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Research Article

SCREENING OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF STEM BARK OF BAUHINIA VARIEGATA IN RATS

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ABSTRACT

Objective: To study the hepatoprotective activity of ethanolic extract of stem bark of *Bauhinia variegata.*

Material and methods: Albino wistar rats of either sex weighing 150-200g were divided into seven groups (n=6). Liver injury was produced by carbon tetrachloride (CCl_4) 1 ml/kg/d dissolved in olive oil (1:1) orally. Silymarin (100mg/kg) orally was used as standard drug. Test groups received Ethanolic extract of stem bark of *Bauhinia variegata* (BVEE) in the doses of 100, 200,400 and 600mg/kg/day orally along with CCl_4. Treatment was given to all the groups daily for 7 days. The hepatoprotective effect of BVEE was evaluated by assessment of biochemical parameters [Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin] and the antioxidant activity in the liver tissue was estimated by determining the activities of antioxidant enzymes: reduced glutathione (GSH), catalase (CAT) as well as the level of lipid peroxidation by Malondialdehyde (MDA). Histopathological examination of the liver was also done.

Results: BVEE (400mg/kg and 600mg/kg) exhibited highly significant reduction ($p<0.001$) in AST, ALT, ALP and total bilirubin. BVEE (200mg/kg and 100mg/kg) exhibited highly significant reduction ($p<0.001$) in AST, ALT and ALP, just significant reduction ($p<0.05$) in total bilirubin. (BVEE) in doses of 200,400 and 600mg/kg/d showed highly significant reduction ($p<0.001$) in MDA and rise in ($p<0.001$) in CAT and GSH. BVEE in dose of 100 mg/kg/d showed highly significant reduction ($p<0.001$) in MDA and rise in ($p<0.001$) in CAT but only significant rise ($p<0.01$) in GSH. Histopathological examination of the liver suggested hepatoprotective effect of the extract by decreasing the extent of centrilobular necrosis, fatty changes and congestion of sinusoids when compared to carbon tetrachloride group. **Conclusion**: BVEE showed significant dose dependent protection against carbon tetrachloride induced liver injury in rats.

Keywords: Carbon tetrachloride (CCl_4), Bauhinia variegata ethanolic extract(BVEE), Lipid peroxidation, Malondialdehyde (MDA), Catalase (CAT), Reduced glutathione (GSH)

INTRODUCTION

Liver is an organ of paramount importance which plays an essential role in regulating homeostasis in the body. It is involved in almost all the biochemical pathways related to metabolism, excretion and body defense [1]. Liver which is the key organ of metabolism and excretion is exposed to a variety of xenobiotics and therapeutic agents continuously. Thus the disorders associated with this organ are numerous and varied [2]. Liver diseases, such as jaundice, cirrhosis and fatty liver are very common worldwide [3]. Several environmental toxins and carcinogens are also converted into reactive intermediates during metabolism, resulting in tissue damage. Since the metabolic function of the liver is primarily responsible for detoxification of diverse therapeutic agents, toxins and carcinogens, drug-induced liver injury may manifest as acute hepatitis, cholestasis which may also lead to development of cirrhosis [4]. There is increasing evidence that free radicals and reactive oxygen species play a crucial role in various steps that initiate and regulate the progression of liver diseases independently of the original agent [5].

Treatment options for common liver diseases such as drug induced hepatitis, fatty liver and chronic hepatitis are very few and only supportive. The effectiveness of agents available for the treatment of liver disease are inconsistent and have greater incidence of side-effects [6]. *Bauhinia variegata* commonly known as Kachnar is a medium-sized, deciduous tree, found throughout India. Its stem bark, flowers, flower buds, leaves and root are used in folklore medicine for the treatment of various problems of gastrointestinal tract as carminative, antihelminthic and liver tonic. In ayurveda it is used in the treatment of diarrhoea, dysentery, goitre, lymphadenitis, worm infestation, rectal prolapse and as depurative (blood purifier) , alterative for improving detoxifying function of liver [7,8]. Various biological activities such as antimicrobial, anti-inflammatory, analgesic, cytotoxic, antiobesity and nephroprotective effect of this plant have also been reported [9-14], but there are few reports regarding hepatoprotective activity of the plant [15].

Bauhinia variegata has been reported to have Tannis, Total phenols, Flavonoids and other polyphenolic compounds in stem bark [16-18].

