Hepatoprotective and Antioxidant Activities of Ziziphus rotundifolia (Linn.) against Carbon Tetrachloride-Induced Hepatic Damage in Rats

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ABSTRACT
Many hepatoprotective herbal preparations have been recommended in alternative system of medicine for the treatment of hepatic disorders. There are few or no systemic studies have been done on protective efficacy of Ziziphus rotundifolia (Rhamnaceae) to treat liver diseases. In this study, hepatoprotective and antioxidant activity of ethanolic extracts was evaluated against CCl4-induced liver damage in rats. Liver damage was evidenced by elevated levels of biochemical parameters such as serum glutamate oxaloacetic acid transaminase, glutamate pyruvic transaminase and serum alkaline phosphatase. Treatment with ethanolic extracts of Z. rotundifolia (300 mg/kg, p.o.) produced a significant reversal in the above biochemical parameters, and reducing power, superoxide anion scavenging activity and reduced histopathological scores. These findings suggest that the extracts of Ziziphus rotundifolia possess significant hepatoprotective and antioxidant properties.

KEYWORDS: Hepatoprotective activity; antioxidant activity; Ziziphus rotundifolia; silymarin; carbon tetrachloride.

Introduction
Plant-based medicines have been an important source for treatment of various ailments. A variety of plants are known to possess hepatoprotective properties. Spermatophytes are said to be primitive vascular plants whose members present considerable diversity in habit, fruit and floral features and shows the medicinal utility. Ziziphus rotundifolia (Rhamnaceae) is a tropical plant that grows throughout the India and other tropical regions of the world (Trease et al., 2001). However, it has not been widely explored for its medicinal properties. There are few systemic studies on protective efficacy of the Z. rotundifolia plant.

The present study was conducted to evaluate the effect of ethanolic extract of leaves of Z. rotundifolia (ZRF) against CCl4-induced hepatic damage in rats.

Materials and Methods

Drugs
CCl4 was purchased from S.S Pharma Chem Ltd. (Warangal AP, India). Silymarin was obtained as gift sample from Micro Labs, Bangalore, India. SGOT, SGPT, and SALP kits were obtained from Span diagnostics. Lactate dehydrogenase was obtained from Hyderabad. All other chemicals and reagents were of analytical grade.

Animals
Male albino rats (150-200 g) were obtained from the National Institute of Nutrition, Tarnaka, Hyderabad. They were kept in the animal house at room temperature of 25°C-30°C and at 45%-55% relative humidity for 12 hours, each of dark and light cycle. The animals were feed with rat pellets (Hindustan Lever Limited, Bangalore, India) and filtered water. Animal studies in the work have been strictly performed as per the Institutional Animal Ethical Committee (IAEC) constituted under the guidelines of Committee for the Purpose of Control and Supervision on Experimental Animal (CPCSEA), Ministry of Environment, Government of India.

Preparation of Extracts
The leaves of Z. rotundifolia (ZRF) collected from Shathavahana university campus, Karimnagar Andhra Pradesh, India between November and December. The plant was authenticated by the Professor R. Odaiah, SSR Government Degree and PG College Karimnagar, Andhra Pradesh, India. A voucher specimen (SSR
synthesis, which are often implicated to assess the extent their serum levels and to a reduced total protein the liver into the blood stream, leading to an increase in ZRF was evaluated using CCl4-induced model 5. Group consisting of six animals. Hepatoprotective activity of the rats were divided into four groups, each group between 150 g and 175 g were used as animal models. of CCl4 induced liver damage. Male albino rats weighing 1776 Int J Pharm Sci Nanotech Vol 5; Issue 3 • October–December 2012

Ethanolic Extract

The powder of whole plant (800 g) was wetted over night with ethanol (95%) and Soxhlet extracted with 450 ml of 95% ethanol at controlled temperature (Jothy et al., 2009). The collected extract was concentrated under reduced pressure using vacuum pump (Rotary Evaporator) complete removal of solvent. The resulting ethanol extract was suspended in 0.5% Tween 80 to required concentrations and used for the experiments.

