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ISSN: 2321-2624
Research Article

International Journal of Medicine and Pharmaceutical Research
www.pharmaresearchlibrary.com/ijmpr
IJMPR, 2013: Vol.1(4): 342-347

Potential Hepatoprotective Activity from Extracts of *Mimusps Elengi* Linn

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Available online 5 October 2013

Abstract
Modern therapeutic alternatives for the hepatoprotection are providing symptomatic control and offering many side effects to the liver which in turn can make damage to liver more pronounced. The drugs in the modern medicine are not able to provide protection to the liver in multifacet activity as by antioxidant activity, hepatocyte regenerative activity etc. The Indian Traditional Medicine is predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. The present study was aimed to evaluate the potential of Methanolic extract of bark of *Mimusops elengi* in in-vivo hepatoprotective activity.
Key words: Mimusops elengi, hepatoprotective, CCl_4, hepatoprotection, detoxification

Introduction

Liver is an important organ actively involved in many metabolic functions and is the frequent target for a number of toxicants [Meyer et al., 2001]. Hepatic damage is associated with distortion of these metabolic functions [Wolf, 1999]. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects [Guntupalli et al., 2006]. Modern therapeutic alternatives for the hepatoprotective activity are providing symptomatic control and offering many side effects to the liver which in turn can make damage to liver more pronounced. The drugs in the modern medicine are not able to provide protection to the liver in multifacet activity as by antioxidant activity, hepatocyte regenerative activity etc. These are some of the limitations of the modern therapeutics to solve the problem of hepatotoxicity. Since time immemorial, mankind has made the use of plants in the treatment of various ailments. The Indian Traditional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases. One of the important and well-documented uses of plant-products is their use as hepatoprotective agents. Hence, there is an ever in-creasing need for safe hepatoprotective agent [Babu et al., 2001].
Mimusops elengi, [ME] commonly called 'Bakul' is a medicinally important plant of family sapotaceae. All parts of the tree have medicinal properties. *Mimusops elengi*, commonly called as 'Bakul', is a medicinal plant belonging to family Sapotaceae. It has many medicinal properties as antiulcer, wound healing, antioxidant etc. ME is reported in Ayurveda for providing the hepatoprotection. ME is enriched with saponins and rich source to antioxidants such as tannins etc **[**Chopra et al., 2000**]**.The present study was aimed to evaluate the potential of Methanolic extract of bark of ME [MEP1] in in-vivo hepatoprotective activity.

Materials and Methods

Collection and Authentication of Plant Material

Mimusops elengi bark was collected during May and June from Rajgurunagar, Pune District, Nagpur and Mumbai [Maharashtra State, India]. The plants was identified and authenticated by Botanical Survey of India and a voucher specimen was deposited at Botanical Survey of India.

Preparation of Methanolic Extract of Mimusops Elengi [MEP1]

The stem barks of *Mimusops elengi* collected from different regions [MEP, MEM, MEN for ME collected from Pune, Mumbai and Nagpur respectively] were shade-dried and powdered in a grinder. The powdered material [100 g] was extracted with methanol using soxhlet extraction at 40°C. The extracts were dried on a tray dryer at 40°C [yield – 10.2, 11 and 10.91 % w/w respectively for MEP, MEM and MEN].The extracts were denoted as MEP1, MEM1 and MEN1 respectively. Animals: Wistar rats of either sex rats weighing between 130 to 145 gm were used in this evaluation. Animals were housed in well ventilated stainless-steel cages at room temperature [24±2°C] in hygienic condition under natural light and dark schedule and were fed on standard laboratory diet. Food and water were given ad libitum.

Experimental Design for Evaluation of Potential Hepatoprotective Activity of MEP1 in CCL4 Induced Hepatotoxicity in Rats [CHATERRJEE,T.K.,2000]:

The rats were divided randomly into five groups of six rats each. Group I [normal control] received neither the plant extract nor CCl_4 for 72 hours that is they receive only food and water only; Group II [induction control] was given a single intraperitoneal dose of CCl4 [3ml/kg] [17]. Group III-V received crude MEP1 respectively at an oral dose of 100, 200 and 400 mg/kg b. Wt

Experimental Design for Evaluation of Potential Hepatoprotective Activity of MEP1 in Paracetamol Induced Hepatotoxicity in Rats [**CHATTOPADHYAY ET AL., 2003**]**:**

