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Hepatoprotective activity of *Euphorbia neriifolia* against paracetamol induced hepatotoxicity in rats

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ABSTRACT

The objective of the present study was to investigate the hepatoprotective activity of methanol extract of *Euphorbia neriifolia* (MEEN) against paracetamol induced hepatotoxicity. Hepatotoxicity was induced in Wistar rats by oral administration of paracetamol (640 mg/kg suspended in 1% carboxy methyl cellulose), once during the 16 days treatment period. MEEN was administered orally at the doses of 200 and 400 mg/kg daily for 16 days. Silymarin (25 mg/kg) was used as standard drug. Hepatoprotective activity was evaluated by the biochemical estimation of liver function parameters (SGPT, SGOT, ALP, total protein and total bilirubin), antioxidant assays of liver homogenate (lipid peroxidation, reduced glutathione content, superoxide dismutase and catalase activity) and histological study of liver tissue. In MEEN treated animals, the toxic effect of paracetamol was controlled significantly by restoration of the biochemical parameters, such as, SGPT, SGOT, ALP, total protein and total bilirubin, as well as by the improvement of the antioxidant status to/towards near normal values. Histology of the liver sections of the animals treated with the extracts showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration, which further evidenced the hepatoprotective activity of MEEN. The results show that the methanol extract of *Euphorbia neriifolia* possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

Keywords: *Euphorbia neriifolia*, paracetamol, hepatoprotective, silymarin and antioxidant

INTRODUCTION

Herbs play a major role in the management of various liver disorders along with other system associated diseases. Liver is a key organ regulating homeostasis within the body by various functions. Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Hepatotoxicity is very common ailment resulting in serious debilities ranging from severe metabolic disorders to even mortality (Patel et al., 2008). Plant derived natural products such as flavonoids, terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity (DeFeuils, 2003; Banskota et al., 2000; Takeoka and Dao, 2003).

Euphorbia neriifolia Linn (Euphorbiaceae) commonly known as “Sehund or thohar” in Hindi, is found throughout the Deccan Peninsula of India and grows luxuriously around the dry, hilly, rocky areas of North, Central and South India. Ayurveda describes the plant as bitter, pungent, laxative, carminative, improves appetite useful in abdominal

troubles, bronchitis, tumors, loss of consciousness, delirium, leucoderma, piles, inflammation, enlargement of spleen, anaemia, ulcers and fever (Hernández et al., 2003; Chellaiah et al., 2006). As far as our literature survey could ascertain, no information was available on the hepatoprotective activities of the stem of *E. neriifolia*. Therefore, the aim of this current investigation was to explore the hepatoprotective potential of methanol extract of stem of *E. neriifolia* against paracetamol induced hepatotoxicity in rats.

Paracetamol (acetaminophen) is a widely used antipyretic and analgesic which produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates (Jollow et al., 1974; Wong et al., 1981). However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450 to a highly reactive metabolite *N*-acetyl-*P*-benzoquinone imine (NAPQI) (Savides and Oehne, 1983; Vermeulen et al., 1992). NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid (Moore et al., 1985). However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or SH group of protein and alters the homeostasis of calcium after depleting GSH. Silymarin is marketed as one of the standard hepatoprotective herbal formulation.

MATERIALS AND METHODS

Plant material

The stem of *Euphorbia neriifolia* was collected from the rural region of Midnapore, West Bengal, India. The plant was authenticated by the Botanical Survey of India (BSI), Shibpur (W.B), India. Air dried whole stem (500 g) were powdered in a mechanical grinder and the powdered materials was extracted by methanol using Soxhlet extraction apparatus. The solvent was completely removed under reduced pressure in a rotary vacuum evaporator. The concentrated extract (yield 35.42%) was stored in vacuum desiccators for further use.

Animals:

Healthy Wistar albino male rats (180 g $\pm$ 20) were used for the present study. They were maintained at standard laboratory conditions and fed with commercial pellet diet and water ad libitum. The experiments were performed following the animal ethics guidelines of Institutional Animals Ethics Committee.

Acute toxicity study:

Healthy rats were starved overnight and then were divided into five groups (n=4). Group I-IV animals were orally fed with MEEN in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 g/kg b.wt, while group V (untreated) served as control. The animals were observed continuously for first 2 h for any gross change in behavioral, neurological and autonomic profiles or any other symptoms of toxicity and mortality if any, and intermittently for the next 6 h and then again after 24 h, 48 h and 72 h for any lethality or death. One-tenth and one-fifth of the maximum safe dose of the extract tested for acute toxicity were selected for the experiment (OECD, 2008).

