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Evaluation of hepatoprotective activity of ethanolic extract of *Butea monosperma* against thioacetamide induced hepatotoxicity in rats

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Abstract
Due to damage of Hepatic tissue, there may impairment in liver function which may have deleterious effect on human health. Management of liver diseases by using natural herb is recent need. In this research we evaluated the hepatoprotective activity of ethanolic extract of SBBM against TTA induced hepatotoxicity in rats. (Group I received 0.1% CMC, Group II received only TTA, Group III received sily marin, Group IV received ethanolic extract of SBBM orally 200 mg/kg &Group V received ethanolic extract of SBBM orally 400 mg/kg)Group II-V received intraperitonial injection of TAA. On 16th day of the experimental period, animals were sacrificed, the serum was separated for the estimations of histopathalogical, biochemical& morphological parameters, results suggest that it may contribute to the chemopreventive effect of SBBM showed a significant recovery in the level of glutathione and its metabolizing enzyme in the liver, which is shown by the elevated levels of SGOT, SGPT, GSH and Lipid peroxidation, Hence, on the basis of result obtained it can be said that SBBM exercised powerful free radical scavenging activity and hepatoprotective activity.

Keywords: SBBM (Stem bark of *Butea monosperma*), TAA (Thioacetamide), SGPT, SGOT, GSH and Lipid peroxidation

Introduction
[1, 2, 3] Liver diseases are the most serious ailment and are mainly caused by toxic chemicals (Excess consumption of alcohol, high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents, peroxidised oil, etc.). In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases. In India, more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations. [4]Hepatic tissue damage causes impairment to liver function which may have deleterious effect on human health [4]. Management of liver diseases is still a challenge to the modern scientific community [5]. In view of severe undesirable side effects of synthetic agents; there is needs of attention to follow systematic research to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity, Most of the herbal medicines speed up the natural healing processes of the liver mediated through their antioxidative potential with almost negligible side effects [6-8]. Several medicinal plants are practiced for their traditional use in hepatic disorders/jaundice since long time. The present study aims to investigate the hepatoprotective activity ethanolic extract of SBBM against TTA induced hepatotoxicity in rats. *Butea monosperma* (Lamk.) Taub, belonging to family-Fabaceae was commonly known as Palash in Hindi, It is found in greater parts of India, Burma and Sri Lanka. It is capable of growing in water logged situations, black cotton soils, saline, alkaline, swampy badly drained soils and on barren lands except in arid regions [8]. Sharma *et al*., (2010) reported hepatoprotective activity of aqueous extract of flowers of *Butea monosperma* against CCl4 induced liver damage. They found significantly increased in the excretory capacity of liver [9]. Seed contains Palasonin, monospermoside (butein3-e-D-glucoside) and somonospermoside, several flavonoids. butin (α-Amyrin), β- sitosterol, Monospermin. Above researcher reported that the methanolic extract of flower of *Butea monosperma possess* hepatoprotective effects due to presence of flavonoid (butin, butein, butrin and isobutrinchalcones) [10]. However SBBM also contain flavanoid. However no scientific work has been carried out on ethanolic extract of SBBM, *w*hich can prove hepatoprotective activity of SBBM.

Plant material: The stem bark of *Butea monosperma* was identified and authenticated from Agriculture College, Indore; (M.P). The collected stem bark pieces were dried for 7-10 days in dried in the shade. After complete drying, the dried pieces were powered & used for extraction.

Extraction: [11] The powder (260 g) was treated with petroleum ether (Ratio is 1:10, powdered drug Table: 1 and petroleum ether) for three day for the removal of waxy & fatty material. Then it was dried & extracted with 95% ethanol for three days by soxhlet apparatus. After the complete solvent extraction, solvent was removed by solvent evaporator in reduced pressure by rotary evaporator and get dried extract with minimal solvent. After that weigh the accurate amount of dried extract and calculate the percentage yield of extract. The weight of ethanolic extract of SBBM obtained was 6 g (2.3% w/w yield).

Phytochemical Studies

[12] The phytochemical screening of suspension of extract was carried out as described by Khandelwal.

Procurement of animals and housing condition

[13] Albino Wistar rats of either sex weighing around 100-180g were procured from Indore, M.P., All animals were housed in polypropylene cages in a temperature controlled animal house room at 24±1 °C temperature, 60±5% relative humidity and 12 hour light and 12 hour dark cycle. The animals were fed with pelleted feed with standard rat diet and tap water throughout the experiment. All animal experiment was carried out in accordance with the guidelines of CPCSEA with registration number and study was approved by the IAEC.

Acute oral toxicity

[13] The acute oral toxicity study was carried out as per the guidelines set by OECD, revised draft guidelines 423, received from CPCSEA.