Animals were divided into five groups of 6 animals each. The first group received saline 1 ml/kg for one week [control]. The group II received saline 1 ml/kg for one week [positive control]. The groups III, IV and V received crude MEP1 respectively at an oral dose of 100, 200 and 400 mg/kg b. wt respectively once a day for seven days. On the fifth day, after the administration of the respective treatments, all the animals of groups II, III, IV and V were administered with paracetamol 2 g/kg orally. On the seventh day after 2 h of respective treatments the blood samples were collected for the estimation of biochemical marker enzymes. Then animals under ether anesthesia were sacrificed. The livers from all the animals were collected, washed and used for the estimation of tissue GSH levels.

Assessment of Hepatoprotective Activity:

In the present study the hepatoprotective activity was evaluated biochemically and histopathologically. After 72 hours of drug treatment, the animals were dissected under ether paraffin embedding using the standard microtechnique anesthesia. Blood from each rat was withdrawn from [Alan L and Miller ND, 1996]. Sections [5 µm] of livers stained with hemotoxylin carotid artery at the neck and collected in previously and eosin, were observed microscopically for labeled centrifuging tubes and allowed to clot for histopathological studies. 30 min at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 minurtes. Following biochemical analysis were done- The collected blood samples were used for the analysis of biochemical markers SGPT [Bradley DW. Et al., 1972], SGOT [Rej R et al., 1973], ALP [MacComb RB and Bower GN., 1972], biluribin, [Pearlman PC and Lee RT., 1974] cholesterol [Allain CC., 1974], and HDL [Burstein M. et al., 1970] levels. The tissue GSH was evaluated according to Ayake et al. [Aykae G et al., 1985].

Results and Discussion

The present study had been attempted to demonstrate the role of hepatoproctective activity of crude methanol extracts of ME in carbon tetrachloride induced hepatotoxicity at different doses. ME collected from different regions from the Maharashtra had screened for the phytochemical investigations for the chemical constituents. It was observed that all the three specimen contained saponin, flavonoids, tannins and alkaloids. Specimen collected from Rajgurunagar Pune had depicted high amount of saponins and tannins than that of remaining ones.All the three speciments were subjected to extraction by methanol. All the extracts were screened for potential hepatoprotective activity in CCl4 induced toxicity in rats [Data not shown]. Methanolic extract of ME collected from Rajgurunagar Pune MEP1 was found to be showing highest activity and hence selected for further screening using paracetamol induced liver injury in rats. Liver damage induced by CCl 4 is commonly used model for the screening of hepatoprotective drugs [Ma. F., 1991].The CCl_4 is converted into reactive metabolite, halogenated free radical by hepatic cytochrome P450s [Yusuf, M.et al., 1994] which in turn covalently binds to cell membrane and organelles to elicit lipid peroxidation with subsequent tissueinjury.

The measurement of lipid peroxide is also a marker of hepatocellular damage [Journal of Research in Indian Medicine, 1970; Fleurentin, J. et al., 1946]. The methanolic extract of aerial parts of Plumbago zeylanica administered prophylactically exhibited significant protection against CCl_4 -induced liver injury as manifested by the reduction in toxin mediated rise in serum level of SGPT, SGOT, ALP and total bilirubin in rats.The results of hepatoprotective activities of crude methanol extracts of ME at a dose of 100, 200 and 400 mg/kg on rats intoxicated with carbon tetrachloride were illustrated in the Fig. 1 and 2. MEP1 exhibited dose dependent activity in restoring elevated levels of biochemical parameters. Serum SGPT, SGOT and cholesterol levels were normalized by the

treatment [p<0.05] but dose of 400 mg/kg showed highest restoration of elevated enzymatic parameters. The qualitative phytochemical investigation on MEP1 indicated presence of the polyphenols, tannins and alkaloids. MEP1 has significant hepatoprotective activity. This may be probably due to the higher content of the triterpenes, tannins and flavonoids. Hence further work is necessary to elucidate the constituent responsible for hepatoprotective activity along with their mechanism of action.

