

HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF

***BAUHINIA VARIEGATA* LINN. LEAVES**

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Summary

Bauhinia variegata Linn. (Kachnar) is traditionally used in Indian system of medicine as astringent, tonic, anthelmintic, used in ulcer & leprosy. The buds are acrid, used in piles, cough and liver complaints. The objectives of present investigation was to evaluate the hepatoprotective activity of ethanolic extract of *Bauhinia variegata* (EEBV) leaves. Hepatotoxicity was induced by paracetamol in wistar albino rat. Extract was administered orally at different doses (200mg/kg and 400mg/kg). The effect of EEBV on the serum marker enzyme viz. SGOT, SGPT, SALP & serum bilirubin was assessed. Histopathological study was also done to evaluate the hepatoprotective activity. Present study shows the EEBV exhibits hepatoprotective effect by a significant reduction in level of SGOT (53.26%), SGPT (41.64%), SALP (72.30%) and bilirubin (68.18%). It also preventing liver histopathological changes in rats induced with PCM hepatotoxicity. Overall study reveals that the ethanolic extract of *B. variegata* Linn. having significant hepatoprotective activity.

Keywords: *Bauhinia variegata*, Paracetamol, Hepatoprotective, Histopathology, Acute toxicity.

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Introduction

Liver is the vital organ of intense metabolic activities involved in the removal of exogenous & endogenous challenges.^[1] *Bauhinia variegata* Linn. (Kachnar) (Leguminosae) is a medium sized deciduous plant. It is distributed throughout in India, ascending to an altitude of 1300 m. in the Himalayas. It grows best in the full moon or partial shade.^[2-3] The bark is astringent, tonic, anthelmintic & used in ulcer & leprosy. The buds are acrid, used in piles, cough, liver complaints, astringent.^[4] The various flavonoids from leaves have been reported as effective hypoglycaemic agent.^[5-6] Various flavonoids isolated from roots, non woody aerial parts & flowers of *B. variegata* Linn.^[7-8] The most severe clinical consequences of liver diseases are hepatic failure, Liver cirrhosis, Portal hypertension, Jaundice. Hepatic failure develops as the end point of progressive damage to the liver either by destruction of hepatocytes or by repetitive discrete waves of parenchymal damage. Jaundice is a yellow discoloration of skin & sclera, occurs when systemic retention of bilirubin leads to elevated serum level above 2.0mg/dl.^[9] Though there is no scientific evidence to support the hepatoprotective effect of *B. Variegata*, tribal men continue to use the plant in the treatment of liver disorders. The identification of compounds from *B. variegata* with antihepatotoxic activity may also provide an opportunity to develop a new class of hepatoprotective agent. Therefore, the most desired outcome of the present work will be the establishment of *B. variegata* as a hepatoprotective agent.

Materials and Methods

Plant material

The leaves of the *Bauhinia variegata* Linn. were collected from Rath (Distt.- Hamirpur) in the month of July 2010. The leaves were dried in shade. The plant material was identified, authenticated and voucher specimen number (97308) was lodged in the departmental herbarium of National Botanical Research Institute, Lucknow.

Extract Preparation

The freshly collected leaves of *Bauhinia variegata* were first air dried and then dried in tray drier under control conditions and powdered. The powdered leaves were macerated with petroleum ether to remove fatty substances; the marc was further exhaustively extracted with of ethanol for 3 days (3 X 3L) by hot percolation method and centrifugation at 10,000 rev/min. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure. The extract was stored in a desiccator for use in subsequent experiment.

Drugs & Chemicals

Paracetamol (Amrit Pharma Jaipur), Liv. 52 (Himalaya Ltd.) and Aspirin (Amol Pharma. Jaipur) were used. The kits for estimation of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin (TB) were purchased from Beacon Diagnostic Pvt. Ltd. Kabilpore and Loba Chemicals, Mumbai. All other chemicals used for the experiments were of high analytical grade.

Experimental Animals

Healthy Albino rats (Wistar strain) and Swiss albino mice of either sex, weighing about (180-250gm) (20-25gm) were obtained from animal house, Institute of Pharmacy, Bundelkhand University, Jhansi. The animals were housed in specific standard laboratory conditions. The conditions were kept in a temperature controlled environment ($25\pm 1^{\circ}\text{C}$) and with a regular 12h light/ 12h dark cycle. All animals were fed with commercial diet and water *ad libitum*, during the experiment. All protocols of the study was approved by the Institutional Animal Ethical Committee.

