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Hepatoprotective Efficacy of *Swietenia Mahagoni* L. Jacq. (Meliaceae) Bark against Paracetamol-induced Hepatic Damage in Rats

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ABSTRACT

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In present study, the defatted methanol extract of *Swietenia mahagoni* bark (MESM) was evaluated for its protective effect on paracetamol-induced liver damage in Wistar rats. Liver histopathology, serum biochemical parameters viz. serum glutamine oxaloacetate transaminase (SGOT), serum glutamine pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), total serum protein, total bilirubin content; and liver biochemical parameters such as thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) content, superoxide dismutase (SOD), catalase (CAT) were estimated. Biochemical and histopathological observations indicated that MESM exerted remarkable hepatoprotective efficacy against paracetamol-induced hepatic damage in rats that is plausibly attributable to its augmenting endogenous antioxidant mechanisms.

KEY WORDS: *Lipid peroxidation, Glutathione, Biochemical, Silymarin.*

INTRODUCTION

Liver, the key organ of metabolism and excretion is an important organ for detoxification of xenobiotics, environmental toxicants and liver damage is associated with distortion of several metabolic functions; hence liver diseases are of serious health problem. In the absence of reliable liver protective drugs in allopathic medical practices, naturally occurring compounds have been found to have major role in the management of various liver diseases. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional systems of medicine in India. However a satisfactory remedy for serious liver diseases is not still available, so search for effective hepatoprotective drugs are continued.

Swietenia mahagoni L. Jacq. (Meliaceae), commonly called as *Mehgoni* in *Bengali* and West Indian Mehgoni in English, is a large semi-evergreen economically important timber tree native to southern Florida, Cuba, Bahamas, Hispaniola, and Jamaica and cultivated in tropical regions, such as India, Malaysia, and Southern China[1,2]. In India, traditionally it is used for several medicinal purposes. The seeds and bark are used for the treatment of hypertension, diabetes, malaria, and epilepsy as a folk medicine in Indonesia and India[3,4]. The bark is considered an astringent and is taken orally as a decoction for diarrhea, as a source of vitamins and iron, and as haemostyptic. The bark serves as antipyretic and tonic[5]. Traditionally the bark decoction is used orally to increase appetite, to restore strength in cases of tuberculosis, to treat anaemia, diarrhea, dysentery, fever and toothache[6]. The leaf decoction is used against nerve disorders, the seed infusion against chest pain and a leaf or root poultice against bleeding[7].The authors have noticed that local people of East Medinipur, (West Bengal), Balasore (Orissa) traditionally use the aqueous extract of its seed and bark for curing psoriasis, diabetes, diarrhea and also used as an antiseptic in cuts and wounds.

Previous workers reported its bark for its inhibitory activity against HIV-1 protease[8]. Antidiabetic activity was reported for its seeds[9]. Previously the authors have reported antidiabetic, sedative and anticonvulsant effects of its bark[10,11]. For any drug, evaluation of possible hepatotoxicity should be of paramount importance. As there are no reports of *S. mehagoni* bark's effects on liver, present investigation was aimed to evaluate the hepatoprotective potential of *S. mahagoni* bark against paracetamol-induced hepatic damage in Wistar rats in pursuit of newer liver protectants.

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MATERIALS AND METHODS

Plant material: The stem bark of *S. mahagoni* was collected during November 2008 by incision and peeling from the mature plants grown in the hill region of West Mednipur, West Bengal, India. The species was authenticated by Dr. M. S. Mondal, Botanical Survey of India, Kolkata, India and the voucher specimen (PMU-3/JU/2007) has been preserved at Pharmacology Research Laboratory, Department of Pharmaceutical Technology, Jadavpur University for future reference. Just after collection the bark was washed thoroughly with running tap water, cut into small pieces, shade dried at room temperature (24-26^{0}C) and ground mechanically into a coarse powder.

Preparation of extract: The powdered plant material (750 g) was extracted successively with petroleum ether and 80 % aqueous methanol in Soxhlet extraction apparatus. The methanol extract was filtered and evaporated to dryness *in vacuo* (at 35 ºC and 0.8 MPa) in a Buchi evaporator, R-114. The dry extract (MESM, yield 11.85 %) was kept in a vacuum desiccator until use. Preliminary phytochemical analysis revealed the presence of mainly tannins, flavonoids and triterpenoids in MESM[12].