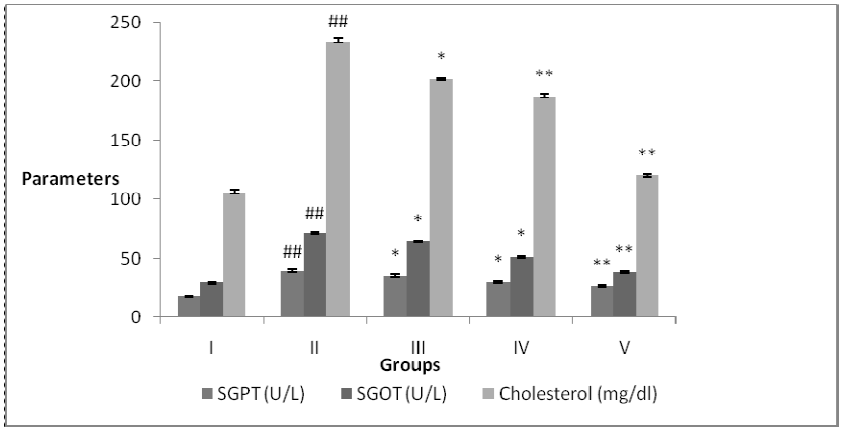


Fig. 1: Effect of MEP1 in CCl_4 induced liver injury

Values are mean±S.E.M., *n* = 6 in each group; statistical analysis by one-way ANOVA followed by *post hoc* Dunett test using Graphpad Instat software; *p* value *<0.05, **<0.01 compared to Group II and ##<0.01 when compared to Group I

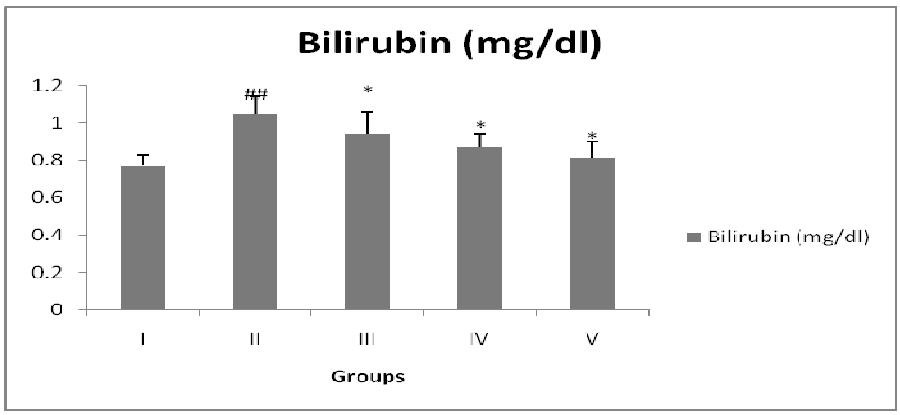


Fig. 2: Effect of MEP1 in CCl4 induced liver injury

Values are mean±S.E.M., *n* = 6 in each group; statistical analysis by one-way ANOVA followed by *post hoc* Dunett test using Graphpad Instat software; *p* value *<0.05, **<0.01 compared to Group II and ##<0.01 when compared to Group I

Paracetamol is normally eliminated mainly as sulfate and glucoronide. Only 5% of the paracetamol is converted into N-acetyl-p-benzoquineimine. However, upon administration of toxic doses of paracetamol the sulfation and glucoronidation routes become saturated and hence, higher percentage of paracetamol molecules are oxidized to highly reactive N-acetyl-p-benzoquineimine [NAPQI] by cytochrome-450 enzymes. Semiquinone radicals, obtained by one electron reduction of NAPQI, can covalently binds to macromolecules of cellular membrane and increase the lipid peroxidation resulting in the tissue damage. Higher dose of paracetamol and NAPQI can alkylate and oxidize intracellular GSH and protein thiol group, which results in the depletion of liver GSH pool subsequently leads to increased lipid peroxidation and liver damage [Diadelis R. et al., 1994]. In our experiments it is observed that tissue GSH levels in the paracetamol group is decreased which clearly indicates that there is a significant hepatic damage due to paracetamol. This is further evident from the fact that there is elevation in the levels of various biochemical markers of hepatic damage like SGPT, SGOT, bilirubin, and cholesterol.

Paracetamol has enhanced the levels of SGPT, SGOT, bilirubin [both total and direct bilirubin levels], Alkaline phosphatase level [ALP], total cholesterol, whereas HDL and tissue GSH levels are decreased significantly.

