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Research Article

Experimental Evaluation of the Hepatoprotective Effect of *Butea monosperma* Extract on Antitubercular Drugs Induced Hepatotoxicity

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ABSTRACT: Context: Haphazard use of drugs is one of the key reason for progression of liver diseases. Drugs such as paracetamol, isoniazid, rifampicin etc cause hepatotoxicity. However, there is currently no single synthetic drug which is effective for the treatment of such conditions. Drugs from natural sources have been used by humans from eternal era. Thus, plants serve to be an important source to explore hepatoprotectives. **Objective:** The current study was designed to assess the hepatoprotective activity of *Butea monosperma* extract. **Materials and methods:** Leaves of *B. monosperma* were dried in shade, powdered and extracted with ethanol and phytochemical screening was performed. The extract phenolic and flavonoids contents were estimated. The extract was also subjected to acute toxicity studies as per OECD guidelines. Hepatoprotective studies were performed using isoniazid- rifampicin induced hepatotoxicity in rats. **Results:** Results of the phytochemical tests and phytoanalytical studies demonstrated that the extract was rich in flavonoids, glycosides and polyphenolics. The extract also demonstrated excellent hepatoprotective activity against isoniazid- rifampicin induced hepatotoxicity in rats. **Discussion and conclusion:** Results of study demonstrate that ethanol extract of *B. monosperma* is potent source of phytochemicals that are responsible to demonstrate hepatoprotective activity.

KEYWORDS: liver, *Butea monosperma*, hepatoprotective, SGPT, SGOT, ALP.

INTRODUCTION

The liver plays an important role in metabolizing chemical entities and reducing their toxicities. Overdose of certain drugs causes detrimental effect on the liver causing its malfunctioning, necrosis and atrophy. Some of these agents cause severe destruction of the mitochondria. Activation of cytochromal enzymes causes oxidative stress.[1] Synthetic drugs including paracetamol, aspirin, rifampicin and isoniazid cause detrimental effect on liver,[2,3] leading to malfunctioning of liver or hepatotoxicity.

Butea monosperma (Fabaceae) or 'Palash' grows widely in all parts of Indian subcontinent. The plant finds multifold use in folk remedies. Leaves, flower, seeds of the tree are known for their anthelmintic, laxative, appetizer and aphrodiasic.[4]

Various important active constituents of B. monosperma are butin, butein, butrin, isobutrin, palestrina, coreopsis and isocoreopsin, chalcones, and aurones along with phenolic compounds.[5]The plant has shown protection against hepatic carcinogenesis and oxidative damage[6] and defensive role against thioacetamide-mediated hepatic alterations in experimental animals.[7]

In view to this, the current work was aimed to determine hepatoprotective potential of *B.monosperma*.

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MATERIALS AND METHODS

Plant material

Butea monosperma leaves were collected from local forest of Jabalpur and authenticated by Dr. A.B. Tiwari, Department of Crop and Herbal Physiology, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur (M.P.) (specimen

voucher no. HD/CHPY/988). Leaves were collected in the month of November and dried in shade.Leaves were coarsely powdered and used for preparation of extract.

Chemicals and Drugs

Isoniazid, rifampicin and silymarin, were purchased from CDH, Mumbai, India. All the chemicals used were of analytical grade.

Extraction

An amount of 100 g of powdered plant material was extracted with ethanol by Soxhlet extraction for 36 hrs. The extraction procedure was repeated thrice in order to have optimum extraction. The extract was filtered using Whatman filter paper and the solvent was removed under reduced pressure using a rotary vacuum-evaporator concentrated at 40 ± 5°C. The dried extract was stored at 4°C until use.

Phytochemical and phytoanalytical studies

Phytochemical screening of ethanol extract was performed by standard methods.[8] Total flavonoid content[9] and total phenolic content[10] were also determined.

Animals

Healthy Albino wistar rats (100-150 g, 8-9 month) of either sex were used. The animals were housed in groups of 5 per cage (standard plastic cages) with 12/12 h light and dark cycle in Institutional animal house prior to pharmacological studies. The animals were fed with a standard diet and provided water *ad libitum*. After one week of acclimatization the animals were used for further experiments. All animals were fasted overnight before testing ; tap water was supplied *ad libitum*. All the protocols were approved by Institutional Animal Ethics Committee.