Drugs and chemicals: Bovine serum albumin: Sigma Chemical Co., St. Louis, USA; Trichloroacetic acid (TCA): Merck Ltd. Mumbai, India; Thiobarbituric acid (TBA), Nitroblue tetrazolium chloride (NBT): Loba Chemie, Mumbai, India; 5,5'-dithio *bis*-2-nitro benzoic acid (DTNB), Phenazonium methosulphate (PMS), Nicotinamide adenine dinucleotide (NADH) and reduced glutathione (GSH): SISCO Research Laboratory, Mumbai, India. Silymarin: Ranboxy Laboratories, Indore, India. All the other reagents used were of analytical reagent grade obtained commercially.

Animals: Adult male Wistar albino rats weighing 170-200 g were used for the present investigation. They were housed in clean polyacrylic cages (38×23×10 cm) with not more than four animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2ºC, relative humidity 55-65%, with dark/light cycle 12/12 h). They were allowed free access to standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experiment. All experimental procedures described were reviewed and approved by the University Animal Ethical Committee, Jadavpur University (Reg. no. 367001/ C/CPCSEA).

Acute toxicity: The acute oral toxicity of MESM in male Swiss albino mice was determined as per reported method[13].

Treatment schedule: The rats were divided into five groups (n = 8). A single dose of 640 mg/kg paracetamol in 1 % methyl cellulose was administered orally to each animals in group II, III, IV and V. After administration of paracetamol suspension, MESM was administered orally (p. o.) at the doses of 25 and 50 mg/kg body weight (b. w.) to groups III and IV respectively daily for 16 days. Group V received reference drug silymarin (25 mg/kg b.w; p. o.) daily for 16 days[14]. Group I served as normal (vehicle) control and group II served as paracetamol control and received normal saline (5 ml/kg b.w., p. o.) for 16 days. After 24 h of last dose, blood was collected from overnight fasted rats of each group by cardiac puncture, for estimation of serum biochemical parameters. Then the rats were sacrificed by cervical dislocation for the study of liver biochemical and histopathological parameters.

Serum biochemical estimations: Serum glutamine oxaloacetate transaminase (SGOT), serum glutamine pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP) and total bilirubin content were estimated by using commercially available kits (Span Diagnostic Ltd., Surat, India). Serum total protein was estimated according to the reported method[15].

Liver biochemical estimations: The levels of lipid peroxidation i. e. thiobarbituric acid reactive substances (TBARS) in the liver tissue were measured as per reported method[16]. The levels of lipid peroxides were expressed as µmoles of malondialdehyde (MDA)/g of liver tissue. The reduced glutathione (GSH) content of liver tissue was determined as per reported method and expressed as µg/g of liver tissue[17]. The superoxide dismutase (SOD) and catalase (CAT) activity in liver tissue were assayed as per the methods described previously[18,19]. The SOD activity was expressed as unit/mg of liver tissue and CAT was expressed in terms of µmol of hydrogen peroxide decomposed/min/mg of liver tissue.

Histopathological study: For histopathological study the fresh liver tissues were collected and immediately fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections (4-5 µm) were prepared and then stained with hematoxylin-eosin dye for photomicroscopic observations.

Statistical analysis: All results were expressed as the mean ± standard error of mean (SEM). The results were analyzed for statistical significance by one-way ANOVA followed by Dunnett's *post hoc* test of significance. $P < 0.05$ was considered as statistically significant.

RESULTS

Acute toxicity: The oral LD_{50} value of the MESM in male Swiss mice was 200 mg/kg body weight.

Serum biochemical parameters: Biochemical parameters like SGOT, SGPT, SALP and serum bilirubin in the paracetamol control group were significantly ($p < 0.001$) elevated as compared to the normal saline group. Treatment with MESM at the dose of 25 and 50 mg/kg significantly ($p < 0.001$) reduced the SGOT, SGPT, SALP and serum bilirubin levels towards the normal values in a dose dependent manner. The total protein was found to be significantly decreased in the paracetamol control group as compared with the normal saline group ($p < 0.001$). Administration of MESM at the dose of 25 mg/kg ($p < 0.05$) and 50 mg/kg ($p < 0.001$) in paracetamol-intoxicated rats significantly increased the total protein content as compared with the paracetamol control (Table 1).

Liver biochemical parameters: The levels of TBARS represented as MDA were significantly ($p < 0.001$) increased in paracetamol control animals as compared to normal control group. Treatment with MESM at 25 and 50 mg/kg significantly ($p < 0.001$) reduced the MDA levels when compared with paracetamol control animals (Table 2). The level of reduced GSH was significantly depleted in paracetamol control group ($p < 0.001$) as compared with normal control group. Reduced GSH level was found to be significantly elevated towards normal level on administration of MESM at 25 ($p < 0.05$) and 50 mg/kg ($p < 0.001$) as compared with paracetamol control group (Table 2). There were significant ($p< 0.001$) reduction in superoxide dismutase and catalase activities in paracetamol control groups compared with normal control group. Administration of MESM at 25 ($p < 0.05$) and 50 mg/kg significantly ($p < 0.001$) recovered SOD and CAT activities towards normal values when compared with paracetamol control animals (Table 3).

