ORIGINAL ARTICLE

Screening of roots of *Baliospermum montanum* for hepatoprotective activity against paracetamol induced liver damage in albino rats

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The objective of the present investigation was to study hepatoprotective activity of alcohol, chloroform and aqueous extract of roots of *Baliospermum montanum* paracetamol induced liver damage model in rats. Liver damage in rats was produced by paracetamol (2 g/kg, po) in tween 80. Alcohol, chloroform, aqueous extracts of roots of the plant was administered to rats daily for seven days. The biochemical parameters were investigated. Histopathological changes in liver were studied. Concurrently silymarin was used as standard hepatoprotective agent. The results indicated that biochemical changes produced by paracetamol were restored to normal by alcohol, chloroform and aqueous extracts. The alcohol and aqueous extract of roots of *Baliospermum montanum* showed significant hepatoprotective effect whereas chloroform extract showed moderate hepatoprotective activity against paracetamol induced liver damage model in rats.

Key words: *Baliospermum montanum*, hepoatoprotective, paracetamol, silymarin

INTRODUCTION

The liver, because of its strategic anatomical location and its large metabolic conversions, is exposed to many kinds of Xenobiotics and therapeutic agents. Moreover, the rapidly growing morbidity and mortality from liver diseases are largely attributable to the increasing number of chemical compounds and environmental pollution. Unfortunately, so far, in the modern era of medicine there is no specific treatment to counter the menacing impact of these dreaded diseases.[1] The therapeutic regimen followed in all these cases up to the present moment is by and large symptomatic and at best palliative, but it still confronts the practitioner with formidable task. Due to this fact efforts to find suitable palliative and /or curative agents for the treatment of liver diseases in natural products of plants and mineral origin are being made.[2] Liver injury induced by paracetamol is the best characterized system of Xenobiotics induced hepatotoxicity in human beings.[3] Large doses of paracetamol, a widely used analgesic-antipyretic drug is known to cause hepatotoxicity in man and laboratory animals. The modern medicines have little to offer for alleviation of hepatic ailments, whereas most important representative are of phytoconstituents.[4]

Baliospermum montanum Muell Arg (Euphorbiaceae) known, as Danti is a well known plant in Ayurvedic system of medicines. The plant is distributed throughout India in plains and low hills. The roots are acrid, thermogenic, anthelmentic, and diuretic. The leaves are good for asthma and bronchitis. The seeds are drastic purgative. Root bark has been reported to possess antileukemic activity. Some important chemical constituents of the roots include baliospermin, montanin.[5,6]

To explore the possibility of using the traditional medicine with proper chemical and pharmacological profile, in recent days, there has been a large volume of work aimed at scientific validation of efficacy of herbal drugs used in the traditional medicine. The modern medicine does not have suitable answer for many conditions such as liver disorder, asthma, cardiovascular disorder etc.

The survey of literature reveals that the root of *Baliospermum montanum* is found to be used in the traditional system of medicine as a liver tonic.[6] However hepatoprotective activity of *Baliospermum montanum* has not been scientifically investigated. Therefore, in the present study, the hepatoprotective effect of different root extracts of *Baliospermum montanum* has been evaluated in paracetamol induced liver damage in the male albino rats (Wistar strain).

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MATERIALS AND METHODS

Plant Material

The roots of *Baliospermum montanum* was collected from local area of Belgaum and authenticated from Botanical survey of India, Koregaon, Pune and voucher specimen (BSI/WC/tech272) was preserved in Pharmacognosy museum at K.L.E.S's college of pharmacy for future details.

Drugs and Chemicals

Paracetamol was obtained as gift sample from Torrent Research center, Ahmedabad, Silymarin was obtained as gift sample from Cadila Pharma Ltd; India. Standard Kit of Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT) and Alkaline phosphatase (ALP) were obtained from Span Diagnostics Ltd. All other reagents used for the experiments were of high analytical grade.

Preparation of Extracts

The Fresh roots of the plant were air dried and powdered to 40 mesh and stored in airtight container till further use. Powder (500 g) was subjected to successive solvent extraction with the solvents in the order of increasing polarity i.e. Petroleum ether (40-60°C), followed by chloroform, alcohol and aqueous. Aqueous extract was performed by cold maceration process.[7] The extracts were subjected to quantitative chemical test such as steroids, tannins, alkaloids, flavonoids. The extracts were dried in hot air oven to obtain semisolid mass. Suspensions of each extract were prepared using 0.1 % between 80 and subjected for hepatoprotective activity against paracetamol-induced hepatotoxicity.

Experimental Animals

Male Wistar albino rats, weighing between 150-200 g were used for the hepatoprotective activity. The animals were housed in polypropylene cages and maintained at 24 ± 2°C under 12h light /dark cycle were fed *ad libitum* with standard pellet diet and had free access to water. They were initially acclimatized for the study protocol and study protocol was approved by Institutional Ethical Committee as per the Requirements of Committee for the purpose of control and supervision of animals (CPCESA), New Delhi. Before conducting the experiment, ethical clearance was obtained from Institutional Animal Ethical Committee K. L. E. S College of Pharmacy, Belgaum, Karnataka.

