Hepatoprotective potential of ethanolic extract of *Caesalpenia crista* leaves against paracetamol induced hepatotoxicity in rats

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ABSTRACT

**Objective:** To investigate the hepatoprotective activity of ethanolic extract of leaves of *Caesalpenia crista* (C. crista) against paracetamol induced hepatotoxicity in rats.

**Methods:** Paracetamol (2 g/kg body weight) was used to induce hepatotoxicity in albino rats. Ethanolic extract of leaves of *C. crista* was administered at the dose levels of 200 and 400 mg/kg body weight orally for 7 d. Silymarin (100 mg/kg) was used as standard drug. The hepatoprotective effect of ethanolic extract was evaluated by assessment of biochemical parameters such as serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, serum alkaline phosphatase, bilirubin (total and direct), and triglycerides content. Histopathological study of rat liver was also done.

**Results:** Administration of ethanolic extract at doses 200 mg/kg and 400 mg/kg body weight exhibited significant reduction in elevated level of serum marker enzymes, bilirubin (total and direct) and triglycerides when compared to positive control group.

**Conclusions:** It is concluded that the ethanolic extract of *C. crista* leaves seems to justify the promising hepatoprotective effect on paracetamol induced liver damage in rats.

**Keywords:** *Caesalpinia crista*, Paracetamol, Hepatoprotective, SGOT, SGPT, Bilirubin, Histopathology

Comment:
The present study reports the toxicology test of a local species from coastal area of India. The work is a good and standard study on ethnopharmacology. New information can be derived and this is useful for further drug research and development. In addition, the information from this work can be added in the database on pharmacology, ethnopharmacology and toxicology.

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1. Introduction

Liver is the main organ which regulates many important metabolic functions. Hepatic injury is directly associated with these altered metabolic functions[1]. Liver diseases such as jaundice, cirrhosis and fatty liver are rampant and large public health problem in the world. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are...
inadequate and sometimes can have serious side effects[2]. In the past, several studies have been carried out to examine the effect of plants used traditionally by herbalists to support normal liver function and treat the diseases of liver. So far, various experimental evidences have confirmed the efficacy of plants such as Silybum marrium (milk thistle), Curcuma longa (turmeric) [3] and Nymphaea stellata [4]. Despite the tremendous strides in modern medicines, there is still a need for a drug that stimulates liver function or offers protection to the liver from damage or helps regeneration of hepatic cells [5].

Caesalpinia crista (C. crista) (Family: Fabaceae), commonly known as karanja in Hindi, is an extensive shrubby perennial climber distributed throughout India in planes on waste land and coastal areas up to the altitude of 1000 m in the hills [6]. C. crista contains various phytoconstituents such as alkaloids, saponin glycosides and flavonoids. The seeds of the plant contain aspartic acid, arginine and citroline. A new diterpine δ-caesalpin is isolated from seeds karnel [7]. This plant has profound medicinal use and is a proved anti-inflammatory, anthelmintic and antimalarial drug. It has also been an effective stomachic, digestive and was used as liver tonic in the treatment of jaundice and various liver disorders. The seeds are useful as antidiabetic, antiperiodic, antipyretic, etc [8].

2. Materials and methods

2.1. Collection and authentication of plant material

The leaves of C. crista were collected in the month of March from local area of Moradabad and authenticated by Dr. Saini DC, Senior Scientist, Palaeobotany, Birbal Sahni Institute of Palaeobotany, Lucknow, India. A voucher specimen No. BSIP 10 was deposited in BSIP herbarium and crude drug sample is preserved in the Department of Pharmacognosy, Teerthanker Mahaveer College of Pharmacy, TMU, Moradabad.

2.2. Preparation of extract

The fresh leaves were cleaned, shade dried and mechanically reduced to moderately coarse powder. The dried powder was defatted with petroleum ether (60-80 °C) and then extracted with ethanol. Both extracts were concentrated by using rotary evaporator at 40 °C under reduced pressure and then the extracts were evaporated on a thermostat-controlled water bath till complete drying. The extracts were subjected to preliminary phytochemical investigation.

2.3. Preliminary phytochemical investigation

In order to identify the presence of various phytoconstituents such as carbohydrate, alkaloids, glycosides, phenolic compounds etc., a preliminary phytochemical study was performed with both extracts [9-11].

2.4. Chemicals

All chemicals and solvents were of analytical grade and procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India. Paracetamol was procured from E. Merck (India) Ltd., Mumbai. Standard kits for biochemical estimation of serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP) and bilirubin were collected from Span Diagnostics Ltd., India.

2.5. Preparation of the drug solution

The ethanolic extract of C. crista was dissolved in normal saline to prepare dose of 200 and 400 mg/kg body weight.

2.6. Acute toxicity study

The acute toxicity study was carried out as per the guidelines set by OECD-423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Govt. of India [12]. The ethanolic extract was orally administered to adult Wistar albino rats. The groups were continuously observed for mortality and behavioral changes during the first 24 h and then daily for a fortnight. The oral LD50 was found to be more than 3000 mg/kg.