Acute Toxicity Studies

Acute toxicity study was carried out according to the Organization of Economic Corporation Development (OECD) guidelines No. 425. EEBV was administered orally in doses of 100, 200, 400, 800, 1000 and 2000 mg/kg to the group of mice (n=3) and the percentage mortality was recorded for a period of 24h then 72h in regular intervals and their after for 14 days. During the first 4h after the drug administration, the mice were observed for any gross behavioral change and the parameters such as hyperactivity, grooming, convulsions, sedation, salivation, and loss of righting reflex respiration.^[10]

Phytochemical Screening

Preliminary phytochemical screening for the presences of alkaloids, flavonoids, tannins, glycosides, resins, phenols, volatile oils and saponins was carried out using standard test procedures.^[11]

Treatment schedule

Animals were divided into five groups of six animals each. In the paracetamol induced liver injury model, paracetamol is used as hepatotoxin. Paracetamol (2gm/kg) suspension prepared using 0.1% Tween 80 was administered to all animals except the animal of control group. Syrup Liv. 52 (0.5ml/100gm) was used as a standard. All the drugs were administered orally.

Group I: served as control & received 1.5% Tween 80 in distilled water as vehicle (10ml/kg) for 7 days.

Group II: received hepatotoxin paracetamol (2 g/kg) single dose on 6th day.^[12]

Group III: received hepatotoxin (2 g/kg) single dose and Liv. 52 syrup (0.5ml/100gm) simultaneously for 7 days.^[13]

Group IV: received hepatotoxin (2 g/kg) single dose and BVEE (200 mg/kg) simultaneously for 7 days.

Group V: received hepatotoxin (2 g/kg) single dose and BVEE (400 mg/kg) simultaneously for 7 days.

Biochemical Studies

On the seventh day of treatment, blood samples of the rats were withdrawn from retro orbital plexus with the help of a glass capillary under light ether anesthesia and were allowed to clot for 30min then centrifuged to separate serum. The serum was utilized for the estimation of SGOT, SGPT^[14], ALP^[15] & Serum-bilirubin^[16].

Histopathological studies

One animal from the treated groups showing maximal activity as indicated by improved biochemical parameters from each test, positive control, hepatotoxin and control groups were utilized for this purpose. Animals were sacrificed & dissected. The liver were taken out, washed with water, dried gently with filter paper & preserved in 10% formalin saline. The liver tissues in each group were preserved in 10% formalin saline & proceed for

histopathology. Sections were stain with haemotoxylin-eosin dye & finally observed under microscope for histopathological changes in liver architecture & their photomicrographs were taken.^[17]

Statistical Analysis

The result was expressed as mean \pm S.E.M. & % protection by drug extract hepatotoxin induced charges. The present protection was calculated by considering the difference in enzyme levels between rats treated with hepatotoxin & control. Statistical evaluation was done by Dunnett's test to compare each group treated with control.

Results

Phytochemical Screening

Preliminary phytochemical studies revealed the presence of phenolic compounds, flavonoids, Phytosterols, proteins and amino acids in ethanolic extract of *B. variegata* Linn. leaves.

Acute Oral Toxicity Studies

Neither lethality nor moribund state of the mice was observed up to 72 hrs after EEBV administration and mice exhibited normal behavioral, neurological and autonomic profiles up to 2000 mg/kg.

Biochemical studies

In experiment, it is observed that the level of hepatic biochemical markers i.e. SGOT, SGPT, SALP & Bilirubin is increased in comparison to the control group, shown in table 1. This clearly indicates that there is significant hepatic damage due to the paracetamol. The toxic effect of PCM was controlled in animals treated with ethanolic extract of *B. variegata* 400 mg/kg/day by way of restoration of the markers levels in the liver with comparison to 200mg/kg/day.

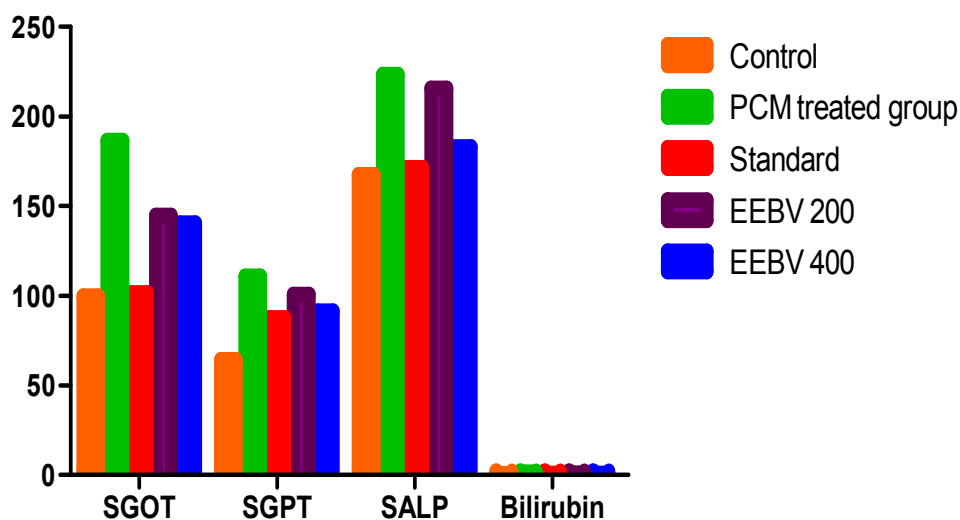
From the bar diagram representation of enzyme level of SGOT, SGPT & ALP shown in figure no. 1, it is concluded that EEBV have potential to reduce the elevated level of biochemical markers when compared with PCM treated group.

