Hepatoprotective effect of *Caesalpinia crista* Linn. against CCl₄ and paracetamol induced hepatotoxicity in albino rats

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This study was done to investigate the hepatoprotective effect of extracts of *Caesalpinia crista* against carbon tetrachloride and paracetamol induced liver toxicity in albino rats. Seeds of *C. crista* were subjected to ethanolic and aqueous extraction. Albino rats were exposed to carbon tetrachloride (3 ml/kg rat b.w) and paracetamol (3 g/kg rat b.w) in two different protocols. Seven groups (n = 6) of animals were used in each protocol. Olive oil was used as vehicle. Rats treated with extracts of *Caesalpinia crista* exhibited a significant reduction in CCl₄ and paracetamol induced increase in serum levels of alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), total bilirubin and also caused a significant increase in serum level of total proteins which was decreased by hepato-toxic compounds used. The protective effect of these extracts was comparable with Silymarin. Ethanolic extract of *C. crista* was able to normalize the biochemical levels and histopathological changes which were altered due to CCl₄ and paracetamol intoxication.

**Key words:** Hepatotoxicity, *Caesalpinia crista*, paracetamol, CCl₄.

**INTRODUCTION**

Liver is one of the largest organs in human body and chief site for intense metabolism and excretion. It has a surprising role in the maintaining, performing and regulating homeostasis of the body (Ward et al., 1999). The liver disorders are a world problem (Bruck et al., 1996). Due to excessive exposure to hazardous chemicals the free radicals generated will be so high such that they overpower the natural defensive system leading to hepatic damage and causes jaundice, cirrhosis and fatty liver, which remain one of the serious health problems (Zimmerman and Hayman, 1976). Hepatotoxicity rate has been reported, much higher in developing countries for example like India (8 to 30%) as compared to advanced countries (2 to 3%) with a similar dose schedule (Sharma, 2004).

The Indian system of medicine (Ayurveda) recommends...
a number of medicinal preparations for the treatment of liver disorders as there is an absence of a reliable liver protective drug in the modern system of medicine (Chatterjee, 2000). Natural remedies from medicinal plants are considered to be effective and safe alternative treatments for hepatotoxicity has been reported to be much higher in developing countries like India (8 to 30%) compared to that in advanced countries (2 to 3%) with a similar dose schedule (Sharma, 2004).

Caesalpinia crista belonging to the plant Caesalpiniiaceae is a medicinal plant growing widely throughout India and tropical countries of the world (Kirtikar et al., 1999). It is a large straggling and very thorny shrub. Traditionally, in Ayurveda, this plant was used for the treatment of gynecological disorders, constipation, piles, skin diseases and ulcers (Williamson, 2002). Most widely used part is seed kernel which is reported as a rich source of norcassane and norcassane type diterpenoids (Kalauni et al., 2005). The stem part and root part constitutes two novel peltogynoids, 6-methoxyfurcularim and pulcherrimin and one novel homoisoflavonoid 8-methoxyfurcularim (Cheenpracha et al., 2005). Its seeds are reported as antipyretic, anti-inflammatory, anthelmintic and antimalarial antidiuretic, antianaphylactic, antibacterial, antiarrhoeal, antiamoebic and antiviral properties (Dhar et al., 1968). It has been reported that the methanol extract of C. crista seed and seed kernel possess anti-feedant and anthelmintic property (Jabbar et al., 2007). So after extensive literature survey, the present study was designed to assess the hepatoprotective activity of the different extracts of seeds parts of C. crista against liver damage and to authenticate the data by using different models for analyzing the hepatoprotective activity.

MATERIALS AND METHODS

Chemicals

All the chemicals used in this research were of analytical grade and obtained from local market of Jaipur and assay kits for the estimation of biochemical factor were purchased from span diagnostics.

Plant

The seeds of C. crista were procured from the local market of Ropar, Punjab in the month of December, 2011. The seeds were authenticated by Mr. Madan Pal, Ex. Eng., Horticulture Division No.-2, Chandigarh.