As Efficacious, safe and cost effective medical therapies for various liver ailments are lacking, natural sources like plants are required to be explored for protection against liver injuries produced by various harmful agents. Therefore the present study was framed to assess the hepatoprotective activity of stem bark of *Bauhinia variegata.*

MATERIAL AND METHODS

Animals

Adult albino wistar rats of either sex weighing 150- 200 grams were obtained from the Central Animal House, JNMC, Aligarh Muslim University. The animals were housed in polypropylene cages bedded with paper strips which were kept in well ventilated room under standard conditions (12 h light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water ad libitum. They were acclimatized to the laboratory conditions for 1 week .

Plant material and Preparation of Extracts

Bauhinia variegata was obtained from botanical garden of A.M.U . The plant was identified and authenticated by Dr. Athar Ahmed, Assistant professor, Department of Botany, Aligarh Muslim University and voucher specimen was submitted (Voucher number :DWS/VS/02). The stem bark was collected, thoroughly washed, shade- dried and pulverized in electric grinder. 100g of powder was extracted in 300 ml ethanol for 72 hours with the help of Soxhlet's apparatus. The extract obtained was collected in Petri dish and evaporated till dryness at 40-50 ºC in autoclave. Yield obtained was 40.68%

Chemicals

Carbon tetrachloride (CCl_4) and Silymarin (Silybon) was obtained from Thomas Baker and Microlabs respectively

IAEC approval

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) on 04-05-2011. All animal experiments were carried out as per the rules and regulations of IAEC & CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) under the "Guidelines for Care and Use of Animals in Scientific Research".

Experimental design

Albino wistar rats of either sex weighing 150-200g were divided into seven groups, containing six animals each.

Group I (Normal control) received distilled water (1ml/kg/d, orally) only.

Group II (Negative Control) was given carbon tetrachloride 1 ml/kg/d dissolved in olive oil (1:1) orally [19].

Group III (Positive control) received Silymarin (100mg/kg) orally along with CCl_4 (1ml/kg) [20].

Test groups (IV-VII) received Ethanolic extract of stem bark of Bauhinia variegata (BVEE) in different doses with CCl4 (1ml/kg) as follows:

IV- BVEE (100 mg/kg) + CCl_4 (1ml/kg).

V- BVEE (200 mg/kg) + CCl_4 (1ml/kg)

VI- BVEE (400mg/kg) + CCl_4 (1ml/kg)

VII- BVEE (600 mg/kg) + CCl_4 (1ml/kg)

All the groups were given the above treatment daily for 7 days.

Assessment of hepatoprotective activity

On 8th day the animals were sacrificed under ether anaesthesia and blood was collected by direct cardiac puncture. Liver was dissected out and 500 mg of liver tissue was taken for determination of the levels of antioxidant Enzymes. Rest of the liver was kept in 10% formalin for histopathological examination.

Determination of serum biochemical parameters

The collected blood was centrifuged at 5000 rpm for 10 minutes and serum was separated. Serum was analyzed for biochemical parameters like Alanine aminotransferase **(**ALT) and Aspartate aminotransferase (AST) [21], Alkaline phosphatase (ALP) [22] and Total bilirubin [23].

Estimation of lipid peroxidation and antioxidant enzymes

The liver was homogenised in 10 % w/v of phosphate buffer (0.2M, pH-6.6) i.e. 500 mg of liver was homogenised with 5 ml of buffer and used for estimation of Malondialdehyde (MDA) [24], Catalase (CAT) [25] and Reduced glutathione Reduced Glutathione(GSH) [26].

Histopathological examination

Liver tissue was fixed in 10% formalin, dehydrated in graded ethanol and embedded in paraffin wax. Sections were prepared and stained with hematoxylin and eosin. The slides thus prepared were observed for histopathological features under the microscope.

Statistical Analysis

The results were expressed as Mean ± Standard Error of Mean (SEM). The groups were compared by one way analysis of variance (ANOVA) followed by post hoc "Dunnett's Multiple comparison test" to analyze statistical significance. $p < 0.05$ was considered to be significant.

RESULTS

Biochemical parameters

Biochemical parameters of all the control and test groups are presented in table 1. The normal control group which was given only distilled water served as a baseline for all the biochemical parameters. Negative control showed highly significant rise ($p< 0.001$) in AST, ALT, ALP and Total bilirubin when compared with normal control group. Positive control showed highly significant decrease ($p<0.001$) in AST, ALT, ALP and Total bilirubin as compared to negative control group. Biochemical parameters in BVEE treated groups were compared with negative control group. BVEE 400 and 600mg/kg/d exhibited highly significant reduction ($p<0.001$) in AST , ALT, ALP and total bilirubin. BVEE 200mg/kg and 100mg/kg exhibited highly significant reduction ($p<0.001$) in AST, ALT and ALP but just significant reduction($p<0.05$) in Total bilirubin (Table 1).

