent. Earlier studies also revealed the antioxidant potential of piperine, the principal alkaloid of *P. longum*[27,28]. Significant reduction was observed in the level of lipid peroxides in serum, liver and kidney of G_T, though the level was significantly higher than that of the normal control group. But G_T failed to produce any significant increase in the level of GSH (Table 1). Glutathione functions as a direct free radical scavenger and can stabilize membrane structure through the removal of products of lipid peroxidation. It appears that administration of *P. longum* extract at the dose of 300 mg/kg along with MSG could protect the tissues only to a certain extent against the oxidative damage induced by MSG, which is evident from the levels of lipid peroxides in G_T compared with the positive control (G_P) and normal control (G_N). Furthermore, ethanol extract of *P. longum* at this dose was found to be insufficient to elevate GSH to a significantly higher level. It has been suggested that antioxidant activity of *P. longum* could be attributed to its ability to maintain high pool of GSH either by decreasing its catabolism or increasing its synthesis/transport [27, 28]. Insignificant increase in the level of GSH and a limited reduction in the level of lipid peroxides, which is not comparable to that of the normal control, suggest that there might have been a rapid utilization of the newly synthesized/transported GSH for the elimination of ROS and quenching the products of lipid peroxidation.

Serum ALT and AST activities were found to be increased significantly in G_P (Table 2), which might be due to oxidative stress and resu ant damage to cell membranes. Co-administration of *P. longum* and MSG showed a significantly low level of AST. This could be attributed to the overall protective effect of *P. longum* to tissues other than hepatocytes. Wakade *et al.*[29] also reported that methanol extract of *P. longum* fruits protected against adriamycin induced cardiotoxicity and decreased the serum AST level. Absence of any reduction in serum ALT activity in G_T (Table 2) might be due to its more specificity to hepatocytes. This observation correlates with the findings on the levels of lipid peroxides and GSH in the treated group. Administration of *P. longum* at 300 mg/kg along with MSG could not offer a complete protection against the oxidative stress induced alteration in the membrane integrity, which changed the membrane permeability resulting in the leakage of intracellular enzymes. Such permeability changes may not be identifiable under ordinary microscopical examination of tissue sections.

Table 2—Effect of co-administration of MSG and *P. longum* on serum alanine amino transferase (ALT) and aspartate amino transferase (AST) activity
[Values are Mean ± SE of 6 animals]

Groups	ALT (U/L)		AST (U/L)	
	0^{th} day	21^{st} day	0^{th} day	21^{st} day
G_N	43.43^a ± 1.32	44.38^a ± 1.79	192.47^a ± 3.86	193.56^a ± 3.06
G_P	46.67^a ± 1.63	95.67^b ± 2.11	197.33^a ± 2.74	309.50^c ± 6.83
G_T	48.50^a ± 0.72	100.50^b ± 6.97	206.83^a ± 1.60	218.33^b ± 4.82

Level of significance was determined column wise between G_N, G_P and G_T
Values not bearing a common superscript letter (a, b and c) differ significantly
G_N- Normal control group, G_P- Positive control group, G_T- Treated group

Table 3—Effect of co-administration of MSG and *P. longum* on serum triacylglycerol, total cholesterol, urea and creatinine
[Values are Mean ± SE of 6 animals]

Groups	Triacylglycerol (mg/dl)		Total cholesterol (mg/dl)		Urea (mg/dl)		Creatinine (mg/dl)	
	0^{th} day	21^{st} day	0^{th} day	21^{st} day	0^{th} day	21^{st} day	0^{th} day	21^{st} day
G_N	30.14^a ± 1.67	31.64^a ± 2.12	32.50^a ± 1.05	33.06^a ± 2.52	32.70^a ± 1.87	31.97^a ± 2.30	0.52^a ± 0.02	0.53^a ± 0.03
G_P	36.33^a ± 2.09	105.67^c ± 6.43	39.50^a ± 1.54	82.83^c ± 4.40	32.17^a ± 1.51	56.00^b ± 2.61	0.48^a ± 0.01	0.65^a ± 0.04
G_T	28.83^a ± 1.74	54.83^b ± 4.19	33.50^a ± 1.65	64.67^b ± 6.81	31.83^a ± 2.57	52.00^b ± 2.19	0.50^a ± 0.00	0.50^a ± 0.02

Level of significance was determined column wise between G_N, G_P and G_T
Values not bearing a common superscript letter (a, b and c) differ significantly
G_N- Normal control group, G_P- Positive control group, G_T- Treated group