Acute Oral Toxicity Studies

Wistar albino rats 150-200 g (male), maintained under standard husbandry conditions, and were used for all sets of experiments. The acute oral toxicity study was carried out as per the guidelines set by organization for Economic Co-operation and Development (OECD), received draft guidelines 423, received from committee for the purpose of control and supervision of experiments on Animals (CPCSEA), Ministry of social justice and empowerment, Government Of India. Animals were allowed to take standard laboratory feed and tap water. The alcoholic, chloroform and aqueous extracts were administered to different groups of rats in doses ranging from 100-2000 mg/kg. There is no lethality in any of the groups. One tenth of the maximum dose of the extract, tested for acute toxicity, was selected for evaluation of hepatoprotective activity i.e. 200 mg/kg. The experiments were performed after the experimental protocols had been approved by the Institutional Animal Ethical Committee K. L. E. S's College of Pharmacy, Belgaum, Karnataka.[8]

Evaluation of Hepatoprotective Activity

In the paracetamol induced liver injury model, paracetamol (2 g/kg) suspension prepared using 0.1% Tween 80, was administered to all animals except the animal of the normal control group. Silymarin (100 mg/kg p.o) was used as a standard. The animals were segregated in to six groups of six each. Group 1, which served as normal control receiving 1.5 % Tween 80. Group 2 received paracetamol (2 g/kg, p.o) single dose on 6th day. Group 3 received paracetamol (2 g/kg, p.o) single dose and Silymarin (100 mg/kg, p.o) simultaneously for 7 days. Group 4 received paracetamol (2 g/kg, p.o) single dose and alcoholic extract (200 mg/kg po) simultaneously for 7 days. Group 5 received paracetamol (2 g/kg, p.o) single dose and chloroform extract (200 mg/kg po) simultaneously for 7 days. Group 6 received paracetamol (2 g/kg, p.o) single dose and aqueous extract (200 mg/kg po) simultaneously for 7 days.[9]

On the seventh day of the start of respective treatment the rats were anesthetized by light ether anesthesia and the blood was withdrawn from retro orbital plexus. It was allowed to coagulate for 30 min and serum was separated by centrifugation at 2500 rpm. The serum was used to estimate Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT) and Alkaline phosphatase (ALP).[10]

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. The animals were sacrificed and the abdomen was cut open to remove the liver. Then 5 mm thick piece of the liver were fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12 h and then embedded in paraffin, using conventional methods and cut into 5 m thick sections and stained, using haematoxylin-eosin dye, and finally observed

under microscope for histopathological changes in liver architecture, and their photomicrographs were taken.[11]

Statistical Analysis

The mean values ± SEM was calculated for each parameter. Percentage reduction against the hepatotoxins by the test sample was calculated by considering enzyme level difference between the hepatotoxin treated. For determining the significant inter-group difference, each parameter was analyzed separately, and one way analysis of variance (ANOVA) was carried out. Then the individual comparison of the group means values were done using Dunnett's 't' test procedure.[12]

RESULTS

In the study of the effect of alcohol, chloroform and aqueous extracts of *Baliospermum montanum* on normal liver function, it was found to be non toxic at the selected dose (200 mg/kg) since the parameters SGPT, SGOT and ALP were within like that of control. Paracetamol intoxication in normal rats elevated the levels of SGPT, SGOT and ALP significantly, indicating acute centrilobular necrosis. The rat treated with alcoholic extract and aqueous extract showed a significant reduction in all the biochemical parameter elevated by paracetamol. Whereas, the chloroform extract showed moderate reduction of all three biochemical parameters. This reduction in biochemical parameter exhibited by alcoholic and aqueous extracts is similar when compared with that of silymarin. The percentage reductions of all the three biochemical parameters against hepatotoxin in Table 1.

The histopathological profile of the rat treated with alcohol and aqueous extracts showed no visible changes confirming the safety of the extract at selected dose. Histopathological examination of liver section of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal space. In the liver section of the rats intoxicated with paracetamol [Fig. 1], there is disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis. The liver section of the rats treated with chloroform extract and intoxicated with paracetamol showed moderate hepatoprotective activity [Fig. 2]. While rats treated with silymarin and intoxicated with paracetamol showed less disarrangement and degeneration of hepatocytes, indicating marked regeneration activity [Fig. 3].[13]

Table 1: Effect of root extracts of *Baliospermum montanum* on hepatotoxicity induced by paracetamol

Group (*n*)	SGPT IU/L	SGOT IU/L	ALP IU/L
Control	52.66 ± 2.24	58.05 ± 1.60	58.83 ± 1.27
Paracetamol treated	130.66 ± 3.77	132.66 ± 1.50	131.33 ± 1.72
Alcohol extract	94 ± 2.32	91.66 ± 2.72	98.66 ± 1.74
Chloroform extract	104.05 ± 2.11	106.5 ± 2.72	105.5 ± 2.43
Aqueous extract	103.33 ± 2.89	102.33 ± 2.29	99.66 ± 1.20
Standard drug silymarin	75.05 ± 1.31	78 ± 1.52	79.166 ± 2.66

n = 6, **P*<0.05; Data analysed by ANOVA followed by Dunnett's 't' test. All groups compared with paracetamol (2 g/kg) alone