Preparation of extract

Shade dried part (seeds) of plant were powdered (200 g) coarsely and firstly extracted with petroleum ether for defatting and then ethanol by Soxhlet apparatus for 72 h and aqueous extracted was prepared by maceration process. The obtained extracts were concentrated until dryness under reduced pressure and controlled temperature (40 to 50°C) using flash evaporator and then preliminary phytochemical screening were performed (Kokate et al., 1997). Percentage yield of all extracts were calculated. The % yield of ethanolic and aqueous extracts was found to be 8.7 and 13.3%. The LD50 determination of C. crista seed extract was reported by Kshirsagar (2011).

Experimental animals

Wistar albino rats weighing 180 to 200 g of either sex maintained under standard husbandry conditions at temperature 23 ± 2°C, relative humidity 55 ± 10% and 12 h light dark cycle. Animals were fed with standard laboratory food and water ad libitum. Experiments on animals were performed after taking the approval of experimental protocols by the institutional animal ethics committee under the registration no.IAEC/NIMS/PH/JPR/12/2011.

Active phytochemical constituents

Crude extracts (ethanolic and aqueous) of the plant C. crista contains phytoesters, alkaloids, triterpenoids, saponins, flavonoids, tannins, carbohydrates, reducing sugars, proteins, etc. (Harborne, 1973).

Experimental design

Hepatoprotective activity against CCl4 induced hepatotoxicity

Animals were randomly divided into seven groups (n=6 animals in each group). The first group served as vehicle control (that is, olive oil - 1.5 ml/kg of rat b.w). The second group served as carbon tetrachloride (CCl4) intoxicated control and received single oral administration of CCl4 mixed with olive oil as vehicle in 1:1 ratio (3 ml/kg of rat body weight). The third group was given standard drug silymarin at a dose of 100 mg/kg and remaining groups were given two different extracts of both (ethanolic and aqueous) of C. crista at a dose of 100 and 200 mg/kg, respectively (group IV to VII) (Mir et al., 2011).

Hepatoprotective activity against paracetamol induced hepatotoxicity

Animals were divided into seven groups (n=6 animals in each group). The animals in group I served as vehicle control. Group II rats served as control and were administered with distilled water by oral administration of paracetamol (PCM) at a dose of 3 g/kg body weight, 1 h after distilled water administration. Group III served as standard and given silymarin at a dose of 100 mg/kg. The animals in group IV, V, VI and VII served as test groups and were treated orally with ethanol and aqueous extract of C. crista of 100 mg and 200 mg/kg body weight, once in a day for 10 days followed by a single oral administration of PCM (3 g/kg body weight), respectively, 1 h after extract administration. After 24 h of PCM administration on 10th day, rats of all groups were sacrificed by decapitation and the blood was collected by retro-orbital method (Ranawat et al., 2010).

Blood collection

Each animal was anaesthetized with diethyl ether. Blood was collected by retro-orbital method in a 5 ml disposable syringe and 2
ml blood was drawn very gently and slowly. The blood collected was immediately shifted to dried clean centrifugation tubes and allowed to clot then serum was separated by centrifugation at 3,000 rpm for 15 min. Serum was separated and then preserved in the cuvettes at -20°C in the freezer until analysis. Biochemical estimations were made the following day.

**Assessment of liver function**

Biochemical parameters like alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), serum bilirubin and total protein were analysed from the serum collected from the different animals of different groups.

**Histopathology**

The liver tissue was dissected out and fixed in 10% formalin solution, dehydrated in ethanol (50 to 100%), cleared in xylene and embedded in paraffin wax. 5 to 6 mm thick sections were prepared and then stained with hematoxylin and eosin dye for microscopic observations.

**Statistical analysis**

The significance of difference among the control group and various treated groups were analyzed by means of analysis of variance (ANOVA) with least significant difference (LSD) post hoc test used to compare the group means and P < 0.05 was considered statistically significant. The experimental results are expressed as mean ± standard error mean (SEM). Statistical package for social sciences (SPSS) for windows (version 15.0, Chicago, IL, USA) was used for statistical analysis.

**RESULTS AND DISCUSSION**

Carbon tetrachloride is one of the most commonly used hepatotoxin in the experimental study of liver diseases and the hepatotoxic effects of it are largely due to its active metabolite, trichloromethyl radical (Ranawat et al., 2010). The activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids, which leads to the formation of lipid peroxides. This lipid peroxidative degradation of bio-membranes is one of the principle causes of hepatotoxicity of CCl₄ (Kaplowitz et al., 1986).