Acute Toxicity Studies

The acute toxicity of ethanol extract was determined in albino Wistar rats. The animals were fasted overnight prior to the experiment and treated with fixed doses as per OECD guideline No. 423.[11] The ethanolextract of *B. monosperma* was devoid of mortality at doses of 2000 mg/kg in rats. The animals were observed continuously for 2 hrs after administration for their behavioral, neurological and autonomic profile. No death was observed at the highest dose. Therefore doses of 200 mg/kg and 400 mg/kg were used for experimental hepatoprotective studies.

Hepatoprotective studies

Isoniazid (50 mg/kg, p.o.) and rifampicin (100 mg/kg, p.o.) were dissolved in distilled water (1 ml/kg) and the pH was adjusted to 3.0 with 0.1 N HCl to give clear solution.[12] Animals were divided into 5 groups of 5 animals in each group and were subjected to treatment for the further *in vivo* hepatoprotective studies as outlined below.

Group A: Normal control (saline solution; 1ml po)

Group B: Toxicant (Isoniazid 50 mg/kg + Rifampicin 100 mg/kg po)

Group C: Toxicant + Silymarin 50 mg/kg po

Group D: Toxicant + Ethanol extract of *B.monosperma* 200 mg/kg po

Group E: Toxicant + Ethanol extract of *B.monosperma* 400 mg/kg po

The vehicle or drug treatments were carried out orally from day 1 to 9 with concurrent administration of isoniazid and rifampicin. During the period of drug treatment, the rats were maintained on anormal diet and water was supplied *ad libitum*. On day 10, blood was collected by cardiac puncture under mild ether anesthesia The serum was separated by centrifugation at 3000 rpm for 15 min, and subjected to estimation of various biochemical parameters including serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (ALP), serum direct bilirubin, serum total bilirubin, cholesterol and HDL. The livers were kept in 10 % formalin solution for *Histopathological* studies. Photographs of liver were examined carefully on the surface for gross visible hepatic necrosis from all the treated groups.

Statistical analysis

Data were expressed as Mean ± SEM (n=5). Hepatoprotective activity was analyzed statistically using one-way analysis of variance (ANOVA) test. Level of significance were set at* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

RESULTS

Phytochemical and phytoanalytical studies

Phytochemical studies of *B.monosperma* extract demonstrated the presence of glycosides, alkaloids, flavonoids, phenolics and carbohydrate. The extract was found to be rich in flavonoids [188.54 ± 3.96 mg QE (Quercetin Equivalent)/100g] and phenolics [598.12 ± 4.06 mg PE (Pyrocatechol Equivalent) /100g].

Hepatoprotective studies

In the present work, restoration in the level of enzymes viz. SGPT, SGOT and ALT in group D and E animals demonstrates progressive improvement in oxidative

stress (Figure 1). Total and direct bilirubin was significantly less (p<0.05; p<0.01) in group D and E when compared to C group (p<0.001), indicating a protective effect of the extract on elimination of bile pigments (Figure 2). Use of isoniazid and rifampicin during the experimental protocol period caused devastating effect on the biological markers bilirubin (Figure 2) and cholesterol (Figure 3). In this study, significant (p < 0.05, p < 0.01) decrease in levels of cholesterol and HDL extract treated group was observed which is comparable to C group (p<0.001) (Figure 3). Along with this, significant reduction in lipid peroxidation (p< 0.01) (Figure 4) and restoration of glutathione (p< 0.01) (Figure 5) levels were observed.

Histopathology of liver of normal control rat demonstrated healthy anatomical features (Figure 6). Isoniazid-rifampicin treated rat's liver showed the presence of inflammatory collections. The extract treated groups showed no necrosis and the presence of only minimal inflammatory surroundings along with normal liver architecture. This demonstrates hepatoprotective action by the extract. Thus, gross *Histopathological* scrutiny demonstrated a protective effect of *B.monosperma* extract on the structural anatomy of liver.