Experimental design

Thioacetamide induced hepatotoxicity: [14] Thioacetamide (TAA) was given to rats intraperitoneally 300 mg/kg body weight on 13th and 14th day. TAA is metabolized by the liver cytochrome P4502E1 enzymes, rendering sulfone and sulfoxide derivatives which are apparently responsible of structural proteins and NADPH cytochrome P450 reductase activity and to nitric oxide synthesis. Besides, TAA toxicity mechanism was related to alterations in enzyme inactivation.

Grouping of animals: Each group contained 5 adult albino Wistar rats (100-150 g). Group I received 0.1% CMC (10 ml/kg, p.o.). Group II received only thioacetamide (300 mg/kg, *i. p.*) on 13th and 14th day. Group III received silymarin (25 mg/ Kg, p.o.) from day 1 to day14.Group IV received ethanolic extract of SBBM orally 200 mg/kg from 1 to 14 days. Group V received ethanolic extract of SBBM orally 400 mg/kg from 1 to 14 day. The total experimental duration was 15 days. On the 13th and 14th day, Group II-V received intraperitonial injection of TAA 300 mg/kg body weight in physiological saline at 24 h interval only. On 16th day of the experimental period, animals were fasted overnight and sacrificed. Blood samples were collected in heparinised tubes and plasma was separated by centrifugation at 2000×g for 10 min. Liver was removed, cleared off blood, photographed and immediately transferred into ice-cold saline. Liver tissue was weighed and homogenized in appropriate buffers and used for various estimations [15], The blood was collected by direct cardiac puncture under light ether anesthesia and serum was separated to estimations of histopathalogical, biochemical and morphological parameter were serum glutamic-pyruvic transaminase (SGPT) & serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP) and [16] Lipid Peroxidation in erythrocytes (TBARS) and liver weight. The data of hepatoprotective were analyzed by one way analysis of variance (ANOVA) used to evaluate differences between groups, followed by Dunnett´s t test, used to compare the means of specific groups, with $p<0.01$ was considered as statistically significant.

Result

Phytochemical Evaluation

The qualitative chemical examinations are shown presence alkaloids, steroid, flavonoid, phenol, triterpenoids and absence of tannin, saponin.

Effect of SBBM on liver weight: In the present study, **(**Table No 1), administration of TTA increased Liver weight which was significantly attenuate by pretreatment with SBBM (200 mg/kg, 400 mg/kg) and Silymar in (25 mg/kg) as compared with the experimental control group. Effect of different doses of SBBM (200 and 400 mg/kg p.o.) and Silymarin (25 mg/kg, p.o.) on liver weight,*Significant $p<0.05$, ** Very Significant $p< 0.01$, *** Highly significant. Significant $p<0.001$ as compared to that of normal control.

Table 1: Effect of SBBM on Liver weight

S No.	Groups	Liver Weight (gm)
1	Normal Control	3.655±0.2155
2	Experimental Control	8.134±0.383$^{###}$
3	*Butea monosperma* 200 mg/kg	7.012±0.5951**
4	*Butea monosperma* 400 mg/kg	5.842±0.6102***
5	Silymarin 25 mg/kg	4.205±0.3687***

Table 2: Effect of ethanolic extract of SBBM Alanine transminase. (N = 5, the value is in mean± SEM.)

Sr. No.	Groups	SGPT (IU/L)
1	Normal Control	84.43±4.763
2	Experimental Control	194.3±6.735$^{###}$
3	SBBM 200 mg/kg	168.9±6.684**
4	SBBM 400 mg/kg	126.2±6.991**
5	Silymarin 25 mg/kg	111.9±3.319***

Effect of SBBM on liver SGPT, SGOT, Lipid peroxidation and Glutathione Reductase

Effect of SBBM on liver SGPT

The present study, **(**Table No 2) administration of TTA increased ALT level which was significantly reversed by pretreatment with SBBM (200 mg/kg, 400 mg/kg) and Silymarin as compared with the experimental control group. Effect of different doses of SBBM (200 and 400 mg/kg p.o.) and Silymarin (25 mg/kg, p.o.) on ALT. *Significant $p< 0.05$, ** Very Significant $p< 0.01$, *** Highly Significant $p< 0.001$ as compared to that of normal control group

Table 3: Effect of ethanolic extract of SBBM *on* Aspartate transminase (AST) (N = 5, the value is in mean± SEM.)

Sr. No	Group	SGOT(IU/L)
1	Normal control	88.68±1.688
2	Experimental control	195.8±4.353$^{###}$
3	SBBM 200 mg/kg	164.3±13.54**
4	SBBM 400 mg/kg	125.8±7.359**
5	Silymarin 25 mg/kg	93.42±3.161***

Effect of SBBM on liver SGOT: In the present study,

(Table3) administration of TTA increased AST level which was significantly reversed by pretreatment with SBBM (200 mg/kg, 400 mg/kg) and silymarin 25 mg/kg as compared with the experimental control group. Effect of different doses of SBBM (200 and 400 mg/kg p.o.) and Silymarin (25 mg/kg, *p.o.*) on AST.*Significant $p<0.05$, ** Very Significant $p<0.01$, *** Highly Significant $p<0.001$ as compared to that of normal control group.

