PHARMACOLOGICAL INVESTIGATION OF LEAVES OF ANTHOCEPHALUS CADAMBA (ROXB) FOR HEPATOPROTECTIVE ACTIVITY

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ABSTRACT

The objective of the present investigation was to study hepatoprotective activity of ethanolic extract of leaves of Anthocephalus cadamba, paracetamol induced liver damage model in rats. In this study, liver damage in rats done by paracetamol (2g/kg b.w.) in Tween 80. The ethanolic extract of the plant was administered in rats daily for seven days. The biochemical parameters were investigated. Histopathological changes in liver were studied. Silymarine was used as a standard hepatoprotective agents. The result indicates that biochemical changes produced by paracetamol were restored to normal by ethanolic extract. The ethanolic extract of leaves of Anthocephalus cadamba (ROXB) showed significant hepatoprotective effect against paracetamol induced liver damage model in rats.

Keywords: Anthocephalus cadamba, hepatoprotective, Silymarine, Paracetamol

INTRODUCTION

Anthocephalus cadamba (ROXB) widely distributed throughout india, and is used as a folk medicine in the treatment of fever, anaemia, uterine complaints, blood diseases, skin diseases, leprosy, dysentery and for improvement of semen quality ⁷. The traditional uses of Anthocephalus cadamba (ROXB) bark use in the treatment of hoarseness of throat. The traditional healers use cadamba bark in the treatment of eye diseases. They also prefer the decoction of leaves in place of bark in some purpose. The fruit juice is given to the children to treat gastric irritability. A decoction of leaves is good for ulcers and wounds. The liver play a major role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents when taking a overdose and sometime even when introduced within the therapeutic ranges may cause of injury of the organ. Other chemical agents such as those used in the laboratories and industries, natural chemicals (Microcystine) and herbal remedies can also induced hepatotoxicity. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Liver injury induced by paracetamol is the best characteristic system of xenobiotic induced hepatotoxicity in the human beings ⁶. Large doses of paracetamol, a widely used analgesic-antipyretic drug is known to cause hepatotoxicity...
in men and laboratories animals 4.

**MATERIAL AND METHOD**

**Plant collection**

The fresh leaves were collected during the month of August 2011, from the District Jhansi U.P.). The plant material authenticated by Dr. Neelima Sharma (research officer) NBRI, Lucknow, with the assesion number 5620.

**Drug and Chemical**

All the chemicals were Analytical Grade. Paracetamol was obtained from Amol Pharma, Jaipur Rajasthan) and Silymarine from Krypten Pharma Ltd., India. Standard kit of Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT) and Alkaline Phosphate(ALP).

**Preparation of Extract**

The leaves of *Anthencephalus cadamba* (ROXB) should be air dried and make coarse powder by grinder. The coarsely powdered leaves(250 gm) was packed in Soxhlet Appratus and continuously extracted with ethanol(60°-80°C) till the complete extraction. After completion of extraction, the solvent was removed by distillation and then concentrated extract obtained was dried under reduced pressure using Rotatory Evaporator at the temperature not exceeding 40°C and then the residue dried in the vacuum oven at 760 mm Hg pressure at 37°C, the residue found in powder form. The ethanolic extract obtained green in colour, kept in petri dish and stored in dessicator.

**Experimental Animals**

Healthy Wistar albino rats weighing about (180-250gm) of either sex were obtained from animal house, Institute of Pharmacy, Bundelkhand University, Jhansi. The animal were housed in specific standard laboratory conditions. The conditions were kept in a temperature-controlled environment (25±1°C) and with a regular 12h light/12hr dark cycle. All animals were fed with commercial diet and water during experiment. All protocols of the study were approved by Instititutional Animal Ethical Committee with reference number BU/PHARM/IAEC/11/O26. The IAEC is approved by committee for the purpose of control and supervision of experiments of animals (CPCSEA) with registration number 716/02/a/CPCSEA.

**Evaluation of Hepatoprotective Activity**

In the Paracetamol induced liver injury model, Paracetamol (2g/kg) suspension prepared using 0.1% Tween80, was administered to all animals except the animal of the normal control group. Silymarine (100mg/kg) was used as a standard. The animals were divided in to six group of five in each group. Group1, which served as normal control receiving 1.5% Tween80. Group2 received Paracetamol (2g/kg) single dose on 6th day. Group3 received Paracetamol (2g/kg) single dose and Silymarine (100mg/kg) simultaneously for 7 days. Group 4 received Paracetamol ([2g/kg] single dose and ethanolic extract (200mg/kg) simultaneously for 7 days. Group 5 received Paracetamol (2g/kg) single dose and ethanolic extract (400mg/kg) simultaneously for 7 days. Group 6 received Paracetamol (2g/kg) single dose and ethanolic extract (600mg/kg) simultaneously for 7 days. On the 7th day from the start of respective treatment, the rats were anaesthetized by light ether anaesthesia and the the blood was withdrawn from the retro arterial plexus. It was allowed to coagulate for 30 min and serum was separated by centrifugation at 2500rpm. The serum was used to estimate Serum Glutamate Piruvate Transaminase (SGPT) and Alkaline Phosphate (ALP).