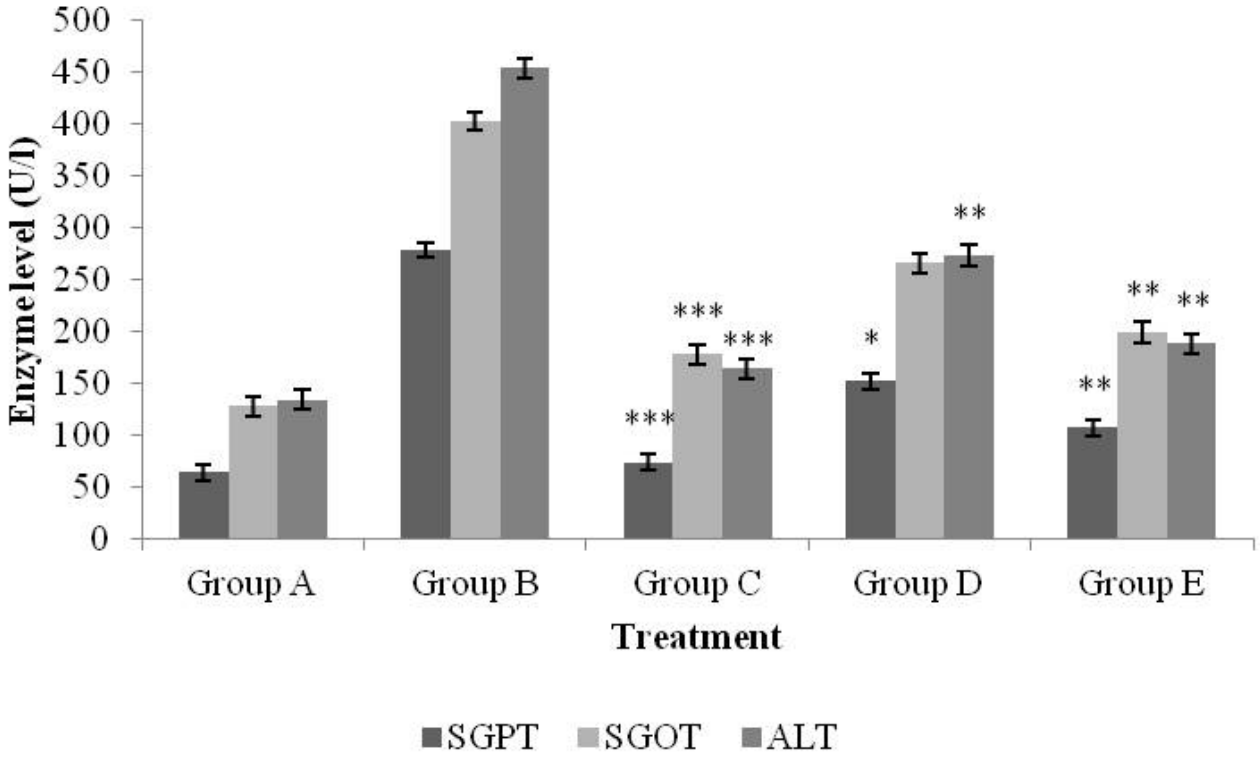

Figure 1. Effect of ethyl acetate extract of *B. monosperma*on SGPT, SGOT and ALP in isoniazid- rifampicin treated rats. Results are given as mean ± SEM of five animals in each group. Drug treated group compared with rest the extract treated groups.Significance at *p < 0.05; **p< 0.01; ***p < 0.001.