In the present study, it was observed that the administration of CCl₄ increased serum bilirubin, decreased the levels of proteins and increased the levels of serum marker enzymes such as AST, ALT, ALP significantly (P < 0.001) which is an evidence of existence of liver toxicity when compared to normal group animals (Table 1). These elevated marker enzymes were brought back and the total protein levels were elevated in case of silymarin treated animals and was found to be highly significant (P < 0.001) and the effects of these can be easily seen in histopathology (Figure 1). C. crista at a dose of 200 mg/kg produced highly significant (P < 0.001) reduction in the elevated marker enzymes like ALT, AST, ALP and
Figure 1. Effect of extracts of Caesalpinia crista Linn. on AST, ALT and ALP in CCl₄ induced hepatotoxicity.
(a) Normal Liver showing a normal portal triad, sinuoids, and arrangement of hepatocytes; (b) CCl₄ treated liver showing marked fatty changes around portal tract & Hepatocytes are laden with fat vacuoles; (c) Liver (CCl₄ + silymarin at the dose of 100mg/kg b.w.) showing normal appearing hepatocytes and no fatty change, or absence of fatty change in hepatocytes, there is also no necrosis; (d) Liver exposed to CCl₄ and pretreated with CCED at the dose 100mg/kg body wt. showing less fatty changes; (e) Liver exposed to CCl₄ and pretreated with CCED at the dose 200 mg/kg b.w. showing almost normal appearing hepatocytes. Fat vacuoles are seen only in some hepatocytes; (f) Liver exposed to CCl₄ and pretreated with CCAD at the dose 100 mg/kg body wt. showing moderate degree of fatty changes; (g) Liver exposed to CCl₄ and pretreated with CCAD at the dose 200 mg/kg body wt. showing mild degree of fatty changes. Histopathology of Liver (CCl₄ hepatotoxicity) (magnification 10×45).
Table 2. Effect of extracts of seeds of *Caesalpinia crista* on PCM-induced Hepatotoxicity.

<table>
<thead>
<tr>
<th>Group</th>
<th>Aspartate Transferase (AST) (IU/L)</th>
<th>Alanine Transferase (ALT) (IU/L)</th>
<th>Alkaline Phosphatase (ALP) (IU/L)</th>
<th>Total protein (g/dl)</th>
<th>S. Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Vehicle Control</td>
<td>13.2±0.407</td>
<td>15.74±0.551</td>
<td>36.21±0.262</td>
<td>6.69±0.012</td>
<td>0.68±0.012</td>
</tr>
<tr>
<td>II PCM+Vehicle</td>
<td>67.1±0.13*</td>
<td>83.2±0.003*</td>
<td>109.9±0.927*</td>
<td>2.69±0.048*</td>
<td>2.63±0.048*</td>
</tr>
<tr>
<td>III PCM+Standard (Silymarin)</td>
<td>22.2±0.006***</td>
<td>19.2±0.03***</td>
<td>41.8±0.195***</td>
<td>6.49±0.015***</td>
<td>0.87±0.015***</td>
</tr>
<tr>
<td>IV PCM+CCED (100 mg/kg)</td>
<td>42.3±0.26**</td>
<td>25.6±0.002**</td>
<td>50.89±3.34**</td>
<td>4.64±0.027**</td>
<td>1.15±0.027**</td>
</tr>
<tr>
<td>V PCM+CCED (200 mg/kg)</td>
<td>41.8±0.22***</td>
<td>23.2±0.002***</td>
<td>57.6±1.155***</td>
<td>5.48±0.021***</td>
<td>1.46±0.021***</td>
</tr>
<tr>
<td>VI PCM+CCAD (100 mg/kg)</td>
<td>61.2±0.792*</td>
<td>31.3±0.003*</td>
<td>51.43±1.163*</td>
<td>3.79±0.037*</td>
<td>1.99±0.037*</td>
</tr>
<tr>
<td>VII PCM+CCAD (200 mg/kg)</td>
<td>53.8±0.008***</td>
<td>28.8±0.003***</td>
<td>61.96±5.167***</td>
<td>4.5±0.029***</td>
<td>1.57±0.029***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n = 6); ***p ≤ 0.0001 when compared with PCM control group, **p ≤ 0.001 when compared with PCM control group, *p ≤ 0.01 when compared with PCM control group, #p ≤ 0.0001 when compared with vehicle control group, CCED (100 mg/kg) - *Caesalpinia crista* ethanolic extract at a dose of 100 mg/kg, CCED (200 mg/kg) - *Caesalpinia crista* ethanolic extract at a dose of 200 mg/kg, CCAD (100 mg/kg) - *Caesalpinia crista* aqueous extract at a dose of 100 mg/kg, CCAD (200 mg/kg) - *Caesalpinia crista* aqueous extract at a dose of 200 mg/kg.