Treatment with MEP1 has significantly brought down the elevated levels of SGPT, SGOT, ALP, bilirubin, cholesterol. Results are reported in Fig. 3 and 4. Treatment with MEP1 has restored levels of above mentioned biochemical markers to the near healthy levels in the dose dependent manner. The treatment has also demonstrated the reduced hepatic damage or improvement in the hepatic architecture [Fig. 5]. Glutathione [GSH] is one of the most abundant naturally occurring tripeptide, non-enzymatic biological antioxidant present in liver [Alan L and Miller ND, 1996]. Its functions are concerned with the removal of free radicals such as H_2O_2 and superoxide radicals, maintenance of membrane protein, detoxification of foreign chemicals and biotransformation of drugs 30. In the present study,the decreased level of GSH has been associated with an enhanced level of lipid peroxidation in Paracetamol intoxicated groups of rats.

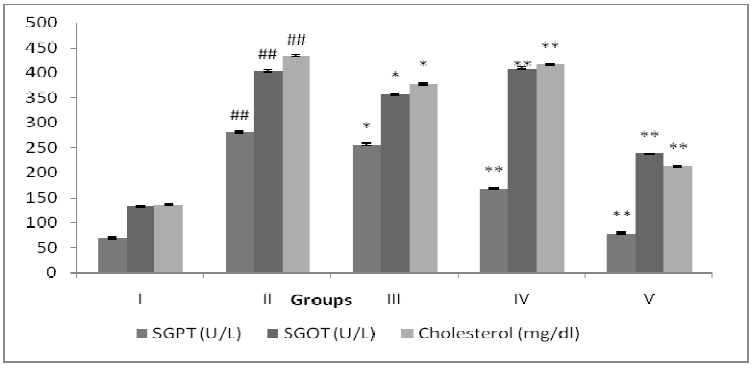


Fig. 3: Effect of MEP1 in paracetamol induced hepatic injury

Values are mean±S.E.M., *n* = 6 in each group; statistical analysis by one-way ANOVA followed by *post hoc* Dunett test using Graphpad Instat software; *p* value *<0.05, **<0.01 compared to Group II and ##<0.01 when compared to Group I

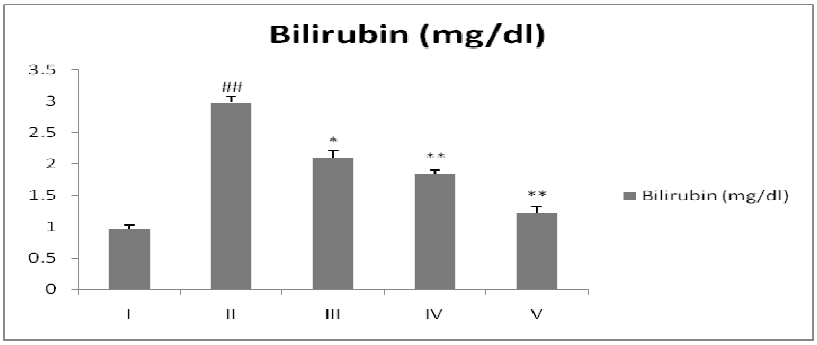
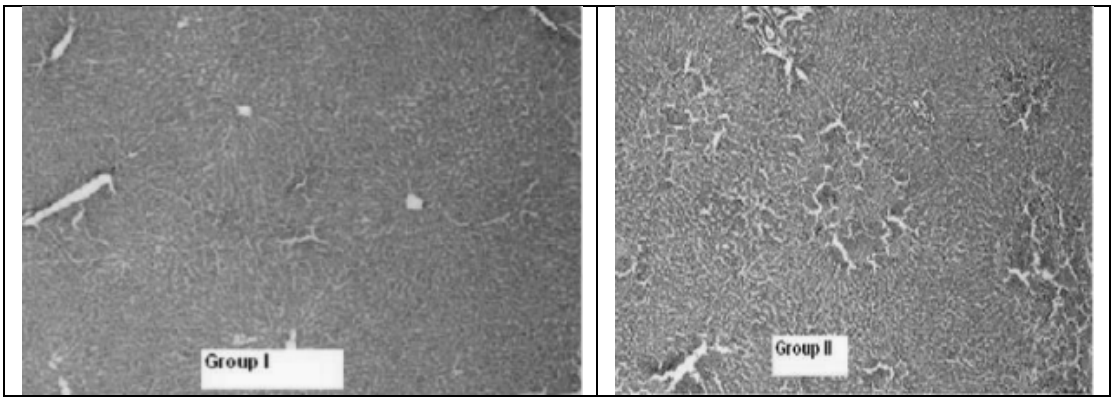