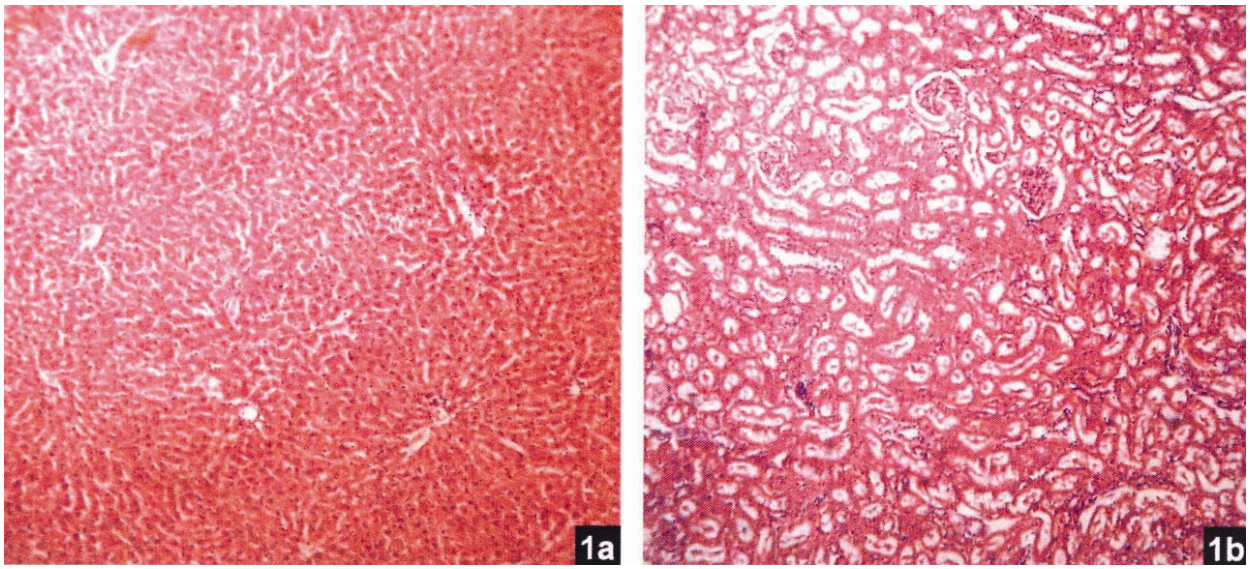

Fig. 1—(a): Liver section of normal control group, G_N (H&E × 100); (b): Kidney section of normal control group, G_N (H&E × 100)

Hyperlipidemia with significantly elevated levels of serum triacylglycerol and cholesterol was noticed in G_P while in G_T its level decreased significantly, though the levels were higher than that of the normal control (Table 3). A shift in glucose metabolism towards lipogenesis might account for the hyperlipidemia in G_P[7]. Hypolipidemic activity of the ethanol extract could explain the substantial reduction in the level of triacylglycerol and cholesterol in G_T, which is in agreement with the findings of Vijayakumar and Nalini[28]. They observed that piperine supplementation lowered the plasma cholesterol and cholesterol/phospholipids ratio in rats fed with high fat diet.

Serum urea level was found to be elevated significantly in both G_P and G_T and did not vary significantly between these groups while creatinine level did not show any significant change between the three groups (Table 3). Increased intake of the amino acid, glutamate in the form of monosodium glutamate might be the reason for this. Histopathological examination of tissues of liver and kidney also supports this view. 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[30]. According to Newman[31] high urea nitrogen to creatinine ratio with normal creatinine levels may occur with hypercatabolic states, pre-renal uremia or high protein intake. Histopathological examination of the positive control group in comparison with the normal control (Fig. 1a & 1b) revealed central venous congestion, diffuse degeneration and necrosis of hepatocytes in para cortical and midzonal areas of liver (Fig. 2a) and cortical tubular degeneration in kidney (Fig. 2b). The treatment group showed an apparently normal histological architecture of liver with regenerative changes such as multinucleated cells and pyknotic nuclei (Fig. 2c). Kidney sections also showed a normal histological architecture except for mild tubular degeneration (Fig. 2d). Regenerative changes and an apparently normal histological architecture of the tissues along with other biochemical observations suggest that administration of ethanol extract of *Piper longum* along with MSG, reduces MSG induced oxidative stress on tissues.

The present study reveals that although the ethanol extract of *P. longum* at 300 mg/kg when administered along with MSG (8 mg/g) could offer significant protection against MSG toxicity, the dose is not sufficient to provide a complete protection against the oxidative injury.