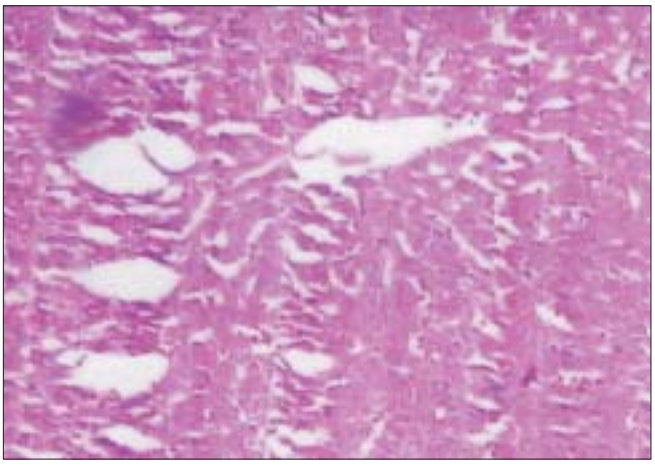

Figure 1: Liver of rat treated with paracetamol

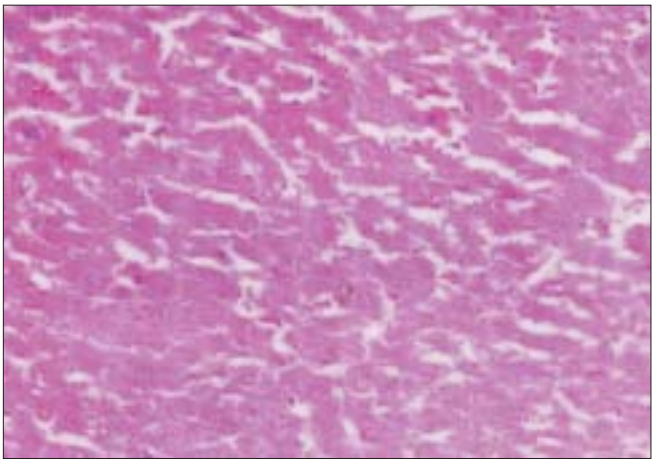

Figure 2: Liver of rat treated with chloroform extract of *B. montanum*

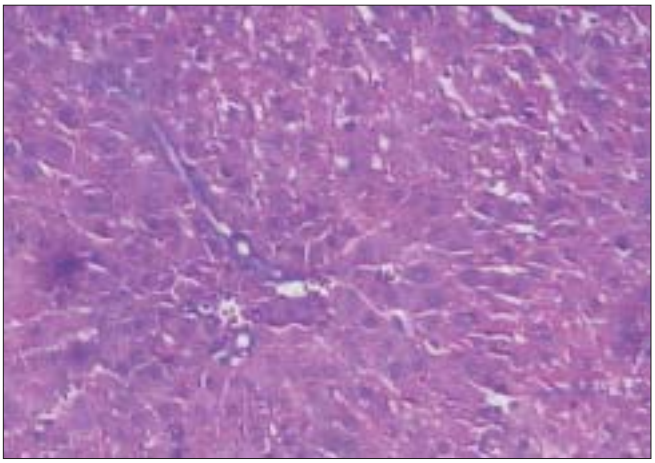

Figure 3: Liver of rat treated with silymarin

DISCUSSION

Paracetamol, an analgesic and antipyretic, is assumed to be safe in recommended doses; overdoses, however, produce hepatic necrosis. Small doses are eliminated by conjugation followed by excretion, but when the conjugation enzymes are saturated, the drug is diverted to an alternative metabolic pathway, resulting in the formation of a hydroxylamine derivative, by cytochrome P_{450} enzyme. Hydroxylamine derivative a reactive electrophillic agent reacts non-enzymatically with glutathione and detoxifies. When the hepatic reserves of glutathione depletes, the hydroxylamine reacts with macromolecules and disrupts their structure and function.

Extensive liver damage by paracetamol itself decreases its rate of metabolism and other substrates for hepatic microsomal enzymes. Induction of cytochrome P_{450} or depletion of hepatic glutathione is a prerequisite for paracetamol-induced toxicity. The Alcoholic and aqueous extract of *Baliospermum montanum* reduced the elevated levels of all the three biochemical parameters by paracetamol. Paracetamol induced liver necrosis was inhibited significantly by *Baliospermum montanum* root extract, which confirms the protective action of the alcoholic and aqueous extract of *Baliospermum montanum* against experimentally induced liver damage in rats. SGPT, SGOT and ALP are the most sensitive tests employed in the diagnosis of hepatic disease. It can be concluded from this investigation that roots of *Baliospermum montanum* possess hepatoprotective activity. Further detailed studies may, however, confirm the utility profile of this drug.

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