Histopathological study: Histopathological study of livers of saline control group showed normal hepatocellular architecture (Fig.1). Livers challenged with paracetamol showed disarrangement of normal hepatic cells with massive interlobular necrosis, inflammatory infiltration of lymphocytes and fatty changes (Fig. 2). The MESM (50 mg/kg) treated rats exhibited significant protection against

Table.1. Effect of MESM on serum biochemical parameters of normal and paracetamol-intoxicated rats.

Treatment	SGOT (IU/L)	SGPT (IU/L)	SALP (IU/L)	Total bilirubin (mg/dL)	Total protein (mg/dL)
Normal saline (5 ml/kg)	24.96 ± 3.22	20.66 ± 5.20	9.82 ± 0.73	0.52 ± 0.07	7.8 ± 0.23
PCM control (640 mg/kg)	85.55 ± 2.37 ¶	84.66± 10.72¶	22.46± 3.10 ¶	2.04± 0.34 ¶	4.93± 0.35 ¶
PCM (640 mg/kg) + MESM (25 mg/kg)	44.49± 2.49**	40.66± 5.45**	14.28± 0.26**	0.67± 0.11**	5.73 ± 0.41*
PCM (640 mg/kg) + MESM (50 mg/kg) .	38.54± 1.55**	33.33± 2.40**	11.42± 0.08**	0.63± 0.13**	6.74 ± 0.17**
PCM (640mg/kg) + Silymarin (25 mg/kg)	31.62± 1.51**	34.66± 5.81**	11.53± 0.44**	0.59± 0.15**	7.53 ± 0.29**

Values are mean ± SEM (n = 8), PCM: Paracetamol. PCM control group vs. normal saline group, ¶ $p < 0.001$. Treated groups vs. PCM control group, ** $p< 0.001$; * $p < 0.05$ where the significance was performed by one-way analysis of variance (ANOVA) followed by Dunnett ' s test.

Table.2. Effect of MESM on MDA and GSH levels in normal and paracetamol-intoxicated rats.

Treatment	MDA (µM/g wet liver tissue)	GSH (µg/g wet liver tissue)
Normal saline (5 ml/kg)	40.42 ± 3.29	23.87 ± 1.72
PCM control (640 mg/kg)	92.65 ± 1.90 ¶	11.80 ± 0.69 ¶
PCM (640 mg/kg) + MESM (25 mg/kg)	58.95 ± 3.83**	16.47 ± 1.29*
PCM (640 mg/kg) + MESM (50 mg/kg)	43.95 ± 1.04**	20.97 ± 1.74**
PCM (640 mg/kg) + Silymarin (25mg/kg)	39.37 ± 2.58**	22.55 ± 3.01**

Values are mean ± SEM (n = 8), PCM: Paracetamol. PCM control group vs. normal saline group, ¶ $p < 0.001$.Treated groups vs. PCM control group,* $p < 0.05$, ** $p < 0.001$, where the significance was performed by one-way analysis of variance (ANOVA) followed by Dunnett ' s test.

Table 3. Effect of MESM on superoxide dismutase (SOD) and catalase (CAT) activities in normal and paracetamol-intoxicated rats.

Treatment	SOD (IU/mg of wet liver tissue)	CAT (µM of H 2 O 2 decomposed/min/mg
Normal saline (5 ml/kg)	7.93 ± 0.27	2.8 ± 0.14
PCM control (640 mg/kg)	3.74 ± 0.20 ¶	0.97 ± 0.20 ¶
PCM (640 mg/kg) + MESM (25 mg/kg)	5.22 ± 0.34*	1.64 ± 0.21*
PCM (640 mg/kg) + MESM (50 mg/kg)	6.76 ± 0.52**	3.07 ± 0.29**
PCM (640 mg/kg) + Silymarin (25 mg/kg)	6.49 ± 0.68**	2.21 ± 0.42**

Values are mean ± SEM (n = 8), PCM: Paracetamol. PCM control group vs. normal saline group, ¶p < 0.001.Treated groups vs. PCM control group,*p < 0.05, **p < 0.001, where the significance was performed by one-way analysis of variance (ANOVA) followed by Dunnett ' s test.

