HEPATO PROTECTIVE ACTIVITY OF BAUHINIA RACEMOSA LINN

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ABSTRACT

Present work was undertaken to evaluate hepatoprotective activity of Bauhinia racemosa stem bark. The biochemical parameters such as SGOT, SGPT, Total bilirubin, ALP were estimated, the histopathological paraffin sections of rat liver were performed, analyzed to assess the hepatoprotective effect. The test material was found effective as hepatoprotective, through in vivo and histopathological studies. The hepatoprotective effect of ethyl acetate extract was comparable to that of silymarin, a standard hepatoprotective agent.

KEY WORDS: Bauhinia racemosa, histopathological studies, silymarin

INTRODUCTION

The plant Bauhinia racemosa Linn (Fabaceae) is the most widely cultivated variety of the genus Bauhinia and is distributed in the sub-Himalayan ranges of India, Sri Lanka, Mexico, Arabia and South Western Africa. The use of stem bark of Bauhinia racemosa has been reported in all kinds of pain, good vesicant, expectorant, stimulant and abortifacient. The decoction of the root bark is used as a stimulant, analgesic and diuretic. The stem bark is used in the treatment of jaundice1. The pods are edible, seeds are useful as purgative, antipyretic, cures eye diseases, head complaints and are used in venereal affections2. A investigated flavones glycoside has been investigated from the stem bark of Bauhinia racemosa3. The present study was undertaken to screen hepatoprotective activity of the stem bark of Bauhinia racemosa.

MATERIALS AND METHODS

Collection of Plant material

Bauhinia racemosa Linn stem bark were collected from Mangalore D.K.District, Karnataka, India. The plant was identified and authenticated by Dr. Gopalkrishna Bhat, Department of Botany, Poornaprajna College, Udupi. A voucher specimen (NGSM 049) was deposited in Pharmacognosy Department of N.G.S.M. Institute of Pharmaceutical Sciences, Mangalore.

Preparation of extract

The stem bark was collected locally it was air dried under shade, powdered mechanically and stored in airtight containers. About 3 kg of the powdered material was boiled with 10 liter of distilled water for 30 min and filtered to obtain the aqueous extract. The extract was concentrated under reduced pressure and finally dried using the water bath. Another 1 kg of the powdered material was subjected for soxhlation. It was refluxed with ethanol for 48 h in batches of 250 g each. The extracts were pooled together and concentrated in vacuum using rotary flash evaporator (Buchi, Flawil, Switzerland).

Animals

Male Wistar albino rats weighing 150-200 g were obtained from animal house located at K.S.Hegde Medical academy Deralakatte, Mangalore and Karnataka. They were maintained at standard housing conditions and fed with commercial diet (Hindustan Lever Ltd., Bangalore) and provided with water ad libitum during the experiment.

Evaluation of hepatoprotective activity

The acute toxicity studies were carried out as per staircase method4. Fifty male rats were divided into five groups of 10 each and were administered with aliquot doses of the extracts orally (200, 400, 800, 100 and 2000 mg/kg). Mortality was not noticed up to 2000 mg/kg. The LD50 of the extracts was found to be 2000 mg/kg body weight. One-tenth of this dose was selected as the therapeutic dose for the evaluation5. Five groups of animals containing six each were used for the study. The animals from Group I served as the control and received the vehicle 1% w/v gum tragacanth at a dose of 1 ml/kg/day of p.o. for 14 days. Groups II–IV received 0.1...
ml/kg/ day i.p. of CCl₄ (E-Merck, Mumbai, India) for 10 days. The standard drug Silymarin (Ranbaxy Lab. Dewas) was administered to Group III animals in the dose of 100 mg/kg/day p.o. for 14 days. While, Groups IV was treated with ethyl acetate extract of Bauhinia racemosa Linn in the dose of 25 mg/kg/day, p.o. (as per acute toxicity studies) for 14 days, respectively. The CCl₄, silymarin and the extracts were administered concomitantly to the respective groups of animals.