**Histopathological Studies**

One animal from the treated group showing maximal activity as indicated by improved biochemical parameters. From each test positive control, hepatotoxins and control groups were utilized for this purpose. The animals were sacrificed and the abdomen was cut open to remove the liver, then 5mm thick piece of the liver were fixed in Bounis Solution(mixture of 75ml of saturated picric acid, 25ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12 hr, and then embedded in paraffin using conventional method and cut in to 5mm thick section and stained using haematoxylin-eosin dye, finally observed under microscope for histopathological changes in liver architecture and thin photomicrographs were taken.

**Statistical Analysis**

The mean value + 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 differences, each parameter was analyzed separately and one way ANOVA was carried out.
Table 1: Effect of ethanolic extract of leaves of *Anthacephalus cadamba* on hepatotoxicity induced by paracetamol

<table>
<thead>
<tr>
<th>Group 1(n)</th>
<th>SGPT IU/L</th>
<th>SGOT IU/L</th>
<th>ALP IU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>68.20± 3.31</td>
<td>72.04± 1.70</td>
<td>73.52± 2.12</td>
</tr>
<tr>
<td>Paracetamol treated</td>
<td>170.62± 3.72</td>
<td>172.33± 1.20</td>
<td>171.18± 1.23</td>
</tr>
<tr>
<td>Standard drug(Silymarine)</td>
<td>86.04± 1.32</td>
<td>88.10± 2.01</td>
<td>87.14± 1.62</td>
</tr>
<tr>
<td>Ethanolic extract(200mg/kg)</td>
<td>121.12± 1.18</td>
<td>118.68± 1.49</td>
<td>120.27± 2.38</td>
</tr>
<tr>
<td>Ethanolic extract(400mg/kg)</td>
<td>112.12± 1.10</td>
<td>114.01± 2.20</td>
<td>113.28± 1.08</td>
</tr>
<tr>
<td>Ethanolic extract(600mg/kg)</td>
<td>104.20± 2.14</td>
<td>103.30± 1.19</td>
<td>103.41± 1.28</td>
</tr>
</tbody>
</table>

N=6, p< 0.04; data analysed by ANOVA followed by dunnett ‘t’ test. all group compared with paracetamol(2g/kg) alone.

Figure 1: Histopathology of liver of rat with vehicle, paracetamol, silymarine & ACEE*(with different doses)
A - liver of rat treated with vehicle, B - liver of rat treated with paracetamol, C - liver of rat treated with silymarine, D - liver of rat treated with ACEE*(200mg/kg ), E - liver of rat treated with ACEE*(400mg/kg ), F - liver of rat treated with ACEE* (600mg/kg )
ACEE* - *Anthacephalus cadamba* ethanolic extract.
RESULT

Microscopic observation of cell necrosis, fatty change, hyaline degeneration, ballooning degeneration and infiltration of Kupffer cell and lymphocytes were performed. In the study of the effect of ethanolic extract of Anthocephalus cadamba with different doses (200, 400, 600 mg/kg) on normal liver function were observed. It was found to be non toxic at given doses. 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. Group 3 (which received Silymarine and Paracetamol) shows normal architecture of liver tissues and cells of hepatocytes and also indicating regeneration activity. Group 4 (which received plant extract 200 mg/kg b.w. and Paracetamol) showing mild hepatoprotective activity. Group 5 (which received plant extract 400mg/kg b.w. and Paracetamol) showing significant protection and normal architecture tissues and cells against given hepatotoxins. Group 6 (which received plant extract 600mg/kg b.w. and Paracetamol) showing significant protection of normal architecture of liver tissues and cells against given hepatotoxins.

DISCUSSION

Paracetamol, an analgesic and antipyretic is assumed to be safe in recommended doses. Overdose however produce hepatic necrosis. Small doses are eliminated by conjugation followed by excretion. Overall study reveals that the ethanolic extract of leaves of Anthocephalus cadamba shows the hepatoprotective activity in comparnings to silymarine shows that SGOT, SGPT, ALP were elevated in animals receiving Paracetamol and reduce the serum enzyme level. In group 3, Silymarine group as positive control at the dose (100mg/kg b.w.). Group 4, received Paracetamol and ethanolic extract of Anthocephalus cadamba leaves at a dose (200mg/kg b.w.), and group 5, Paracetamol and ethanolic extract of Anthocephalus cadamba leaves at a dose (400mg/kg b.w.). In group 6, Paracetamol and ethanolic extract of Anthocephalus cadamba leaves at a dose (600mg/kg b.w.) ethanolic of Anthocephalus cadamba shows significant reduction in elevated levels of biochemical marker as compared with Silymarine.

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