Fig.1. Liver section of normal control rat

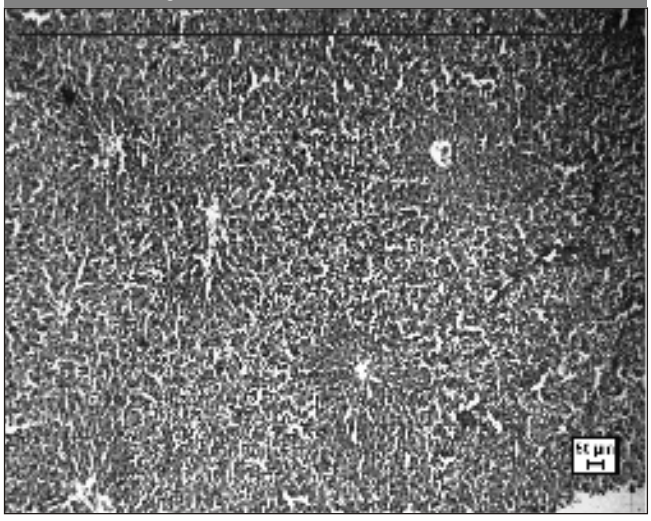
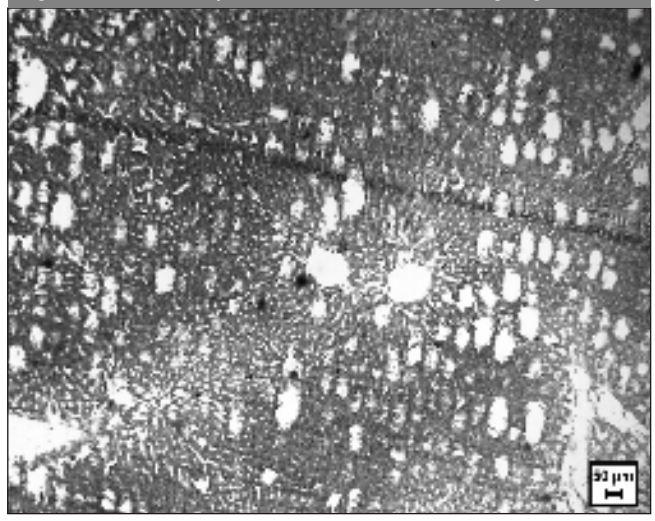


Fig.2. Liver section of paracetamol control rat showing large necrosis.



paracetamol intoxication as evident by presence of normal hepatic cords and absence of necrosis with minimal inflammatory conditions around the central vein (Fig. 4). However, moderate protection was observed in case of low dose (25 mg/kg) group animals (Fig.3).

DISCUSSION

Paracetamol is a widely used antipyretic and analgesic drug which is safe in therapeutic doses but can cause fatal hepatic damage in human and animals at higher toxic doses[20]. Bioactivation of paracetamol by hepatic cytochrome P-450 leads to formation of a highly reactive and toxic metabolite N-acetyl-*p*-benzoquinone imine (NAPQI). NAPQI is normally detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid which is excreted in urine. Toxic overdose of paracetamol depletes hepatic reduced glutathione (GSH) content so that free NAPQI binds covalently to cellular macromolecules causing acute hepatocellular necrosis. The NAPQI then causes acylation or oxidation of cytosolic and membrane proteins and generation of reactive oxygen species (ROS). This leads to further oxidation of protein thiols, lipid peroxidation, DNA fragmentation and ultimately cell necrosis[21,22,23].

It has been well established that elevated levels of SGOT, SGPT and SALP are indicative of cellular leakage and loss of functional integrity of the hepatic cell membranes implying hepatocellular damage. Serum total protein and bilirubin levels on the other hand are related to the function of the hepatic cells revealing the functional status of the hepatic cell[14]. The MESM decreased the elevated serum enzyme and bilirubin levels with elevation of total protein content in the paracetamol treated rats which are comparable to the normal control group. It appears that the MESM preserved the structural integrity of the hepatocellular membrane which is evident from the significant reduction in paracetamol-induced rise in serum marker enzymes in rats. It was further supported by the histopathological studies showing recovery of hepatocellular lesions by MESM in a dose related manner.