Table 1: Effect of Ethanolic extract of stem bark of *Bauhinia variegata* (BVEE) on biochemical parameters against CCl_4 induced liver injury.

Groups (n=6)	AST (IU/L)	ALT (IU/L)	ALP (KAU/dl)	Total Bilirubin (mg/dl)
Normal control	25.66 ± 1.33	23.08 ± 1.30	29.50 ± 1.93	0.51 ± 0.04
Negative control	151.50 ± 0.50***	158.33 ± 1.08***	81.00 ± 1.12***	0.78± 0.03***
Positive control	43.33 ± 1.18***	49.16 ± 2.57***	47.33 ± 2.30***	0.55 ± 0.02***
BVEE100	109.66 ± 1.11***	115.0 ± 1.93***	68.66 ± 2.10***	0.65 ± 0.03*
BVEE200	100.66 ± 1.62***	109.00 ± 1.91***	67.16 ± 1.72***	0.65 ± 0.02*
BVEE400	65.66 ± 1.74***	70.66 ± 1.33***	54.66 ± 1.11***	0.56 ± 0.02***
BVEE600	64.33 ± 2.07***	69.16 ± 1.95***	54.00 ±1.26***	0.56 ± 0.02***

Data are expressed as Mean ± SEM Negative control group was compared with Normal control group and all other groups were compared with Negative control group, * $p< 0.05$, **$p<0.01$ and ***$p<0.001$ were considered significant.

Effect on lipid peroxidation and antioxidant enzymes

Levels of Lipid peroxidation and antioxidant enzymes of negative control group were compared with normal control group. Negative control showed highly significant rise ($p<0.001$) in MDA and reduction ($p<0.001$) in CAT and GSH values as shown in table 2. Positive control group treated with Silymarin showed highly significant reduction ($p<0.001$) in MDA, and rise ($p<0.001$) in CAT and GSH values when compared to negative control group. Test groups treated with BVEE in doses of 200,400 and 600mg/kg/d showed significant reduction ($p<0.001$) in MDA, and highly significant rise ($p<0.001$) in CAT and GSH when compared with Negative control group. BVEE in dose of 100 mg/kg/d showed similar changes but only significant rise ($p<0.01$) in GSH.

Histopathological examination

Histological study of liver sections of normal control animals showed normal hepatic architecture with hepatocyte cords, central vein and sinusoids (Fig 1) whereas animals treated with CCl_4, revealed distortion of hepatic architecture with extensive fatty changes, inflammatory cells infiltration and massive necrosis around central vein (Fig. 2). Positive control and BVEE treated groups were compared with Negative control group. The section of liver tissue treated with standard drug Silymarin showed normal hepatocytes with few inflammatory cells and edema of sinusoids but maintained normal hepatic architecture (Fig.3) BVEE treated groups showed improvement in histology of liver by reducing fatty changes and inflammatory infiltrate in dose dependent manner (Fig.4,5,6,7)

Table 2: Effects of Ethanolic extract of stem bark of *Bauhinia variegata* (BVEE) on lipid peroxidation and Antioxidant enzymes in CCl_4 induced liver injury

Groups (n=6)	MDA (nmol/mg)	Catalase (U/ min/mg)	GSH (µmol/mg)
Normal control	119.76 ± 2.47	96.74± 3.91	10.20 ± 0.81
Negative control	482.13 ± 3.06***	36.13 ± 1.47***	1.50 ± 0.34***
Positive control	152.25 ± 3.52***	78.95 ± 2.87***	7.70 ± 0.20***
BVEE100	261.93 ± 3.71***	55.51 ± 1.37***	3.91 ± 0.38**
BVEE200	224.79 ± 3.22***	61.63 ± 1.45***	4.49 ± 0.50***
BVEE400	172.89 ± 1.58***	72.13 ± 1.45***	6.46 ± 0.45***
BVEE600	168.68 ± 4.52***	72.14 ± 2.59***	6.60 ± 0.36***

Data are expressed as Mean ± SEM Negative control group was compared with Normal control group and all other groups were compared with Negative control group, * p< 0.05, **p<0.01 and ***p<0.001 were considered significant