2.7. Experimental animals

Wistar rats weighing 180 to 200 g were housed in standard laboratory conditions of temperature (22±2 °C), 12 h light and dark places with food and water ad libitum. Animal experiment was approved by Institutional Animal Ethical Committee (IAEC) of Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad (Reg No. 1205/c/09/CPCSEA).

2.8. Assesment of hepatoprotective activity

Wistar rats were divided into five groups with six animals in each group (Control, Positive control, Standard, Test low and high dose). Group I: control group received saline 1 mL/kg for 1 week; Group II: positive control group received saline 1 mL/kg for one week; Group III: standard group received silymarin (100 mg/kg p.o.); Groups IV and V: test low and high doses groups received 200 and 400 mg/kg oral dose of ethanolic extract respectively once a day for 1 week. On Day 5, after the administration of respective treatments, all the animals of Groups...
II, III, IV and V were administered paracetamol at a dose of 2 g/kg p.o. On Day 7, i.e. after 48 h of pharmacological treatments, blood was withdrawn by retro orbital puncture for the estimation of biochemical parameters[13].

2.9. Biochemical estimation

Blood samples were centrifuged for 10 min at 7 000 r/min using micro-centrifuge to separate the serum. The biochemical parameters such as SGOT, SGPT, SALP, bilirubin (total and direct) and triglycerides were estimated. The animals were sacrificed by overdose of ether and autopsied[14]. Livers from all animals were removed, washed with ice cold saline, weighed. Small piece of liver tissue was collected and preserved in 10% formalin solution for histopathological studies.

2.10. Histopathological studies

The liver samples of all experimental groups were preserved in 10% neutral formalin, dehydrated in graded alcohol and then embedded in paraffin. Microtome sections (5 μm) were prepared from each liver sample and stained with haematoxylin-eosin dye[5]. The reactions were examined for the pathological findings of hepatotoxicity.

2.11. Statistical analysis

The results were expressed as mean±SEM. The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Bonferroni’s Multiple Comparison Test.

3. Results

3.1. Phytochemical screening

Preliminary phytochemical screening of ethanolic extract revealed the presence of carbohydrate, alkaloids, glycosides, flavonoids and phenolic compounds. Based on these phytoconstituents, ethanolic extract was selected for pharmacological evaluation.

3.2. Acute toxicity

The extract was found to be safe in the dose used and no mortality up to a dose of 3 000 mg/kg, body weight for ethanolic extract was observed. Hence, 200 and 400 mg/kg body weight p.o. were selected for the activity.

3.3. Hepatoprotective activity

The results of hepatoprotective activity of ethanolic extract of leaves of C. crista on paracetamol treated rats are shown in Table 1. The hepatic enzymes SGPT (125.35±1.90) IU/L, SGOT (244.67±0.35) IU/L, SALP (190.50±2.03) IU/L, direct bilirubin (0.980±0.020) mg/dL, total bilirubin (1.880±0.130) mg/dL and triglycerides (195.20±1.88) mg/dL in serum was significantly increased in paracetamol treated animals when compared to control. The ethanolic extract of C. crista on 200 and 400 mg/kg dose treatments significantly reversed the levels of SGPT [(92.44±1.12) and (84.79±0.40) IU/L], SGOT [(138.28±0.86) and (126.80±0.22) IU/L], SALP [(199.50±1.68) and (83.60±2.05) IU/L], direct bilirubin [(0.310±0.040) and (0.280±0.040) mg/dL], total bilirubin [(0.742±0.050) and (0.680±0.050) mg/dL] and triglycerides [(159.50±0.58) and (148.40±1.27) mg/dL] respectively when compared to paracetamol alone treated rats. Silymarin (25 mg/kg) treated animals also showed significant decrease in SGPT (74.64±0.90) IU/L, SGOT (121.13±0.60) IU/L, SALP (79.66±1.18) IU/L, direct bilirubin (0.200±0.010) mg/dL, total bilirubin (0.640±0.040) mg/dL and triglycerides (145.70±0.88) mg/dL levels when compared to paracetamol alone treated rats.