Experimental designs

Wistar albino rats were divided into five groups (n=6). Group I served as saline control (0.9% w/v sodium chloride, orally). Group II-V received paracetamol suspension (640 mg/kg suspended in 1% methyl cellulose; orally) once and group II served as paracetamol control. After administration of paracetamol suspension, group III and IV received MEEN 200 and 400 (mg/kg body weight; orally) respectively daily for 16 days. Group V received standard drug silymarin (25 mg/kg body weight; orally) daily for 16 days. After 24 h of last dose and 18 h of fasting all animals were sacrificed by cervical dislocation. The blood and liver were collected for biochemical estimation, antioxidant assay and histopathological observation.

Biochemical Estimation

Serum glutamine oxaloacetate transaminase (SGOT), serum glutamine pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), total protein and total bilirubin content were estimated by using commercially available kits from Span Diagnostic Ltd., Surat, India.

Antioxidant estimation

For assessment of antioxidant activities, immediately after collection of blood the rats were sacrificed and livers were dissected out and washed in ice cold normal saline, blotted dry and weighed. Required quantity of the tissue was weighed and 25% (w/v) of each tissue homogenate was then prepared using KCl solution (1.15% w/v) and

centrifuged at 3000 g for 1 hr. The supernatant was used for the determination of lipid peroxidation (LPO) (Ohkawa et al., 1979) and endogenous antioxidant systems such as reduced glutathione (GSH) (Ellman, 1959);, superoxide dismutase (SOD) (Kakkar et al., 1984) and catalase (Aebi, 1974).

Histological observation

Liver is dissected out and the liver samples were excised from the experimental animals of each group and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin and then with bovine solution. They were processed for paraffin embedding following the microtome technique. The sections were taken at 50 μ thickness processed in alcohol-xylene series and were stained with alum-haematoxylin and eosin. The sections were examined microscopically for the evaluation of histopathological changes.

Statistical Analysis

Values were presented as mean $\pm$ S.E.M. Data were statistically evaluated by one way analysis of variance (ANOVA) followed by post hoc Dunnett's test using SPSS software. *P*-values of <0.001 were considered as statistically significant.

RESULTS

In acute toxicity study, MEEN did not show any mortality or toxic effect up to the dose of 2 g/kg body weight, accordingly 200 and 400 mg/kg body weight were taken as low and high dose of MEEN for the experiment.

Administration of paracetamol to the animals resulted in a marked elevation of serum transaminases (SGOT and SGPT), serum alkaline phosphatase (ALP) and total bilirubin (TB), when compared with those of normal control animals. However serum total protein level was decreased. The rats treated with MEEN and with silymarin showed a significant decrease ($P<0.001$) in all the elevated serum marker levels, SGOT, SGPT, ALP and TB, and significant increase ($P<0.001$) in total protein (Table 1) which showed the restoration of the level of liver function biochemistry to the near normal values.

Toxic dose of paracetamol significantly reduced the activities of enzymes (SOD and catalase) and non-enzymatic (GSH) antioxidant system and enhanced lipid peroxidation (LPO) level of liver tissue, as were found in group II animals. MEEN treatment significantly raised both of the enzymatic and non-enzymatic antioxidant systems as was found in case of silymarin treated group, while the elevated LPO level were found to be reduced back to/towards the normal level in MEEN as well as silymarin treated rats (Table 2).

Table1: Effect of MEEN on serum enzyme levels, total bilirubin and total protein of paracetamol intoxicated rats

Treatments	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/100 ml)	Total protein (mg/dL)
Normal control	54.23 $\pm$ 1.26	24.0 $\pm$ 1.84	9.51 $\pm$ 1.12	1.10 $\pm$ 0.19	7.16 $\pm$ 0.70
Paracetamol (640 mg/kg)	138.87 $\pm$ 10.1 [#]	125 $\pm$ 9.44 [#]	44.66 $\pm$ 4.02 [#]	3.12 $\pm$ 0.20 [#]	4.21 $\pm$ 0.38 [#]
MEEN 200mg/kg	117.23 $\pm$ 2.52 [*]	101 $\pm$ 4.62 [*]	35.41 $\pm$ 2.55 [*]	2.81 $\pm$ 0.25 [*]	4.85 $\pm$ 0.42 [*]
MEEN 400 mg/kg	85.93 $\pm$ 4.11 [†]	74.1 $\pm$ 4.36 [†]	26.22 $\pm$ 2.53 [†]	2.11 $\pm$ 0.09 [†]	5.23 $\pm$ 0.56 [†]
Silymarin (25 mg/kg)	58.65 $\pm$ 1.66 [†]	31.2 $\pm$ 2.12 [†]	14.24 $\pm$ 1.21 [†]	1.32 $\pm$ 0.16 [†]	6.86 $\pm$ 0.64 [†]

Table 2. Effect of MEEN on LPO, GSH and CAT levels of paracetamol intoxicated rats