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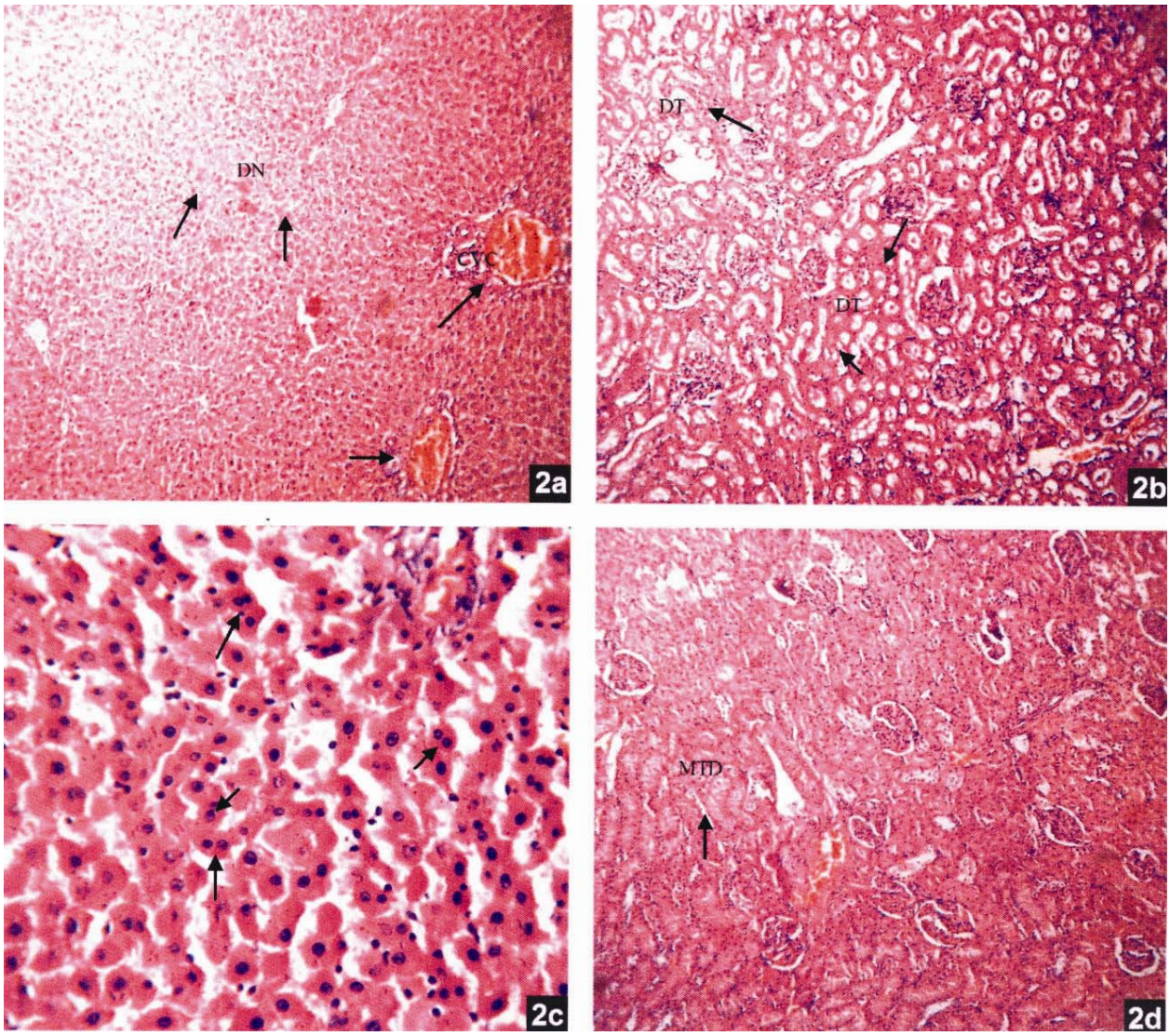

Fig. 2—(a): Liver section of positive control group (G_P) showing central venous congestion (CVC) and diffuse necrosis (DN) of hepatocytes (H&E × 100); (b): Kidney section of positive control group (G_P) showing degeneration of renal tubules (DT) (H&E × 100); (c): Liver section of treated group (G_T) with regenerative changes such as multinucleated cells and cells with pyknotic nuclei (H&E × 400); (d): Kidney section of treated group (G_T) showing normal architecture except for mild tubular degeneration (MTD) (H&E × 100)

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