Paracetamol is a commonly and widely used analgesic antipyretic agent, but over doses of paracetamol deplete the normal levels of hepatic glutathione. Cytochrome P450 enzyme system metabolizes paracetamol and forms a minor but significant alkylating metabolite known as NAPQI (N-acetyl-p-benzo-quinone imine), which in turn is irreversibly conjugated with the sulfhydryl groups of glutathione (Jollow et al., 1973). Production of NAPQI (responsible for the toxic effects of paracetamol) is mainly because of two isoenzymes of cytochrome P450 (CYP2E1 and CYP3A4). Gene of cyp450 is highly polymorphic, however, an individual differences in paracetamol toxicity were believed to be due to a third isoenzyme that is, CYP2D6, which metabolises paracetamol into NAPQI to a lesser extent than other P450 enzymes, its activity may contribute to paracetamol toxicity in ultra rapid metabolizers and when it is taken at high and chronic doses. In the liver, the cytochrome P450 enzymes CYP2E1 and CYP3A4 were primarily responsible for the conversion of paracetamol to NAPQI which undergoes conjugation with glutathione. This in combination with direct cellular injury by NAPQI leads to cell damage and death (Wendel et al., 1979). Excess production of paracetamol metabolite causes the initial hepatic damage and subsequent activation of inflammatory mediator TNF-α which in turn contribute to tissue necrosis (Borne, 1995).

In the present investigation it was observed that the administration of paracetamol increased serum bilirubin, decreased the levels of proteins and increased the levels of serum marker enzymes like AST, ALT, ALP significantly (P < 0.001) which may be due to acute hepato-cellular damage and biliary obstruction (Table 2). Ethanolic extract of *C. crista* at a dose of 200 mg/kg produced highly significant (P < 0.001) reduction in the elevated marker enzymes like AST, ALP, ALT and serum bilirubin in a dose dependant manner and also by silymarin at a dose of 100 mg/kg. The difference in the effects of induction drug, standard drug and test drug at different dose level can be easily seen in histopathology of livers as shown in Figure 2.

In accordance with these results, it may be stated that tannins, saponins and flavonoids which are present in the seed extracts could be considered responsible for the hepatoprotective activity (Takate et al., 2010). Based on the results observed in this study, it is well evident that *C. crista* is pharmacologically effective for the treatment of liver disorders at a higher dose levels when compared to silymarin.

**Conclusion**

The present results provide strong evidence that ethanolic extract of seeds of *C. crista* inhibits
Figure 2. Effect of extracts of Caesalpinia crista Linn on paracetamol induced hepatotoxicity on rat liver (histopathology). 
(a) Normal Liver showing a normal portal triad and normal arrangement of hepatocytes; (b) Paracetamol treated liver showing marked fatty changes around portal tract as well as around central vein. Hepatocytes are laden with fat vacuoles; (c) Liver exposed to PCM and pretreated with silymarin at the dose of 100mg/kg body wt, showing normal appearing hepatocytes and no fatty change, or absence of fatty change in hepatocytes; (d) Liver exposed to Paracetamol and pretreated with CCED at the dose 100mg/kg body wt. showing moderate degree of fatty changes; (e) Arrangement of hepatocytes is nearly similar as that in normal at dose of 200 mg/kg b.w; (f) Liver exposed to paracetamol and pre-treated with CCAD at a dose of 100 mg/kg showing very less effect; (g) Liver exposed to paracetamol and pretreated with CCAD at a dose of 200 mg/kg b.w. Histopathology of Paracetamol induced hepatotoxicity.
hepatotoxicity induced by carbon tetrachloride and paracetamol. The hepatoprotective action was much more significant at the dose of 200 mg/kg when compared to 100 mg/kg. So, further research work is required to isolate the compound responsible for this activity.

REFERENCES


