Hepatoprotective Activity of Aqueous and Methanolic Extracts of Capparis decidua Stems Against Carbon Tetrachloride Induced Liver Damage in Rats

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Abstract: The aqueous and methanolic extracts of Capparis decidua stems locally known as Altoundob were screened for their hepatoprotective activity against CCl₄-induced hepatotoxicity in rats. This plant is used in traditional system medicine in the treatment of jaundice. Yet, no systematic studies on its hepatoprotective activity have been reported. The hepatotoxicity produced by administration of CCl₄ in paraffin oil (1:9 v/v) at a dose of 0.2 mL kg⁻¹ for 10 days, was found to be inhibited by simultaneous oral administration of aqueous and methanolic extracts of C. decidua stems (200, 400 mg kg⁻¹ b.wt.) for 10 days, with evidence of decreased level of serum aspartate amino transferase, alanine amino transferase, alkaline phosphatase and bilirubin. In addition, the concurrent administration of both extracts with CCl₄ for 10 days masked the liver fatty changes induced by the hepatotoxic compound observed in the intoxicated control rats. The results were compared with the hepatoprotective effect of the standard drug silymarin. The preliminary phytochemical screening of the powdered plant showed the presence of alkaloids, flavonoids, tannins, sterols, saponins, cyanogenic glycosides and cumarins as major constituents of the studied extracts. The results of this study indicated that aqueous and methanolic extracts of C. decidua stems could afford a significant protection against CCl₄-induced hepatotoxicity in rats.

Key words: Hepatoprotective activity, Capparis decidua, rats, CCl₄

INTRODUCTION

Liver disease is a worldwide problem. Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Therefore, it is necessary to search for alternative drugs for the treatment of liver disease in order to replace currently used drugs of doubtful efficacy and safety (Ozbek et al., 2004).

Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents (Sharma and Kumar, 2009). Capparis decidua (Family Capparaceae), is a shrub or small tree up to 3 m height. It is widely distributed in Northern and central Sudan especially on sandy soils and in low rainfall savanna on clays (El-Amin, 1990).

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Preparations of the different parts of the plant were claimed to have various medicinal uses among the local population. The bark extract is used in asthma and cough and as diuretics, anticoagulant, anti-inflammatory, astringent, stomachic, laxative, antidote and used for skin diseases (Rahman et al., 2004; Al-Yahya, 1986). The decoction of fresh twigs is kept for 2-3 days and then taken against jaundice, the fumigation of the stems are used as anti-rheumatic (El Ghazali et al., 1997).

Ageel et al. (1986) reported that the ethanolic extract of Capparis decidua and aqueous extract of Capparis spinosa were found to possess significant anti-inflammatory activity against carrageenan induced edema in rats, these two plants were also tested for their antipyretic and analgesic activity. Capparis decidua was found to possess significant antipyretic effect.

Hypoglycemic effect of C. decidua was seen in alloxanized rats when they were fed with 30% extracts of C. decidua fruit powder for 3 weeks. This extract also reduced alloxan induced lipid peroxidation significantly in erythrocytes, kidney and heart. Capparis decidua was also found to alter superoxide dismutase and catalase enzyme levels to reduce oxidative stress. Moreover, C. decidua showed hypolipidemic activity (Modak et al., 2007). Recently, Chahla (2009) reported that the alcoholic extracts of every tested part of C. decidua, manifested significant hypoglycemic activity. The alcoholic extract of C. decidua fruit displayed the best hypoglycemic activity, followed by that of bark and flower.

The alcoholic extract of Capparis decidua fruit was investigated for its antiatherosclerotic activity by Purohit and Vyas (2006). Hyperlipidemia was induced by atherodiet and cholesterol feeding to animals. Rabbits were fed Capparis decidua (500 mg kg⁻¹ b.wt.) or pitavastatin (0.2 mg kg⁻¹ b.wt.) in distilled water along with standard laboratory diet and atherodiet for 60 days. Capparis decidua fruit extract and pitavastatin were found to lower serum cholesterol, LDL-cholesterol, triglyceride, phospholipid and atherogenic index, but found to increase the HDL to total cholesterol ratio as compared with hyperlipidemic control group.