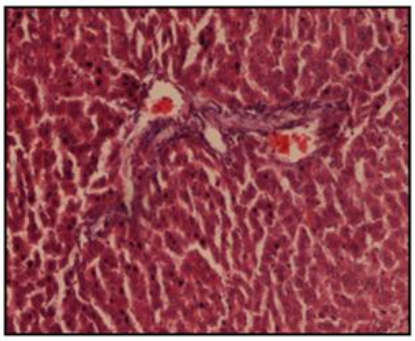

Fig. 1: Normal control

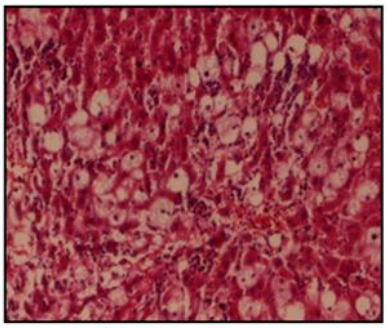

Fig. 2: Negative control,

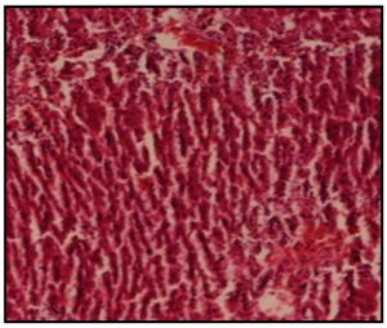

Fig. 3: Positive Control

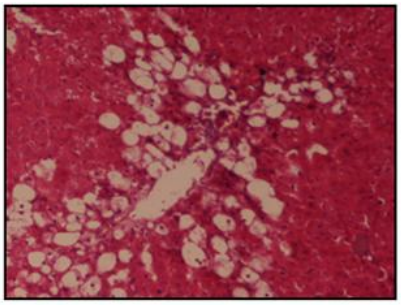

Fig. 4: BVEE 100

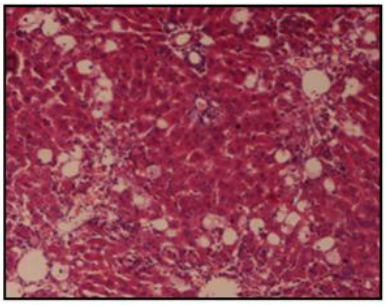

Fig. 5 : BVEE 200

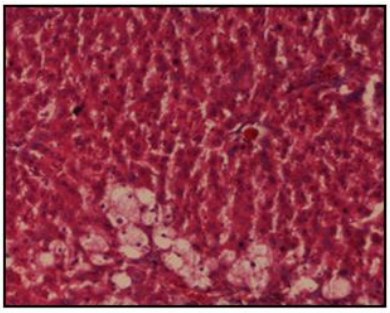

Fig. 6: BVEE 400

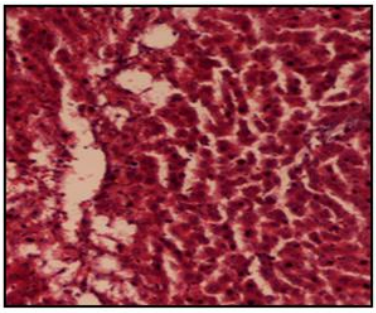

Fig. 7: BVEE 600

DISCUSSION

Carbon tetrachloride (CCl_4) is a well known toxicant and exposure to this chemical is known to induce oxidative stress by the formation of free radicals. Carbon tetrachloride induced hepatotoxicity results from its bioactivation by Cytochrome P450 which leads to formation of reactive intermediate trichloromethyl free radical (CCl_3*) which in presence of oxygen forms Trichloromethylperoxy radical (CCl_3OO*). These reactive intermediates covalently bind to cellular macromolecules, produce lipid peroxidation resulting in the membrane injury and leakage of cytosomal enzymes [27,28].

Aminotransferases are normally present in the hepatocytes and found in serum in low concentrations. These enzymes are released into the blood in greater amounts when there is damage to the liver cell membrane resulting in increased permeability. Alkaline phosphatase (ALP) is found in the bile canalicular membrane of hepatocytes and is usually elevated in intrahepatic cholestasis. Bilirubin conjugation and excretion takes place in liver, both metabolic and excretory functions of the liver can be assessed by estimating total bilirubin. Elevated total serum bilirubin in patients with drug-induced liver disease indicates more severe injury [29].

The efficacy of any hepatoprotective drug is dependent on its capability of either reducing the harmful effects of a hepatotoxin or of maintaining the normal physiological functions that are unbalanced by a hepatotoxin [30].

