PROTECTIVE EFFECT OF CAESALPENIA BONDUCELLA L. LEAF AGAINST ACETAMINOPHEN-INDUCED LIVER DAMAGE IN WISTAR RATS

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ABSTRACT
In the present study, methanol extract of Ceasalpinia bonducella leaf was evaluated against acetaminophen-induced liver damage in Wistar rats for its hepatoprotection action. Animals were divided into 5 groups of six rats each. Oral administration of CB (800 mg/kg) along with Acetaminophen (AA; 2.5 g/kg) showed a significant increase in serum enzyme AST, ALT, Total Bilirubin, Direct Bilirubin and Alkaline Phosphatase levels with a decrease in Total Protein levels (P<0.01). The results were similar to Silymarin (SL), a standard hepatoprotective drug. The present study reveals that the administration of CB can be beneficial for the suppression of liver toxicity. Based on present findings, it can be concluded that methanol extract of Caesalpenia bonducella L. protects the liver from acetaminophen-induced liver damage.

Keywords: Paracetamol, Hepatotoxicity, Liver enzymes.

INTRODUCTION
Liver diseases due to exposure of different drug molecules are very common today1. An attention must be paid to treat the hepatic disorders. The basis of treatment is supportive care, with careful monitoring for signs of acute liver failure or progression to chronic liver disease 2.

The use of herbs, herbal extracts or plant-derived pure chemicals to treat disease is a therapeutic modality, which has stood the test of time 3. In spite of phenomenal growth of modern medicine; there are no synthetic drugs available for the treatment of hepatic diseases. However, there are numerous herbs/herbal formulations claimed to have possess valuable activity in treating liver disorders. Several hundred plants have been examined for use in a wide range of liver disorders. Just a handful has been reasonably well researched 4.

Caesalpinia bonducella (CB; Karanjwa) is an important medicinal plant widely distributed throughout the coastal region of India, Burma, Sri Lanka, and in other tropical and subtropical regions of the world 5,6. Plant is claimed to have numerous therapeutic properties like, antidiuretic, anthelmintic and antibacterial, anti-anaphylactic and antiviral, antiasthmatic, antiamoebic and anti-estrogenic 7,8. Blood sugar lowering action of CB has been primarily evaluated with significant result in rabbit and rat models 9,10. The present study was conducted to evaluate the effect of the methanol extract of the CB leaf against acetaminophen-induced liver damage in Wistar rats.

MATERIALS AND METHODS

Chemicals
Acetaminophens (AA), Silmarin (SL) (Micro Labs Ltd., Bangalore, India). Ethanol (S.D. Fine Chemicals Ltd. Mumbai, India) were procured respectively. AA was suspended in 0.5% gum acacia before use. All other chemicals and reagents were of the highest analytical grade available.

Plant
The leaf of CB was procured from Chidambaram, Cuddalore, Tamil Nadu, India. The plant was identified and authenticated by Chief Botanist, Department of Botany, Annamalai University, Annamalai Nagar Chidambaram, Cuddalore, Tamil Nadu, India. A voucher specimen for plant has been kept at the herbarium of the University.

Preparation of extract
The leaf of CB were dried in shade, powdered and passed through a 40-mesh sieve. Dried powder (500 g) was taken and subjected to successive extraction with petroleum ether, chloroform, methanol and water in Soxhlet apparatus. The extracts were concentrated to dry residue by distillation (temperature 60 °C without vacuum) and dried completely in desiccators and weighed. The extract was freeze dried and stored at –80°C until further use. The dried mass was diluted with normal saline and used in experiments. The methanol extract of CB was found to be effective as hepatoprotective.

Preliminary Phytochemical Screening
Petroleum ether, chloroform, methanol and aqueous extracts of CB were subjected to preliminary phytochemical screening for their presence or absence of active constituents utilizing standard method of analyses11.

Animal
The study was conducted after obtaining institutional (MESCO College of Pharmacy, Mustaidpura, Hydenbad, Andhra Pradesh, India) ethical committee clearance bearing the number 1185/A/08/OPCSEA (Committee for Control and Supervision on Experiments on Animals).

Female Wistar rats (100–150 g; 4–6 weeks old) were maintained under controlled conditions of light (12 h/12 h), temperature (26±2 °C) and relative humidity (44–56%) for 1 week before and during the experiments. The animals had access to standard laboratory feed (Gold Mohur, Hindustan Lever Ltd., Mumbai, India) and water ad libitum. For experimental purposes, animals were kept fasting overnight but were allowed free access to water.