Table No. 1: Effect of leaves extract of *Bauhinia variegata* on hepatotoxicity induced by Paracetamol in serum enzyme level

Enzyme group	SGOT	SGPT	SALP	Bilirubin
Control group	99.78 \pm 0.95	64.243 \pm 1.111	167.45 \pm 0.7125	0.785 \pm 0.01607
PCM treated group	186.70 \pm 1.340**	110.84 \pm 0.9324**	223.46 \pm 1.152**	1.53 \pm 0.1028**
Standard group	101.54 \pm 1.201(ns) 97.97%	87.572 \pm 0.7345** 49.93%	171.35 \pm 1.315** 93.03%	0.9150 \pm 0.02277 (ns) 82.55%
EEBV 200mg/kg B.W.	144.84 \pm 1.082** 48.15%	100.75 \pm 0.7978** 21.65%	215.73 \pm 1.644** 13.80%	1.183 \pm 0.05702** 46.57%
EEBV 400mg/kg B.W.	140.40 \pm 2.578** 53.26%	91.433 \pm 2.118** 41.64%	182.96 \pm 3.345** 72.30%	1.022 \pm 0.05199* 68.18%

N=6, **P<0.01 and *P<0.05; Data analyzed by ANOVA followed by Dunnett's test. All groups compared with control group.

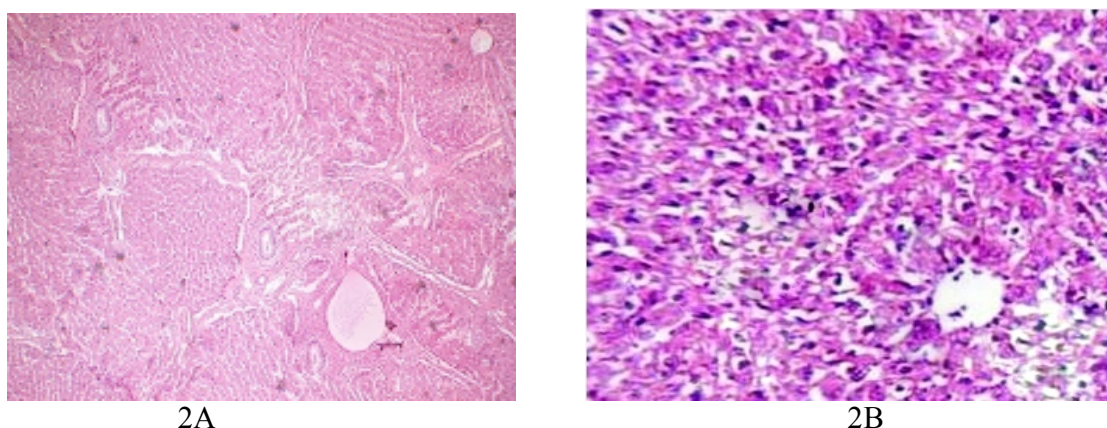
Figure 1: Effect of EEBV on serum enzyme level.