Treatments	LPO (MDA, nano m/mg)	GSH (μ g/mg wet tissue)	CAT (μ M of H ₂ O ₂ decomposed/min/mg wet tissue)	SOD (U/mg wet tissue)
Normal control	20.78 $\pm$ 0.75	5.21 $\pm$ 0.29	1.04 $\pm$ 0.04	10.6 $\pm$ 0.9.6
Paracetamol (640 mg/kg)	99.31 $\pm$ 5.59 [#]	2.06 $\pm$ 0.08 [#]	0.47 $\pm$ 0.025 [#]	6.4 $\pm$ 0.62 [#]
MEEN 200 mg/kg	78.16 $\pm$ 5.58 [*]	2.59 $\pm$ 0.33 [*]	0.51 $\pm$ 0.02 [*]	7.1 $\pm$ 0.56
MEEN 400 mg/kg	64.55 $\pm$ 2.76 [*]	3.44 $\pm$ 0.31 [*]	0.65 $\pm$ 0.01 [*]	7.8 $\pm$ 0.69
Silymarin 25 mg/kg	51.23 $\pm$ 3.03 [†]	5.01 $\pm$ 0.21 [†]	0.96 $\pm$ 0.01 [†]	10.3 $\pm$ 0.92 [†]

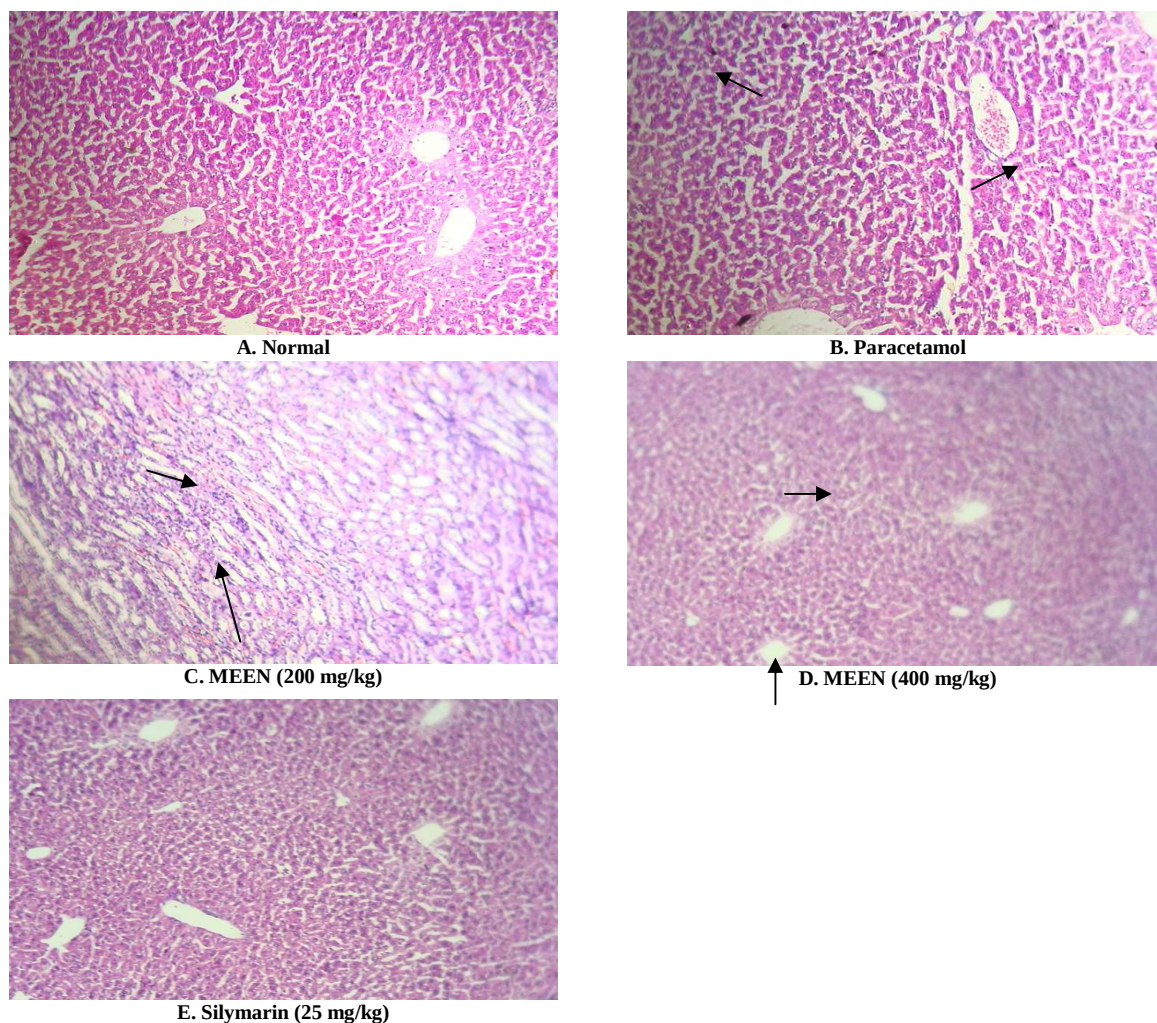


Fig.1. Liver section A; normal control), B; Liver section of Paracetamol-induced rat showing large necrosis, C; Liver section of MEEN (200 mg/kg) treated rat, showing reduction in necrosis, D; Liver section of MEEN (400 mg/kg) treated rat showing sign of recovery and E; Liver section of standard silymarin (25 mg/kg) treated rats showing sign of recovering.

Histopathological examination of liver sections of normal control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Fig.1A). Disarrangement of normal hepatocytes with centrilobular necrosis, vacuolization of cytoplasm and fatty changes were observed in paracetamol intoxicated rat livers (Fig.1B). The liver sections of the rats treated with MEEN low and high dose (Fig.1. C and D) and silymarin (Fig.1. E) showed a sign of protection against paracetamol intoxication as evident by presence of normal hepatic cords and absence of necrosis with minimal inflammatory conditions around the central vein.

DISCUSSION

Liver is largest organ and it is target for toxicity because of its role in clearing and metabolizing chemicals through the process called detoxification. Drug induced liver disorders occurred frequently can be life threatening and mimic all forms of liver diseases (Watkins and Seef, 2006). Paracetamol being a drug capable of causing liver disorders if overdoses are consumed. The covalent binding of N-acetyl-P benzoquinoneimine, an oxidation product of paracetamol, to sulphhydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity have been reported earlier (Boyd and Berczky, 1966).

Estimating the activities of serum marker enzymes, like SGOT, SGPT and ALP can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol, are

released in to the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage (Mitra et al., 1998). The enhanced levels of these serum marker enzymes observed in paracetamol treated rats in our study correspond to the extensive liver damage induced by the toxin. Restoration of the levels of these enzymes to/towards near normal values in the MEEN, low and high dose and silymarin treated animals is a clear manifestation of anti-hepatotoxic effect of the extract and the drug. Increase in serum bilirubin in group II animals reflected the depth of jaundice, which was attenuated to the normal level in MEEN treated groups, indicating its hepatoprotective effect further. The lowered level of total protein recorded in the serum of paracetamol intoxicated rats reveals the severity of hepatopathy, while the attainment of near normalcy in total protein content of serum of the treated groups potentiates the hepatoprotective effect.