DISCUSSION

The current work was designed to evaluate possible hepatoprotective effect of an ethanol extract of *B. monosperma* in isoniazid-rifampicin induced hepatotoxicity. Isoniazid and rifampicin are two antitubercular agents used in the treatment of tuberculosis. Metabolism of isoniazid produces hydrazine/acetyl-hydrazine, which play a key role in hepatic damage.[13] When used in combination with rifampicin, isoniazid causes reduced biliary secretion along with increment in lipid peroxidation and damage to hepatocytes. Thus, this deadly synergistic combination results in enhanced and persistent liver damage.[14]

Bilirubin and serum ALP are closely associated with the functioning of liver cells. Increased level of serum ALP

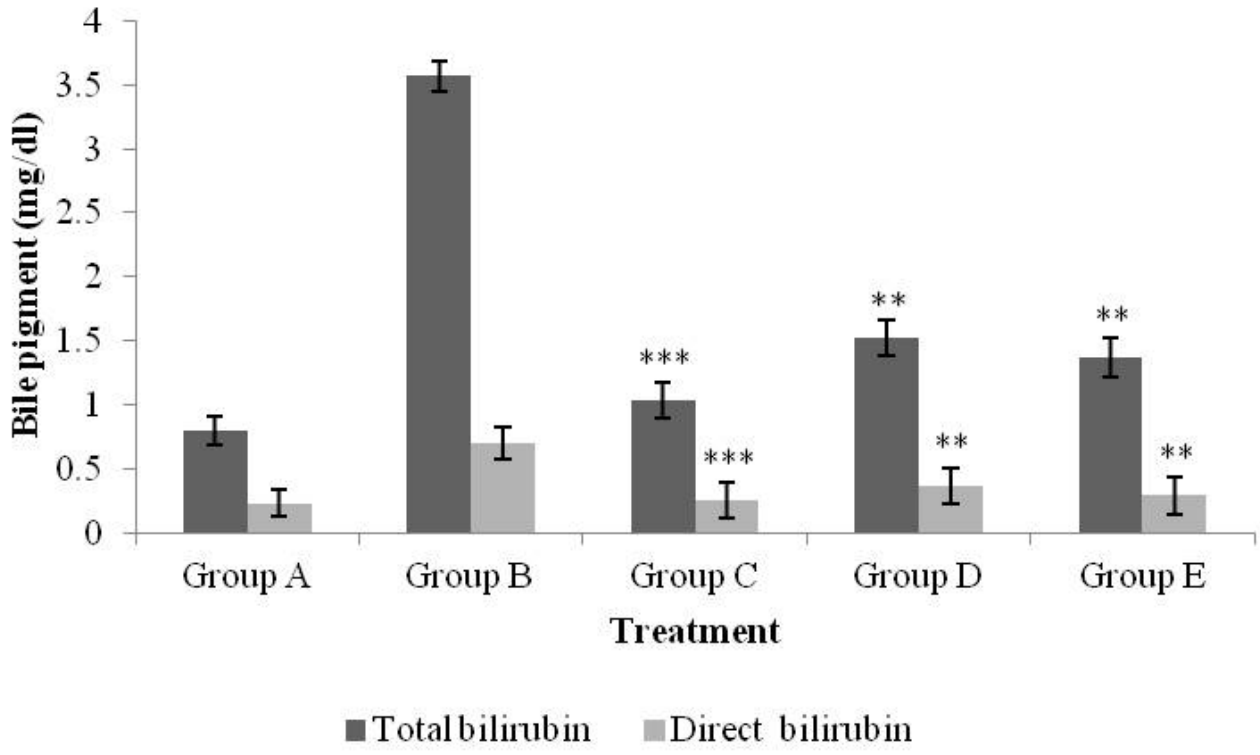

Figure 2. Effect of ethyl acetate extract of *B. monosperma* on total and direct bilirubin in isoniazid- rifampicin treated rats. Results are given as mean ± SEM of five animals in each group. Drug treated group compared with rest the extract treated groups.Significance at $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