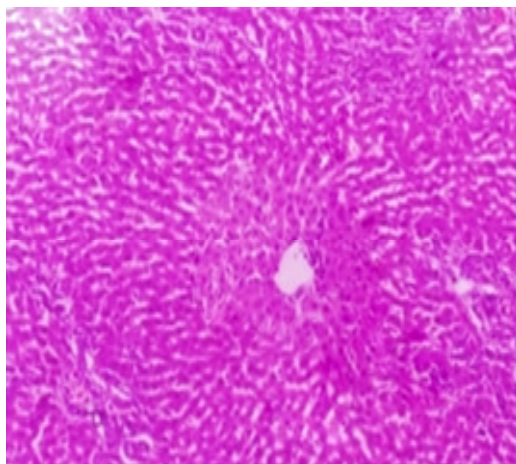


Histopathological Study

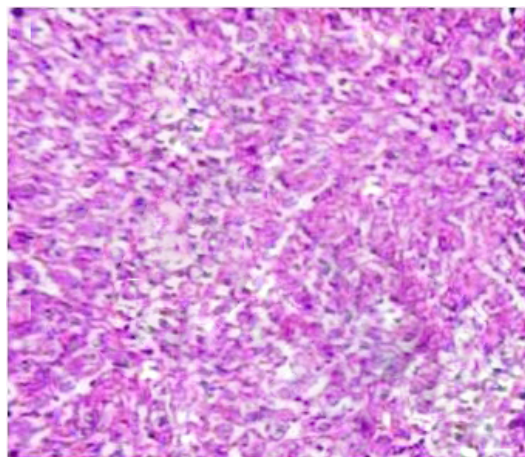
In the histopathological studies, the liver sections of rats (Figure 2) treated with vehicle showed normal hepatic architecture [2A], whereas that of PCM treated group showed total loss of hepatic architecture with intense peripheral central vein necrosis, fatty changes, congestion of sinusoids, Kupffer cell hyperplasia, crowding of the central vein, and apoptosis [2 B]. In case of rats treated with Liv-52 syrup [2C], 200 and 400 mg/kg extract [2D & 2E], respectively, a normal hepatic architecture was seen with only moderate accumulation of fatty lobules and mild degree of cell necrosis, clearly indicating the protection offered by standard drug Liv-52 syrup and the plant extract.

Figure 2: Histopathology of Liver

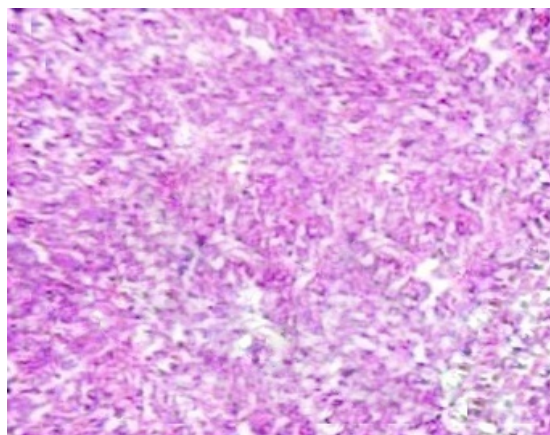




2C



2D



2E

Discussion

Paracetamol is a well known antipyretic & analgesic agent, which is safe in therapeutic doses but can produce fatal necrosis in experimental animals and is employed as an experimental hepatotoxic agent. A sign of hepatic injury is the leaking of cellular enzymes into the plasma due to the disturbances caused in the transport functions of hepatocytes. The estimation of enzymes in the serum is a useful quantitative marker of the extent and type of hepatocellular damage.^[18] The mode of action of paracetamol on the liver is by covalent binding of its metabolite, n-acetyl-p-benzoquinone-amine to sulfhydryl group of protein resulting in cell necrosis and lipid peroxidation.^[19] Due to liver injury caused by PCM overdose, the transport function of the hepatocytes gets disturbed resulting in the leakage of plasma membrane thus causing an increase in serum enzyme levels.^[20]

Chronic administration of paracetamol produced a marked elevation of the serum levels of enzymes in treated animals when compared with that of control group. Treatment with EEBV at dose of 400mg/kg significantly reduced the elevated levels of those enzymes. Treatment with EEBV decreased the serum levels of SGOT & SGPT towards the respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by PCM. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchymal cells. Effective control of ALP, SGOT & SGPT levels points towards an early improvement in the secretory mechanism of the hepatic cells. Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic disease. Hyperbilirubinemia was observed due to excessive heme destruction & blockage

of biliary tract. As a result of blockage of the biliary tract there is a mass inhibition of the conjugal reaction & release of unconjugated bilirubin from damaged & dead hepatocytes.

In this experiment, it is observed that the level of hepatic biochemical markers i.e. SGOT, SGPT, SALP & Bilirubin is increased due to PCM in comparison to the control group. This clearly indicates that there is significant hepatic damage due to the paracetamol. The toxic effect of PCM was controlled in animals treated with ethanolic extract of *B. variegata* Linn. 400 mg/kg by way of restoration of the markers levels in the liver with comparison to positive control Liv 52.

Preliminary phytochemical studies of ethanolic extract of *B. variegata* Linn. leaves shows the presence of flavonoids and phenolic compounds. Earlier investigations have proved the hepatoprotective^[21-23] property of flavonoids and phenolic compounds. The results indicate that EEBV has significant hepatoprotective activity; this may be probably due to the higher content of flavonoids & phenolic compounds in ethanolic extract of *B. variegata* Linn. leaves.

Conclusion

In conclusion, it can be interpreted that EEBV possesses promising hepatoprotective properties, which are probably presences of flavonoids & phenolic compounds. Further studies on isolation and fractionation of the active components from the leaf of *Bauhinia variegata* Linn. are needed.

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