Experimental procedure
Acetaminophen was suspended in 0.5% gum acacia and administered p.o., at a single dose of 2.5 g/kg. The dose is known to bring liver damage in rats 12. Animals were divided into 5 groups of 6 animals each. Group I (normal control) received saline (0.9% NaCl, p.o.) 1 ml/kg and group V (CB per se) received CB 800 mg/kg, p.o. for six days. The group II (AA control), group III (CB + AA), group IV (SL + AA) received 0.5% gum acacia in distilled water (1 ml/kg, p.o.), methanol extract of CB (800 mg/kg, p.o.) and Silymarin (100 mg/kg, p.o.) respectively, once a day for six days. On the fourth day, 30 min after the administration of the respective treatments, all the animals of groups II, III and IV were administered with acetaminophen (2.5 g/kg, p.o.). On the sixth day after 2 h of respective treatments, all the animals each. Group I (normal control) received saline (0.9% NaCl, p.o.) 1 ml/kg and group V (CB per se) received CB 800 mg/kg, p.o. for six days. The group II (AA control), group III (CB + AA), group IV (SL + AA) received 0.5% gum acacia in distilled water (1 ml/kg, p.o.), methanol extract of CB (800 mg/kg, p.o.) and Silymarin (100 mg/kg, p.o.) respectively, once a day for six days. On the fourth day, 30 min after the administration of the respective treatments, all the animals of groups II, III and IV were administered with acetaminophen (2.5 g/kg, p.o.). The study was conducted after obtaining institutional (MESCO College of Pharmacy, Mustaidpura, Hydenbad, Andhra Pradesh, India) ethical committee clearance bearing the number 1185/A/08/OPCSEA (Committee for Control and Supervision on Experiments on Animals).

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Biochemical analysis
The serum was separated after centrifuging the blood sample at 3000 rpm for 15 min and the following enzyme analyses were quantified: aspartate aminotransferase; AST and alanine aminotransferase; ALT 14, total bilirubin 15, alkaline phosphatase (ALP) 16 and total protein 17 respectively.
Statistical analysis

The obtained raw data in each experimental group was computed into mean and standard error of mean (SEM). Groups of data were compared with the analysis of variance (ANOVA) followed by Dunnett’s test for multiple comparison of the two treatment groups with the control. Values were considered statistically significant when p < 0.01.

RESULTS

Preliminary Phytochemical Screening

Alkaloids, Flavonoids & Saponins were found to be present in methanol & aqueous extract as a major constituents while Tannins were absent in all extracts. Chloroform extract also showed the presence of Alkaloids.

Biochemical observations

Effect of AA on serum biochemical parameters

As shown in Table 1 & 2, administration of AA (2.5 g/kg, p.o.) induced a significant increase in the serum enzyme AST, ALT, total bilirubin, direct bilirubin and alkaline phosphatase levels as compared to the normal control group (P<0.01). Total protein levels were markedly reduced in the AA control group as compared to the normal control group (P<0.01).

Table 1: Effect of Caesalpenia bonducella L. administration on serum levels of AST, ALT and ALP in rats exposed to acetaminophen.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>52.4±0.86</td>
<td>26.2±0.49</td>
<td>94.3±0.20</td>
</tr>
<tr>
<td>AA</td>
<td>139±0.30**</td>
<td>63.3±0.25**</td>
<td>380.45±1.25**</td>
</tr>
<tr>
<td>CB+AA</td>
<td>63.4±0.28**</td>
<td>28.4±0.52**</td>
<td>231.20±1.40**</td>
</tr>
<tr>
<td>SL+AA</td>
<td>57.8±0.33**</td>
<td>22.00±0.32**</td>
<td>224.42±0.66**</td>
</tr>
<tr>
<td>CB per se</td>
<td>50.1±0.52**</td>
<td>25.5±0.30**</td>
<td>93.15±0.09**</td>
</tr>
</tbody>
</table>

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; NC: Normal control; 0.9% NaCl, 1 ml/kg, p.o., AA: acetaminophen control (2.5 g/kg, p.o.), CB: Caesalpenia bonducella L. (800 mg/kg, p.o.)+acetaminophen (2.5 g/kg, p.o.), SL+AA: Silymarin (100 mg/kg, p.o.) +acetaminophen (2.5 g/kg, p.o.).

Values are mean±S.E.M. of six animals in each group.