Animals treated with CCl_4 , developed significant liver damage in negative control group , as evident from a significant increase ($p < 0.001$) in the serum levels of AST, ALT , ALP and Total bilirubin, when compared with normal control rats, indicating acute hepatocellular damage and biliary obstruction.(Table1) Silymarin (100mg/kg) significantly decreased ($p < 0.001$) the levels of all biochemical parameters viz. AST, ALT, ALP and Total bilirubin. BVEE in doses of 100,200,400 and 600mg/kg/d decreased all biochemical parameters significantly in a dose dependent manner as shown in Table 1. indicating stabilization of plasma membrane. BVEE 600mg/kg/d showed ceiling effect and showed almost similar changes in the biochemical parameters. BVEE exhibited significant restoration of serum markers, indicating its protection against CCl_4 induced liver injury.

Lipid peroxidation by CCl_4 leads to formation of reactive aldehydes such as Malondialdehyde biomarker of oxidative stress which is used for detection of free radical injury [31,32]. GSH protects against CCl_4 induced microsomal lipid peroxidation and liver injury begins when GSH stores are markedly depleted [33].CAT is active in neutralizing reactive oxygen species and so removes cellular superoxide and peroxides before they react with metal catalysts to form more reactive species [34]. Negative control group showed highly significant raised levels of MDA in liver homogenate of rats indicating excessive formation of free radicals and activation of lipid peroxidation. Reduced glutathione (GSH) and Catalase were significantly decreased in liver homogenate of CCl4 treated rats indicating oxidative stress produced by CCl_4 . Silymarin (100mg/kg/d) significantly reduced MDA levels and increased GSH and Catalase as shown in the table 2. Silymarin protects liver against various hepatotoxic drugs by inhibition of lipid peroxidation, free radical scavenging and membrane stabilizing action [35]. Treatment with Ethanolic extract of *Bauhinia variegata* (BVEE) 100, 200,400 and 600 mg/kg/day showed significant reduction in MDA level and significant rise in CAT and GSH activity when compared to CCl_4 group. The observed decline in lipid peroxides in liver samples of rat following co-treatment with CCl_4 and BVEE suggests that protective potential of BVEE is due to scavenging of free radicals produced by CCl_4. BVEE showed antioxidant activity as indicated by elevation of GSH and Catalase in treated groups (Table 2) which might contribute towards scavenging of free radicals generated via bioactivation of CCl_4.

The animals treated with different doses of the Ethanolic extract of stem bark (BVEE) of *Bauhinia variegata* showed dose dependent decrease in centrilobular vacuolization of hepatocytes and infiltration of inflammatory cells. BVEE 100 and 200 mg/kg/d showed small amount of necrosis and macrovesicular fatty changes as compared to negative control. (Fig.4,5) Liver sections of the animals treated with BVEE in dose of 400 and 600mg/kg/d exhibited significant liver protection against CCl_4, as evident by the presence of normal hepatic cords, with few inflammatory cells and vacuolated hepatocytes , which is comparable to the liver sections of animal treated with standard drug Silymarin (Fig.6,7). These findings are suggestive of hepatoprotective activity of BVEE in all the doses (100,200,400 and 600 mg/kg/d), more marked with 400 and 600mg/kg/d.

The toxicity of CCl_4 is dependent on its own metabolism. The formation of the trichloromethyl and trichloromethylperoxy radical and its reaction with membrane lipids may initiate lipid peroxidation which is a prominent biological event observed when CCl_4 is administered to animals. The possible hepatoprotective activity of *Bauhinia variegata* against CCl_4-induced liver damage in rats might be due to its antioxidant activity as indicated by protection against lipid peroxidation and reduced antioxidant levels, thereby minimizing free radical damage of hepatocytes.

Bauhinia variegata has been reported for the presence of Tannis, Total phenols and Flavonoids in stem bark on phytochemical analysis. These polyphenols showed high free radical scavenging activity [16]. New polyphenolic compounds have also been isolated in stem bark [17]. These polyphenolic compounds may be responsible for the action. But the active compounds which are responsible for the observed hepatoprotective effect, have not been isolated in this study. Therefore, further studies may be conducted to determine the active compounds that are responsible for the hepatoprotective effects and the mechanisms of action involved.

CONCLUSION

BVEE showed dose dependent protection against CCl_4 induced acute liver injury, maximal effect was observed in the dose of 400 and 600mg /kg/d. Further studies for longer duration are required to find out the protective potential of *Bauhinia variegata* against chronic liver injury.

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