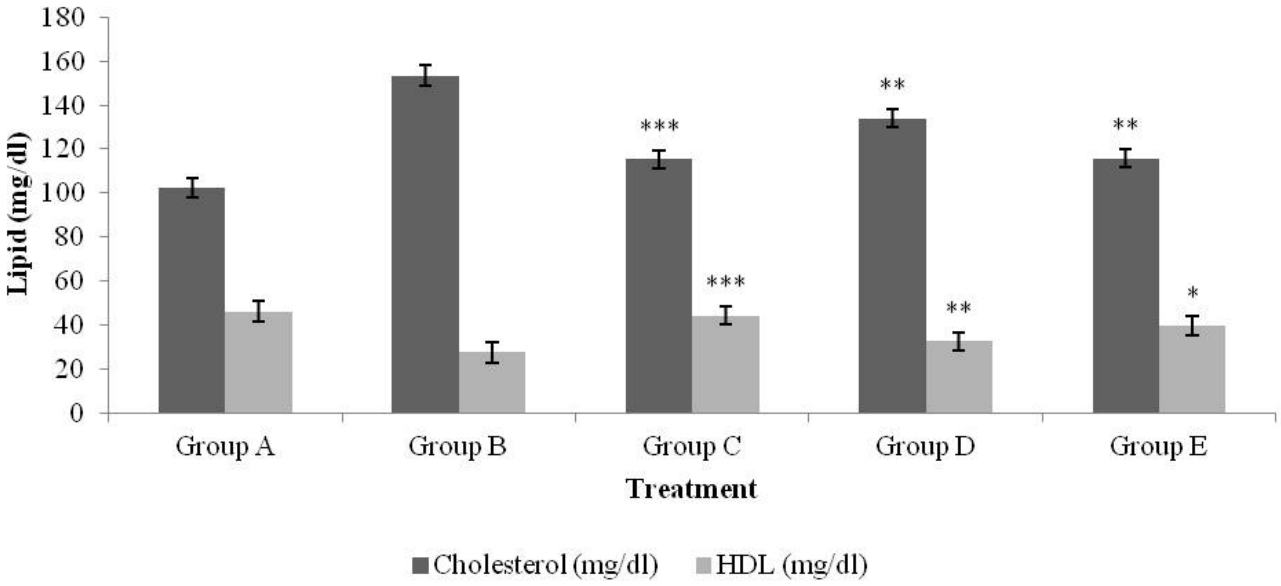

Figure 3. Effect of ethyl acetate extract of *B. monosperma* cholesterol and HDL in isoniazid- rifampicin treated rats. Results are given as mean ± SEM of five animals in each group. Drug treated group compared with rest the extract treated groups. Significance at $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