Assessment of hepatoprotective activity
All the animals were killed on day 14 under light ether anaesthesia. The blood samples were collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 min and biochemical investigations were carried out to assess liver function viz., total bilirubin, serum transaminases, and serum alkaline phosphatase (Raichem, San Diego, CA 92111-1203). The results are expressed as mean±SEM of six animals from each group. The data were evaluated by one-way ANOVA followed by Tukey’s multiple comparison tests. P values <0.01 were considered statistically significant. Histopathology After draining the blood, liver samples were excised, washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Paraffin sections were taken at 5 mm thickness, processed in alcohol–xylene series and were stained with alum haematoxylin and eosin.

RESULTS AND DISCUSSION
The sections were examined microscopically for histopathological changes. Histological profile of the control animals showed normal hepatocytes. Group II animals exhibited intense centrilobular necrosis (N), vacuolization (F) and macrovesicular fatty change. The sections of liver taken from the animals treated with standard drug silymarin showed the hepatic architecture, which was similar to that of control. The animals treated with ethyl acetate extract exhibited significant liver protection against the toxicant as evident by the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration. The administration of CCl₄ to the animals resulted in a marked increase in total bilirubin, serum amino transaminase (SGOT and SGPT) and serum alkaline phosphatase activities. The toxic effect of CCl₄ was controlled in the animals treated with the ethyl acetate extract by way of restoration of the levels of the liver function biochemistry similar to that of the standard drug silymarin (Table 1). The Carbon tetrachloride (CCl₄) has been used to induce hepatotoxicity in experimental animals. This toxic chemical caused peroxidative degradation in the adipose tissue resulting in fatty infiltration of the hepatocytes. The increase in the levels of serum bilirubin reflected the depth of jaundice and the increase in transaminases and alkaline phosphatase was the clear indication of cellular leakage and loss of functional integrity of the cell membrane. Administration of ethyl acetate extract of Bauhinia racemosa Linn also showed positive for flavonoids by ferric chloride, alkaline reagent and Shinoda tests. Further, it has been reported that the flavonoid constituents of the plant possess antioxidant properties and was found to be useful in the treatment of liver damage. The administration of hepatoprotective drugs may induce the hepatocytes to resist the toxic effect of CCl₄. The results indicate that the ethyl acetate extract of Bauhinia racemosa Linn has significant hepatoprotective activity. This may be probably due to the higher content of flavonoids.

REFERENCES
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Table 1: Effect of Bauhinia racemosa on CCl₄ treated rats

<table>
<thead>
<tr>
<th>Design of treatment</th>
<th>Liver (wt/100g body wt)</th>
<th>Dose (mg/kg)</th>
<th>SGPT U/L</th>
<th>SGOT U/L</th>
<th>ALP U/L</th>
<th>Total Bil (mg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>358 ± 0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCl₄</td>
<td>6.5 ± 0.35</td>
<td>1.25 ml/kg</td>
<td>131.45 ± 1.44</td>
<td>45.9 ± 0.85</td>
<td>164.3 ± 3.23</td>
<td>0.74 ± 0.05</td>
</tr>
<tr>
<td>Silymarin + CCl₄</td>
<td>3.4 ± 0.39*</td>
<td>100</td>
<td>134.0 ± 1.15**</td>
<td>82.2 ± 9.33*</td>
<td>213.6 ± 5.47**</td>
<td>0.6 ± 0.06*</td>
</tr>
<tr>
<td>Ethyl acetate extract + CCl₄</td>
<td>4.6 ± 0.45*</td>
<td>400</td>
<td>115.2 ± 1.13*</td>
<td>64.4 ± 5.35*</td>
<td>294.5 ± 5.56*</td>
<td>0.85 ± 0.09</td>
</tr>
</tbody>
</table>

N = 6 animals in each group. *P < 0.001; **P < 0.01 when compared with control.
Values are expressed as mean ± SE.

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