The antioxidant activity or the inhibition of the generation of free radical is important in the protection against paracetamol induced liver lesion, as because, one of the principal causes of paracetamol induced liver injury is formation of lipid peroxides by free radical (Gosselin et al., 1984; Recknagel et al., 1989). The body has an effective defense mechanism, consisting of a set of endogenous antioxidant enzymes including SOD, catalase as well as non-enzymic antioxidants, such as reduced glutathione. In paracetamol intoxicated hepatotoxicity, the balance between ROS production and these antioxidant defenses may be lost, consequently oxidative stress may result which finally may lead to hepatic necrosis (Venkumar and Latha, 2002). MEEN treatment showed significant improvement in the level of these antioxidant systems over those in paracetamol control animals, and this clearly indicates the antioxidant activity of MEEN. The level of lipid peroxide is a measure of membrane damage and alteration in structure and function of cellular membranes. Elevation of MDA level in liver indicates excessive free radical generation and consequently enhanced lipid peroxidation which leads to severe tissue damage (Shenoy et al., 2001), treatment with MEEN significantly reversed these changes.

Histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxin intoxication. In the sections obtained from the rats treated with extract or silymarin and intoxicated with hepatotoxin, the normal cellular architecture was disturbed by hepatotoxin intoxication. In the sections obtained from the rats treated with extract or silymarin and intoxicated with hepatotoxin, the normal cellular architecture was retained as compared to those of the normal control rats, thereby confirming the protective effect of the extract or drug and thus the observation substantiates other results of the experiment.

CONCLUSION

Hence the present study clearly indicates the potent hepatoprotective activity of *E. neliifolia* stem parts against paracetamol induced hepatic damage, which may be due to its antioxidant activity and free radical scavenging property. On preliminary phytochemical screening, MEEN has been found to possess flavonoids, polyphenolics, steroids, and triterpenes. Works are in progress to reveal the details of the mechanism of its potent hepatoprotection as well as to isolate and purify the bioactive principle(s) from the methanol extract of *E. neriifolia* stem parts.

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