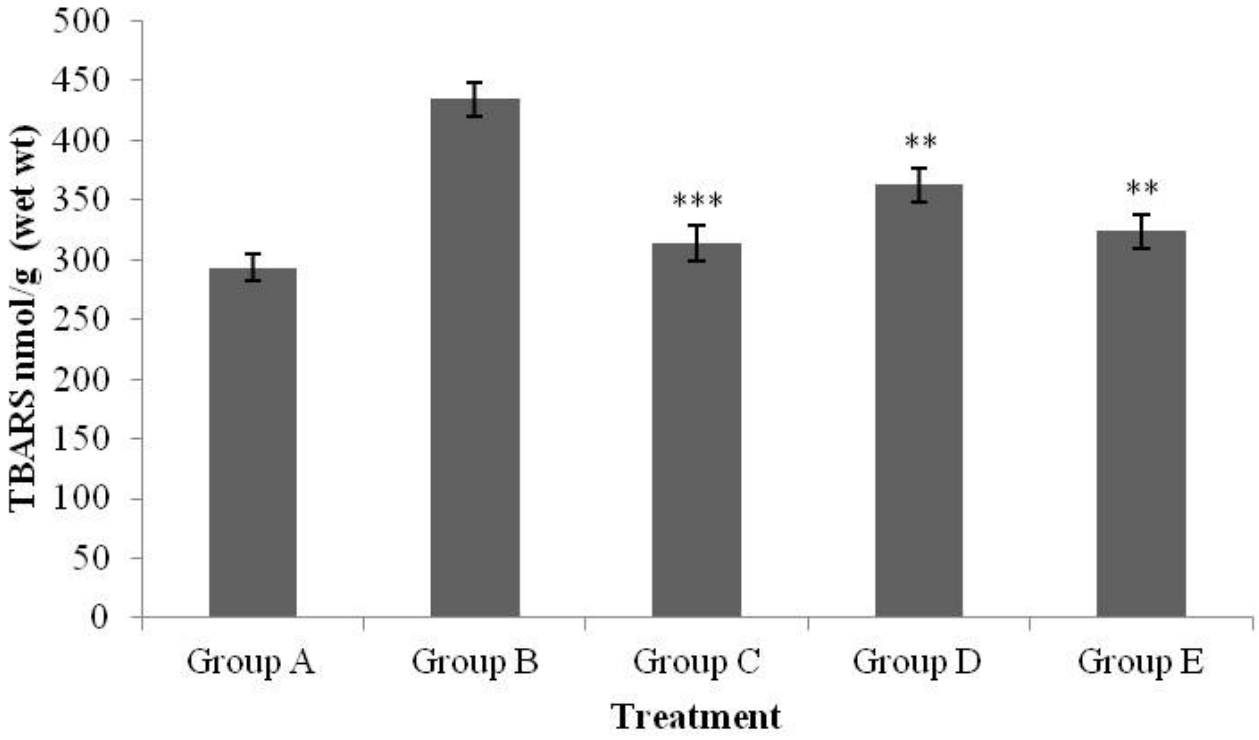

Figure 4. Effect of ethyl acetate extract of *B. monosperma* on TBARS in isoniazid- rifampicin treated rats. Results are given as mean ± SEM of five animals in each group. Drug treated group compared with rest the extract treated groups. Significance at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

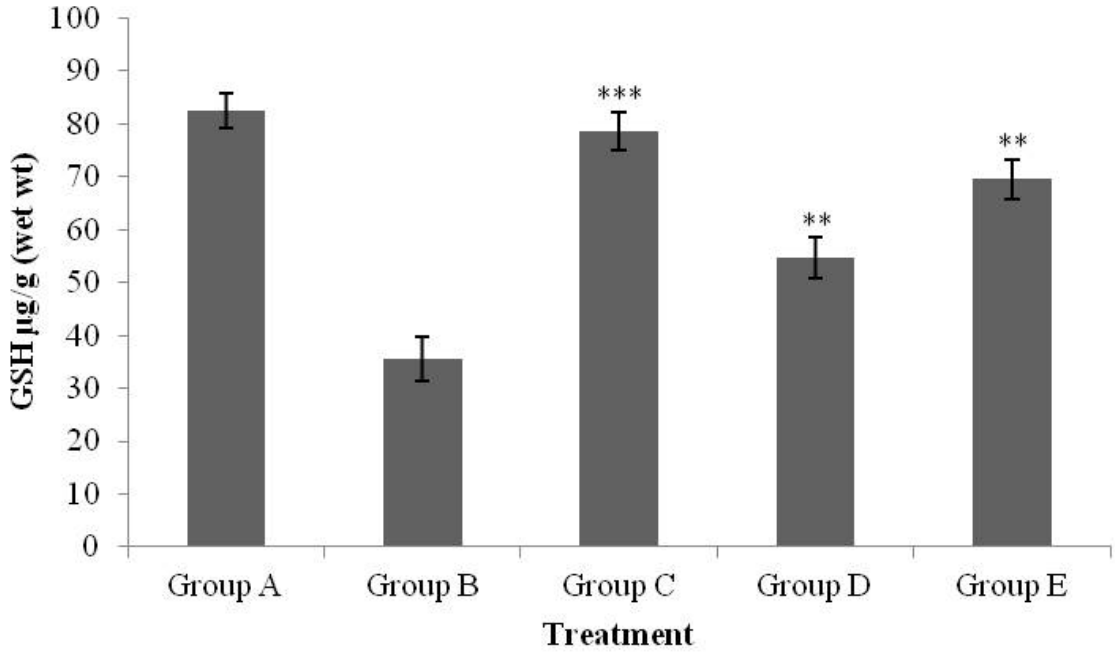

Figure 5. Effect of ethyl acetate extract of *B. monosperma* on GSH in isoniazid- rifampicin treated rats. Results are given as mean ± SEM of five animals in each group. Drug treated group compared with rest the extract treated groups. Significance at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