Table 4: Effect of SBBM Lipid peroxidation. (N = 5, the value is in mean± SEM.)

Sr. No.	Groups	Lipid peroxidation (nmol/gm)
1	Normal Control	38.84±2.649
2	Experimental Control	107.5±8.004###
3	SBBM 200 mg/kg	72.70±1.200*
4	SBBM 400 mg/kg	47.76±1.943**
5	Silymarin 25 mg/kg	40.62±1.478***

Effect of SBBM on Lipid peroxidation

In the present study, (Table 4) the administration of TTA, increased Lipid peroxidation level which was significantly reversed by pretreatment with SBBM (200 mg/kg, 400 mg/kg) and Silymarin 25 mg/kg as compared with the experimental control group. The effect of different doses of SBBM (200 and 400 mg/kg p.o.) and Silymarin (25 mg/kg, p.o.) on lipid peroxidation. *Significant $p< 0.05$, ** Very Significant $p< 0.01$, *** Highly Significant $p< 0.001$ as compared to that of normal control group.

Table 5: Effect of ethanolic extract of SBBM Glutathione Reductase. (N = 5, the value are in mean± SEM)

Sr. No.	Groups	GSH (nmol/gm)
1	Normal Control	20.08±0.692
2	Experimental Control	11.84±0.629###
3	SBBM 200 mg/kg	15.58±0.817**
4	SBBM 400 mg/kg	17.31±0.695**
5	Silymarin 25 mg/kg	19.40±0.968**

Effect of SBBM on glutathione Reductase

In the present study, (Table 5) the administration of TTA decreased GSH level which was significantly attenuate by pretreatment with SBBM (200 mg/kg, 400 mg/kg) and Silymarin 25 mg/kg as compared with the experimental control group.

The statistical analysis is done by using one way ANOVA (Analysis of variance) followed by Dunnett´s t test. In the present study, the administration of TTA decreased GSH level which was significantly attenuate by pretreatment with SBBM (200 mg/kg, 400 mg/kg) and Silymarin 25 mg/kg as compared with the experimental control group. The effect of different doses of SBBM (200 and 400 mg/kg p.o.) and Silymarin (25 mg/kg, p.o.) on GSH.*Significant $p<0.05$, ** Very Significant $p<0.01$, *** Highly Significant $p<0.001$ as compared to that of normal control group.

Conclusion and Discussion

The hepatotoxin is associated with changes at cellular levels that may lead to deterioration of organ functions. Therefore, any improvement in the treatment of hepatic function could be of potentially a great importance. Natural compounds that reduce the chemical activating enzymes might be good candidates for protecting against chemically induced toxicities. The ethanolic extract of SBBM (200 mg/kg, 400 mg/kg) and silymarin 25 mg/kg on TAA leads to oxidative stress, which is characterized by the increased generation of reactive oxygen species. Reactive oxygen species play an important role in many human degenerative diseases. TAA is bio activated by CYP450 and/or flavin containing monooxygenase systems to toxic metabolites TAA sulfine (Sulphoxide) and sulfene (Sulphone), which produce centrilobular hepatic necrosis and apoptosis.14In the present study, we have evaluated the effect of pretreatment of ethanolic extract of SBBM, A induced toxicity and the results suggest that it may contribute to the chemo preventive effect of SBBM showed a significant recovery in the level of glutathione and its metabolizing enzyme in the liver, which is shown by the elevated levels of SGOT, SGPT, GSH and Lipid peroxidation, Lipid peroxidation is accepted to be one of the principal causes of chemically induced liver injury and is mediated by the production of free radicals. Lipid peroxidation level was increased after TAA administration which was significantly decreased by pretreatment with SBBM (200 mg/kg, 400 mg/kg) and silymarin (25 mg/kg).GSH level was also depleted after administration of TAA and significantly increased by pretreatment with SBBM (200 mg/kg, 400 mg/kg) and silymarin (25 mg/kg. The ethanolic extract of stem barks of SBBM & silymarin 25 mg/kg used in the present study seems to offer dose dependent protection and maintain the structural integrity of hepatic cells. This was evident from the significant reduction in TAA-induced enhancement in serum SGOT and SGPT level. These investigations validate the use of SBBM in liver disorders. Major compouds of SBBM include flavanoids and triterpenoids. Combined activities of these compounds might be responsible for increase the antioxidative activities of SBBM.9. Our data, however, provide a substantial amount of mechanistic approach to show the chemopreventive effect of SBBM against oxidative stress. Hence, the present study clearly demonstrates the role of SBBM in the inhibition of biochemical events of Liver damage. On the basis of result obtained we conclude that ethanolic extract of stem bark of SBBM having hepatoprotective potential against TTA. The presence of favonoids and triterpenoids may be responsible for this activity. Further isolation and evaluation of active constituent from SBBM is needed for establishment of mechanism responsible for hepatoprotective activity.

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