CCl4-induced Hepatotoxicity Studies

CCl4 induced liver injury is a common model used for hepatoprotective drug screening (Sunitha et al., 2008). It is postulated that administration of CCl4 could cause cell lyses, resulting in the release of cytoplasmic enzymes of the liver into the blood stream, leading to an increase in their serum levels and to a reduced total protein synthesis, which are often implicated to assess the extent of CCl4 induced liver damage. Male albino rats weighing between 150 g and 175 g were used as animal models. The rats were divided into four groups, each group consisting of six animals. Hepatoprotective activity of ZRF was evaluated using CCl4-induced model 5. Group one was kept on normal diet and served as control, the second group received CCl4 (1.25 mL/kg) by oral route, the third and fourth group received silymarin (100 mg/kg; p.o.) and extract of ZRF (650 mg/kg; p.o.) respectively once daily, for seven days. On the seventh day, CCl4 was given by oral route 30 minutes after the administration of silymarin and test drug. After 36 hours of CCl4 administration, blood was collected and serum separated was analyzed for various biochemical parameters.

Biochemical Estimations for Hepatoprotective Activity

Biochemical parameters like serum glutamate oxaloacetic transaminase (SGOT), serum alkaline phosphatase (SALP), were assayed according to standard methods (Shah et al., 2005).

Biochemical Estimations for Antioxidant Activity

The antioxidant activity was estimated by following in vitro assay methods. Inhibition of DPPH radical, inhibition of nitric oxide radical and superoxide anion scavenging activity (Tepe et al., 2006).

Histopathological Study

All rats were dissected, liver samples were excised, washed with normal saline and processed separately for histological observations, initially the material was fixed in 10% buffered neutral formalin for 48 hours after 7 μm thick paraffin sections of buffered formalin-fixed were stained with haematoxylin-eosin electronic microscopic (5000X) observations of the liver histological architecture of the control and treated rats. (Brijesh et al., 2001).

Statistical analysis

Results were expressed as mean ±SEM. Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by Student's t-test. P values less than 0.05 was considered to be statistically significance when compared with the control.

In vitro Antioxidant Activity

Inhibition of DPPH radical

The free radical scavenging activity of the extract was analyzed by the DPPH (1,1-diphenyl-2-pircyl hydrazyl) assay. A total of 2 mL of the test extract, at concentrations ranging from 1 μg/mL to 100 μg/mL, each was mixed with 1 mL of 0.5 m DPPH (in methanol). The absorbance at 517 nm was taken after 30 minutes of incubation in the dark room temperature. The experiment was done in triplicate the percentage antioxidant activity was calculated as follows:

% antioxidant activity [AA] = 100 – [(Abs sample – Absblank) × 100]/Abs mL of methanol + 2.0 mL of the extract was used as the blank while 1.0 mL of the 0.3 mM.

DPPH solution + 2.0 mL of methanol was used as the negative control. Ascorbic acid was used as the reference standard. (Gupta et al., 2004).

Inhibition of nitric oxide radical

Nitric oxide generated from sodium nitropruside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction the reaction mixture (3 mL) containing sodium nitropruside (10 mM) in phosphate buffered saline (PES) and SM and the reference compound in different concentrations (5, 10, 25, 50 and 100 μg) were incubated at 25°C for 150 minutes in each 30 minutes, 0.5 mL of the Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylene diamine dihydrochloride in 2% H3PO4) was added. The absorbance of the chromophore formed was measured at 546 nm. All the tests were performed in triplicate and the results were averaged. The percentage decrease in absorbance was calculated. Quercetine was used as the standard (Gupta et al., 2004).
Superoxide anion scavenging activity

The measurement of superoxide anion scavenging activity of ZRF was carried by a method of Gupta and colleagues (Gupta et al., 2004). Nitroblue tetrazolium (NBT) solution (1 mL 156 µM NBT in 100 mM of phosphate buffer, pH 7.4), nicotinamide adenine dinucleotide (NADH) (1 mL, 468 µM in 100 mM phosphate buffer, pH 7.4) and 0.1 mL of sample solution of ZRF (5, 10, 25, 50, 100 µg) in water were mixed. The reaction was initiated by adding 10 µL of phenazine methyl sulphate (PMS) solution (60 µM PMC in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction was incubated at 25°C for 5 minutes and absorbance was measured at 560 nm. Decrease in absorbance of the reaction mixture indicated increase in superoxide anion scavenging activity. The percentage decrease in absorbance of the reaction mixture was calculated.