3.4. Histopathology

The histological observations of control rats did not show any alterations in the hepatocytes. The liver of normal rats exhibited normal architecture of hepatocytes with no sign of necrosis and pycnosis and cytoplasmatic vacuolation (Figure 1A). In toxic control group, liver sections showed marked vacuolisation surrounding central vein, mild portal inflammation, feathery degeneration, focal hepatocyte necrosis, sinusoidal dilation

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Biochemical parameters</th>
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<tr>
<td></td>
<td>SGPT (IU/L)</td>
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<tr>
<td>Group I (control)</td>
<td>66.06±0.74</td>
</tr>
<tr>
<td>Group II (paracetamol 2 g/kg)</td>
<td>125.35±1.90</td>
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<tr>
<td>Group III (silymarin 100 mg/kg)</td>
<td>74.64±0.90</td>
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<tr>
<td>Group IV (200 mg/kg C. crista leaves)</td>
<td>92.44±1.12</td>
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<tr>
<td>Group V (400 mg/kg C. crista leaves)</td>
<td>84.79±0.40</td>
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All values are expressed as mean±SEM for six rats in each group. *P<0.05 denote value significantly different from control, **P<0.01, ***P<0.001 denote value significantly different from positive control.
and apoptosis due to paracetamol (Figure 1B). In group treated with silymarin, liver sections showed normal architecture with regenerative activity with mild focal vacuolation, no sinusoidal dilation, necrosis and apoptosis but somewhere diffuse vacuolation in hepatocytes with intervening normal areas were also present (Figure 1C). In group treated with ethanolic extract (200 mg/kg and 400 mg/kg), liver sections showed patchy hepatocyte vacuolation with regenerative activity and intervening large areas of normal hepatocytes. Although the extent of area of necrotic cells was highly reduced and improved histology of liver was less than that of standard silymarin (Figure 1D and E).

Figure 1. Histopathology of liver tissues (×10).
A: Photomicrograph of liver from control group animal showing normal architecture with no necrosis and no cytoplasmic vacuolation; B: Photomicrograph of liver from animal of toxic (positive control) group treated with 2 g/kg, p.o. of paracetamol showing marked vacuolation and portal inflammation; C: Photomicrograph of liver from animal treated with paracetamol and silymarin showing normal architecture with regenerative activity with mild focal vacuolation; D: Photomicrograph of liver from animal treated with paracetamol and low dose (200 mg/kg) of ethanolic extract of C. crista leaves showing patchy hepatocyte vacuolation with regenerative activity and area of normal hepatocytes; E: Photomicrograph of liver from animal treated with paracetamol and high dose (400 mg/kg) of ethanolic extract of C. crista leaves showing patchy hepatocyte vacuolation with regenerative activity and area of normal hepatocytes.

4. Discussion

Overdose of paracetamol causes a potentially fatal, hepatic centrilobular necrosis. The hepatotoxicity of paracetamol has been attributed to the formation of a toxic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) by the action of cytochrome P450E111. NAPQI is normally detoxified by conjugation with reduced glutathione to form mercapturic acid which is excreted in urine. Toxic overdose of paracetamol depletes hepatic reduced glutathione content so that free NAPQI binds covalently to cellular macromolecules causing acute hepatocellular necrosis[15].

Normally, SGOT and SGPT are present in high concentration in liver. The elevated levels of SGOT, SGPT and SALP in the blood are indicative of cellular leakage and loss of functional integrity of hepatic cell membranes implying hepatocellular damage. Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte[16].

The increased triglycerides level in paracetamol induced rats revealed impaired fat metabolism due to hepatic damage. The supplementation of ethanolic extract of C. crista leaves decreased the elevated serum enzyme activities, bilirubin and lipid contents with elevation of total protein content in the paracetamol treated rats which are comparable to the normal control group. It appears that the extracts preserved the structural integrity of the hepatocellular membrane which is evident from the significant reduction in paracetamol-induced rise in serum marker enzymes in rats.

Therefore, on the basis of the results, the possible mechanism of hepatoprotective effect of C. crista might be due to various phytoconstituents such as alkaloids, flavonoids and other phenolic constituents present in ethanolic extract. The histological examination of liver sections revealed that the normal cellular architecture was retained as compared to silymarin, thereby confirming the protective effect of the extract.

Further studies are in progress to isolate the active constituents of C. crista and also evaluate the exact mechanism of action for the hepatoprotective activity.

The ethanolic extract of leaves of C. crista has shown the ability to maintain the normal functional statues of the liver. From the above preliminary study, it can be concluded that the ethanolic extract is proved to be one of the herbal remedies for liver ailment.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background
Liver diseases such as jaundice, cirrhosis and fatty liver are rampant and large public health problem in the world. However, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. Hence it is needed to search for a drug that stimulates liver function or offers protection to the liver from damage or helps regeneration of hepatic cells.

Research frontiers
This report provides new data on ethnopharmacology of local species in coastal area of India. The work can be a further referencing for similar reports in this medical area.

Related reports
There are some similar reports, but those works did not focus on the specific species that is presently studied. This work remains its original and has new data.

Innovations and breakthroughs
This study gives a new knowledge on the specific species. This work is a standard ethnopharmacological study that can give a new information to the followers in this field.

Applications
This work can be further applied in the future biomedical study on this species. The work represents a good example of ethnopharmacology investigation.

Peer review
The present study reports the toxicology test of a local species from coastal area of India. The work is a good and standard study on ethnopharmacology. New information can be derived and this is useful for further drug research and development. In addition, the information from this work can be added in the database on pharmacology, ethnopharmacology and toxicology.

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