Fig. 4: Effect of MEP1 in paracetamol induced hepatic injury

Values are mean±S.E.M., *n* = 6 in each group; statistical analysis by one-way ANOVA followed by *post hoc* Dunett test using Graphpad Instat software; *p* value *<0.05, **<0.01 compared to Group II and ##<0.01 when compared to Group I



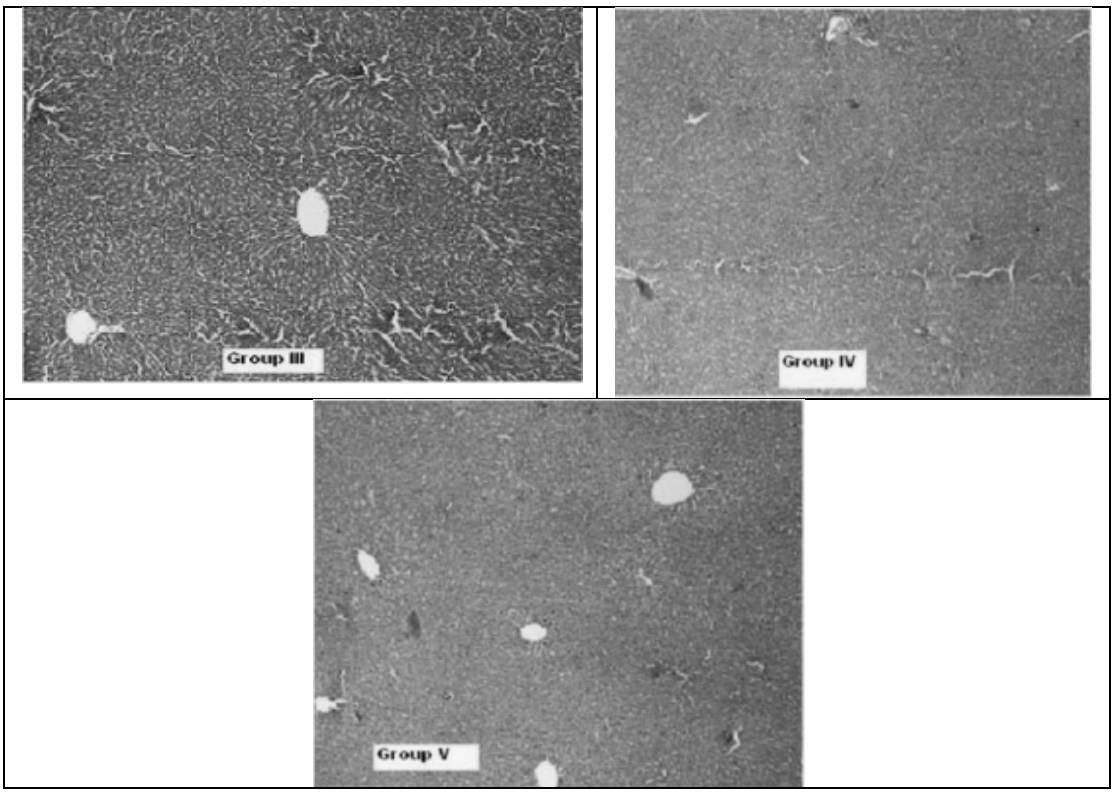


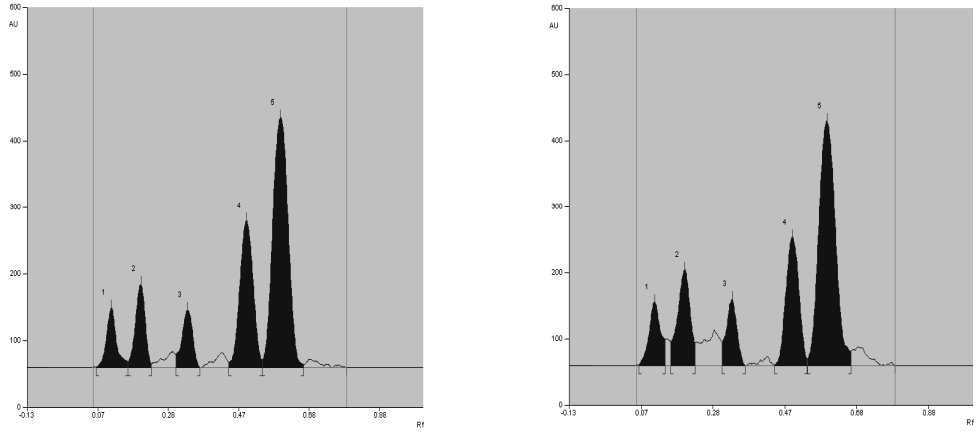
Fig. 5: Effect of MEP1 on histology of liver in paracetamol induced injury in rats

Changes of HE staining in liver tissue of mice 24 h after paracetamol administration [× 100] in control group with a normal central lobular region [Group I], model group with some central lobular hepatocyte necrosis and microvesicular fatty change [Group II], low TP dose group [Group III], medium TP dose group [Group IV] and high TP dose group [Group V]. The pathological change of liver was much milder in different TP dose groups than in model group.

Group	Liver GSH [µ M/g]
I	14.12±0.704
II	7.91±0.385 a
III	9.05±0.401 ns
IV	10.97±0.321 ***
V	12.41±0.615***

Table 1: Effect of MEP1 on liver GSH levels in paracetamol induced hepatic injury