Results

Hepatoprotective Activity

The administration of CCl₄ to the animals resulted in a significant increase in serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP). The oral administration of ethanolic extract of Z. rotundifolia and silymarin significantly reduced the CCl₄-induced increase in the SGOT, SGPT and SALP levels (Table 1 and Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>AST/SGOT</th>
<th>ALT/SGPT</th>
<th>SALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I/normal</td>
<td>-----</td>
<td>43.22±0.11</td>
<td>55.84±4.51</td>
<td>36.22±0.57</td>
</tr>
<tr>
<td>Group-II/CCl₄ toxic</td>
<td>1.00 mL</td>
<td>90.30±4.01*</td>
<td>106±3.57</td>
<td>89.54±3.03</td>
</tr>
<tr>
<td>Group-III/CCl₄ + Silymarin</td>
<td>1.00 mL +25</td>
<td>66.28±2.55</td>
<td>75.53±4.21</td>
<td>42.25±2.02</td>
</tr>
<tr>
<td>Group-IV/CCl₄ + Alcoholic extract</td>
<td>1.00 mL +300</td>
<td>42.35±2.88</td>
<td>63.01±2.57</td>
<td>41.51±4.15</td>
</tr>
</tbody>
</table>

Values are the Mean ± S.D (n = 6).
Significance* P < 0.001 compared to CCl₄.

Ascorbic acid

The toxic effect of CCl₄ was controlled in the animals treated with the extract by way of restoration of the levels of the liver function biochemistry similar to that of standard drug silymarin. Among the extract treated groups, significant hepatoprotective activity was observed (Table 2).

In the histopathological studies, the liver sections of rats treated with vehicle showed normal hepatic architecture (Figure 1), where as that of CCl₄ treated group showed total loss of hepatic architecture with intense peripheral central vein necrosis, fatty changes, congestion of sinusoid, Kupffer cell hyperplasia, crowding of the central vein, apoptosis (Figure 1), in case of rats treated with silymarin (Figure 1) and Z. rotundifolia extracts of 300 mg/kg (Figure 1) respectively, a normal hepatic architecture was seen with only moderate accumulation of fatty lobules and mild degree of cell necrosis, clearly indicating the protection offered by standard drug silymarin and plant extract.
Antioxidant Activity

DPPH Radical scavenging activity of the ethanolic extracts of the leaves of the Z. rotundifolia was compared with those of standard sodium metabisulphate, ascorbic acid. The DPPH radical scavenging abilities of the extracts (89.86%) were found to be less than those of standard sodium metabisulphate, ascorbic acid (93.28%). Table 2 shows the dose response results of nitric oxide scavenging and superoxide anion scavenging of the ethanolic extracts of leaves of Z. rotundifolia. The extract reduced the generation of nitric oxide radical from sodium nitroprusside solution. This showed marked nitric oxide scavenging of the extract (64.32%). Also the extract showed significant superoxide scavenging activity (60.24%) at 100 mg/mL.

Discussion

CCl4 is commonly used for induction of experimental liver toxicity. This toxic chemical causes peroxidative degradation of the adipose tissue, resulting in fatty infiltration of the hepatocytes. Its metabolites such as trichloromethyl radical (CHCl3) and trichloromethyl peroxy radical (CCl3O2) are involved in the pathogenesis. As shown in Figure B, CCl4 causes changes around the central vein in the liver and other oxidative damages with the leakage of marker enzymes like SGOT, SGPT and SALP in the serum. Treatment with ethanolic extract significantly reduced the elevated levels of the enzymes towards the respective normal value that is indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl4.

The qualitative phytochemical investigations on ethanolic extra Z. rotundifolia also showed positive for flavonoids by ferric chloride, alkaline reagent, further more, flavonoids constituents of plant possess antioxidant properties. Administration of ethanolic extract of Z. rotundifolia showed significant super oxide anion, nitric oxide scavenging activities in a dose dependent manner. Simultaneous generation of nitric oxide (NO) and O2− favors the production of a toxic reaction product, peroxynitrite (ONOO−). The scavenging of the superoxide anion and nitric oxide indicate the possibility of preventing the formation of peroxynitrite in the cells. Reducing the nitric oxide generation in the digestive tract was found to be effective in preventing the reaction of nitrite with amines and amides to form carcinogenic nitrosamine and nitrous amides. Hence, NO scavenging activity of Z. rotundifolia extract could play a preventive role against nitrosamine mediated carcinogenesis.

Conclusions

Based on these preliminary studies, it is suggested that Z. rotundifolia shows hepatoprotective and antioxidant actives in animal models. Further studies are needed to confirm these findings.

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References