**p <0.01, as compared to normal control group, ANOVA followed by Dunnett-test

Table 2: Effect of Caesalpenia bonducella L. administration on serum levels of total bilirubin, direct bilirubin and total protein in rats exposed to acetaminophen.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total bilirubin (mg/100ml)</th>
<th>Direct bilirubin (mg/100ml)</th>
<th>Total protein (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.87±0.006</td>
<td>0.2±0.003</td>
<td>5.13±0.032</td>
</tr>
<tr>
<td>AA</td>
<td>2.15±0.021**</td>
<td>0.67±0.011**</td>
<td>3.00±0.021**</td>
</tr>
<tr>
<td>CB+AA</td>
<td>0.93±0.009**</td>
<td>0.26±0.002**</td>
<td>4.98±0.030**</td>
</tr>
<tr>
<td>SL+AA</td>
<td>0.89±0.006**</td>
<td>0.24±0.004**</td>
<td>5.05±0.041**</td>
</tr>
<tr>
<td>CB per se</td>
<td>0.86±0.010**</td>
<td>0.23±0.004**</td>
<td>5.13±0.063**</td>
</tr>
</tbody>
</table>

NC: Normal control; 0.9% NaCl, 1 ml/kg, p.o., AA: acetaminophen control (2.5 g/kg, p.o.), CB: Caesalpenia bonducella L. (800 mg/kg, p.o.), CB+AA: Caesalpenia bonducella L (800 mg/kg, p.o.)+acetaminophen (2.5 g/kg, p.o.), SL+AA: Silymarin (100 mg/kg, p.o.) +acetaminophen (2.5 g/kg, p.o.).

Values are mean±S.E.M. of six animals in each group.

**p<0.01, as compared to normal control group, ANOVA followed by Dunnett-test

**p<0.01, as compared to AA control group, ANOVA followed by Dunnett-test.

Effect of CB on serum biochemical parameters

CB+AA group showed an increased serum enzyme AST, ALT, total bilirubin, direct bilirubin and alkaline phosphatase levels as compared to the AA control group (P<0.01). CB+AA group significantly increased the total protein levels as compared to the AA control group (P<0.01) (Table 1 & 2).

Effect of SL on serum biochemical parameters

SL+AA group showed an elevated serum enzyme AST, ALT, total bilirubin, direct bilirubin and alkaline phosphatase levels as compared to the AA control group (P<0.01). SL+AA group significantly elevated the total protein levels as compared to the AA control group (P<0.01) (Table 1 & 2).

Effect of CB per se on serum biochemical parameters

CB per se group showed non-significant changes in serum enzyme AST, ALT, total bilirubin, direct bilirubin and alkaline phosphatase levels as compared to AA control group (p>0.05). CB per se group did not show a significant change in total protein levels as compared to normal control group (p>0.05) (Table 1 & 2).

DISCUSSION

Treatment and management strategy of liver disease still pretense a significant challenge to the modern medicine due to lack of rationale therapy. The present study focused on the hepatoprotective effect of CB on AA-induced liver damage in Wistar rats and found that administration of CB suppressed the significantly increases in serum enzyme AST, ALT, Total Bilirubin, Direct Bilirubin and Alkaline Phosphatase levels and stimulated the decreases in Total Protein levels (P<0.01).

Acetaminophen, also known as paracetamol, is widely used as an analgesic and antipyretic drug, though proved safe at therapeutic doses; AA is toxic at higher doses and causes acute liver failure illustrated by centrilobular hepatic necrosis. Acetaminophen can also induce renal failure and finally death in severe cases.

Serum AST, ALT, ALP and Bilirubin are the most vulnerable biomarkers directly implicated in the extent of hepatic damage and toxicity. Throughout hepatocellular damage, numbers of enzymes normally located on the cytosol are released into the blood flow. Their quantification in serum is a valuable biomarker of the extent and type of hepatocellular damage. As anticipated, a single oral dose of AA (2.5 g/kg, p.o.) showed significant hepatotoxicity, as evidenced by a dramatic elevation of AST, ALT, ALP, Bilirubin and suppression of Total Protein. Pretreatment along 2 days with CB showed a significant protective effect against AA-induced acute hepatotoxicity in rats. Serum aminotransferase activities have long been regarded...
as as sensitive markers of hepatic injury. Injury to the hepatocytes changes their transport function and membrane permeability, leads leakage of enzymes from the cells. As a result, the significant release of ALT and ALP into the circulation indicates severe damage to the liver cell and alters in the activities of AST, ALT and ALP in the present study may be interpreted as a result of the liver cell destruction or alters in the membrane permeability representing the severity of hepatocellular damage induced by AA, which is in accordance with previous report. The raise in ALT activity is almost always due to hepatocellular injury and is usually accompanied by an increase in AST. An increase in ALP reflects the pathological alteration in biliary flow.

CONCLUSION

In conclusion, the results of the present investigation confirmed that AA causes changes in the levels of several liver components. The CB as well as SL when given orally was able to target the liver enzymes, and found to improve liver enzyme contents in AA-induced Wistar rats. These results present initial evidence that CB may be useful for the treatment of liver damage and raise the possibility of a new application as a hepatoprotective therapeutic modality.

REFERENCES