All values are expressed as MEAN ± SEM, one way analysis of variance [ANOVA] followed by Tukey's Kramer post hock analysis. a $p<0.001$ Vs. vehicle control [Group I]. ns $p>0.05$, *** $p<0.001$ Vs. Paracetamol control [Group II].



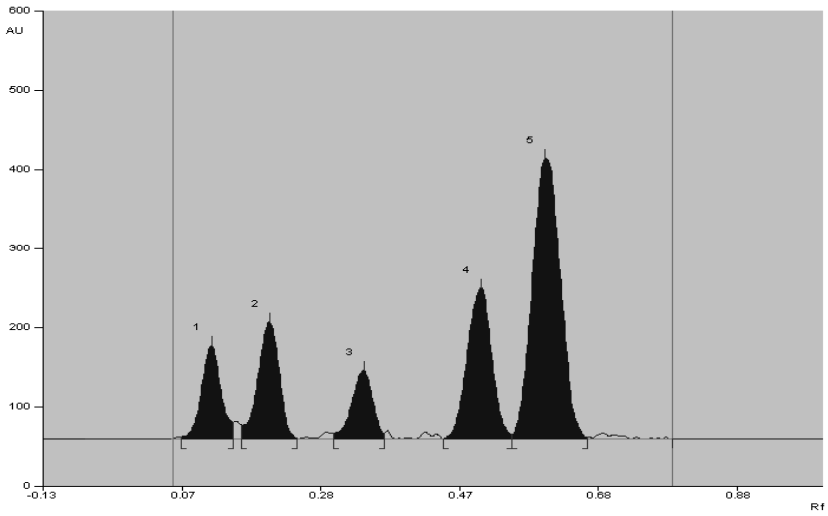


Fig. 6: Identification of extracts using HPTLC

Mobile phase [Methanol: Water: formic acid - 10:5:3] Saturation time 30 minutes HPTLC fingerprinting pattern was developed for Identification of extracts. Treatment with MEP1 increased the level of glutathione in a dose dependent manner [Table 1]. In the present study, it was observed that pre-treatment with MEP1 caused a significant rise in hepatic GSH and which may improve the activities of the liver antioxidant enzymes, thus protecting the liver from Paracetamol. It may be concluded that the hepatoprotective effect of MEP1 is due to the prevention of the depletion in the tissue GSH levels. Upon literature review it is found that the MEP1 possess antioxidant activity possibly by the virtue of the polyphenols present in the extract. Therefore there is a possibility that the antioxidant property of MEP1 may be involved in the hepatoprotectiveproperty [Alan L and Miller ND, 1996].

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