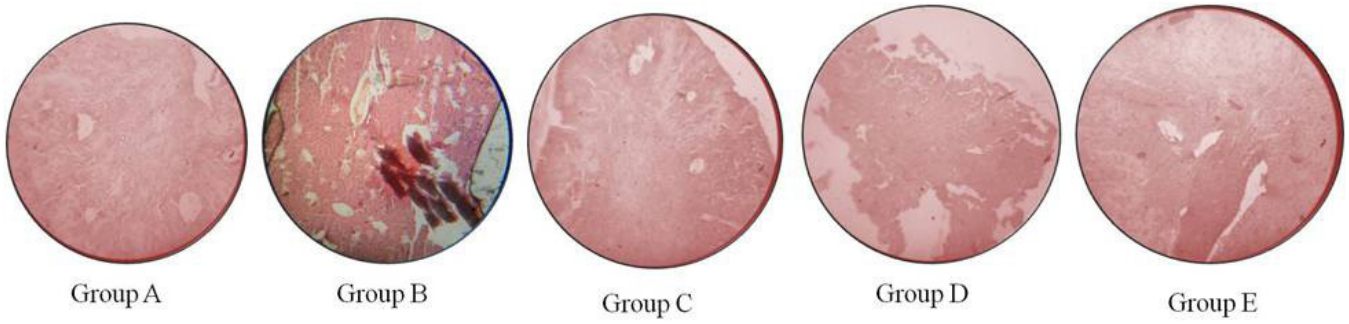

Figure 6. Evidence for the protective effect of *B. monosperma* in rats treated with isoniazid- rifampicin, (a) Control (b) Toxicant (c) Silymarin (d) Extract treated 200 mg/kg, po (e) Extract treated 400 mg/kg, po.

and bilirubin is indicative of enhanced biliary pressure.[15] Elevated levels of SGPT and SGOT are indicative of liver damage.[16] Treatment of rats with *B.monosperma* extract restored enzymes to their respective levels which indicate protective effect of the herb. Cholesterol levels were also restored in animals treated with *B. monosperma* extract.

Lipid peroxidation is observed during destruction of cellular integrity as in case of liver injury.[17] In the current studies, increases in the levels of TBARS was observed in animals only treated with Isoniazid-rifampicin. Such an effect is indicative of increase in lipid peroxidation and failure of antioxidant system leading to formation of excessive free radicals. Administration of the *B. monosperma* extract significantly decreased the production of TBARS, which justifies restoration of cellular integrity in the livers of animals treated with *B. monosperma* extract.[17]

Glutathione is one of the defensive antioxidants that is present in liver. It is associated with removal of free radicals like H_2O_2, O_2^-, and CH_3O^- and also helps to maintain membrane protein thiols.[18] in the current work, isoniazid and rifampicin caused a reduction in the level of this protective antioxidant. On the other hand, administration of *B. monosperma* extract significantly elevated glutathione levels in a dose dependent manner.

The liver controls a number of metabolic functions. Liver injury is related to alteration in activities of these metabolic functions.[17] Thus liver disease is one of the most serious health problems. Historical and recent advances in modern medicine have proved significant health benefits for humans and have increased quality of health. However, no specific drug is available for treatment of liver disease. The Indian traditional medicine system 'Ayurved' recommends a number of herbal remedies and polyherbal preparation for treatment of liver disease.[19]

Experimental studies on phytopharmaceuticals like flavonoids, terpenoids, glycosides etc. have established their role as antioxidant and hepatoprotective agent.[20,21] Hepatoprotectors and antioxidants cease the process of inflammation and thus prevent the progression of inflammatory diseases and carcinogenesis. In the current work, the presence of these phytoconstituents might be responsible for hepatoprotective potential. Thus, the current studies demonstrate profound beneficial effect of *B. monosperma* extract on isoniazid-rifampicin induced hepatotoxicity. A detailed study on its mechanism of action and phytoanalytical studies are in progress in our laboratory.

CONCLUSION

Thus, it can be summed up that *Butea monosperma* is rich source of phytochemicals responsible for hepatoprotective activity.

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CONFLICT OF INTEREST

The authors declare that there was no conflict of interest.

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