Glutathione is the endogenous non-enzymatic antioxidant in

Fig.3. Liver section of MESM (25 mg/kg) treated rat, showing reduction in necrosis

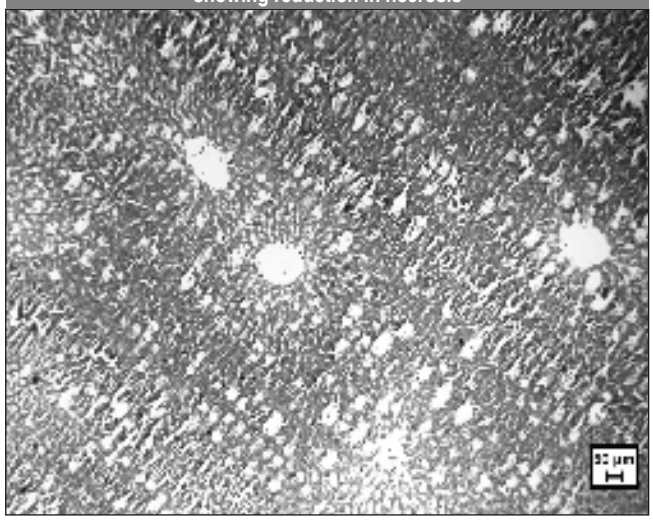
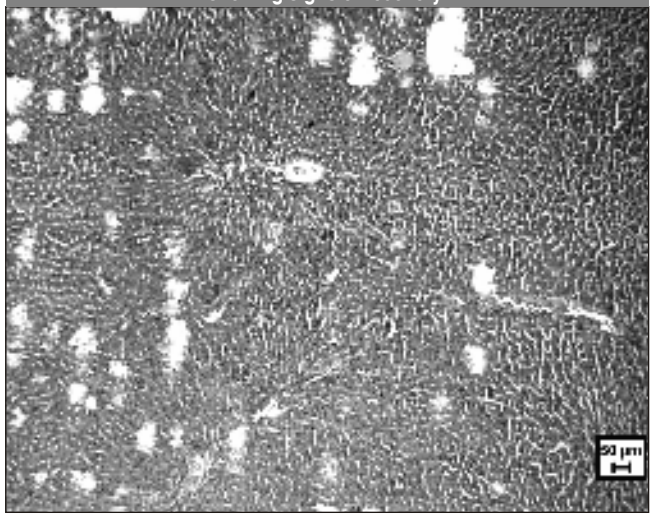


Fig.4. Liver section of MESM (50 mg/kg) treated rat, showing signs of recovery.



our body system and it is protective against chemically induced hepatic damage and oxidative stress[24]. Depleted GSH level with elevated level of lipid peroxidation in paracetamol-induced rats indicated that the experimental dose of paracetamol 640 mg/kg is highly hepatotoxic. It was confirmed from present study that the MESM dose dependently and significantly restored hepatic GSH content towards normal in paracetamol intoxicated rats indicating decreased free NAPQI level in the blood.

Lipid peroxidation is a phenomenon involved in peroxidative loss at unsaturated lipids, thus bringing about cellular lipid degradation and membrane disordering. Elevated lipid perixodation causes tissue injury and damage to cellular macromolecules by generation of reactive oxygen species (ROS) increasing the risk of tissue damage. Lipid peroxidation is usually measured through its catabolite malondialdehyde (MDA) as a marker of oxidative stress[25]. MESM showed ability to prevent paracetamol induced elevation of MDA level, suggesting that MESM inhibited hepatic lipid peroxidation in paracetamol intoxicated rats. This implies the reduction in free radical production and subsequent decrease in damage to the hepatocellular membranes.

Superoxide dismutase (SOD) and catalase (CAT) are endogenous enzymatic antioxidants present in all oxygen metabolizing cells involved in the clearance of superoxide and hydrogen peroxide. The suppression of SOD and CAT activities as a result of liver damage was reported[26]. Similar findings were observed in our present results in paracetamol treated mice. The administration of MESM significantly recovered the SOD and CAT activities towards normal in a dose dependent manner.

Preliminary phytochemical analysis of MESM indicated the presence of flavonoids and tannins and these types of polyphenols are well known natural antioxidants due to their electron donating property which either scavenge the principal propagating free radicals or halt the radical chain[27]. Thus MESM, because of the presence of natural antioxidants, must have exerted protective action against paracetamol-induced hepatic damage, plausibly by increasing the hepatic reduced glutathione content, which would protect the tissue from NAPQI and free radicals, by modulating hepatic lipid peroxidation, by augmenting the activities of cellular antioxidant enzymes viz. SOD and CAT thereby ameliorating the extent of oxidative stress mediated cellular damage caused by paracetamol.

CONCLUSION

From present study it can be inferred that the methanol extract of *S. mahagoni* bark dose dependently offered potential hepatoprotection against paracetamol-induced hepatic damage, normalizing biochemical parameters and liver histology in Wistar rats plausibly by modulating lipid peroxidation and augmenting endogenous non-enzymatic and enzymatic antioxidant defense systems.

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