An alcoholic extract of aerial parts of C. decidua, including flowers and fruits was screened for Central Nervous System (CNS) activity using conventional behavioral animal models. The findings of the study suggested that C. decidua has CNS depressant and anticonvulsant activities (Goyal et al., 2009).

In a recent review (Satyanarayana et al., 2008), it has been stated that many of the Capparis species were reported to possess anti-inflammatory, analgesic, antimicrobial, antihelmintic and hepatoprotective activities. The alcoholic extract of C. decidua root bark possesses significant antibacterial and antifungal activities.

The objective of the present study was to evaluate the hepatoprotective effect of C. decidua stem in carbon tetrachloride induced hepatotoxicity model in rats.

MATERIALS AND METHODS

Plant Material and Extraction

Capparis decidua stems were collected from Arkawet area in Southern Khartoum and dried at room temperature. The plant was authenticated by the botanists in Medicinal and Aromatic Plants Research Institute and a voucher sample was deposited in the herbarium of the Institute.

Aqueous extract was prepared by infusion method and dried using freeze drier apparatus (Harborne, 1973). The coarse powdered part of the plant was also extracted successively with chloroform (60-80°C) and methanol (98%) using soxhlet extractor (Harborne, 1973). The solvent was evaporated under reduced pressure and the yields were calculated. The residue obtained was kept in dry clean bottles till used.
Phytochemical Screening

A general screening was carried out according to the method of Harborne (1973) to determine the chemical constituents of the plant material.

Experimental Design

Thirty five adult male Wister albino rats were used; the animals were grouped randomly into 7 groups of 5 rats each. Group I served as normal control and received only the vehicle liquid paraffin at a dose rate of 0.2 mL kg\(^{-1}\) day \(^{-1}\)/p for 10 days. Group II served as intoxicated control by given CCl\(_4\)-induced hepatotoxicity at a dose rate of 0.2 mL kg\(^{-1}\) day \(^{-1}\)/p in liquid paraffin (1:9) for 10 days. Group III served as hepatoprotective drug control when the rats were given CCl\(_4\) at a dose rate of 0.2 mL kg\(^{-1}\) day \(^{-1}\)/p in liquid paraffin (1:9) for 10 days and at the same time received orally silymarin suspended in 5\% Acacia mucilage at a dose of 100 mL/kg/day. Group IV, V, VI and VII rats were given CCl\(_4\) at a dose rate of 0.2 mL kg\(^{-1}\) day \(^{-1}\)/p in liquid paraffin (1:9) for 10 days and simultaneous oral administration of either aqueous or methanolic extracts of C. decida stem at a dose of 200 and 400 mg kg\(^{-1}\).

Blood was collected by puncturing retroorbital plexus into dry clean bottles and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 2500 rpm for 15 min and analyzed for various biochemical parameters. The biochemical parameters (alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and total bilirubin (TBIL)) were analyzed using commercial kits (Plasmatic Laboratory. Products Ltd. Bridport DT6 5BU U.K.) according to the manufacturers instructions.

At the end of the treatment, the animals were sacrificed by decapitation and post-mortem examination was performed. Small pieces of the livers were collected in 10\% neutral buffered formal saline for histopathological study. Sections of 5-6 \(\mu\) were cut and stained by haematoxyline and eosine.

Statistical Analysis

Statistical analysis of the results was performed using software Statistical Package for Social Sciences (SPSS). Mean values in serum parameters were compared using the student's t-test (Mendenhall, 1971).

RESULTS

Capparis decidae stems were extracted with different solvents (chloroform, methanol, water) and the yield percentages were higher in the methanolic extract (12.61\%) followed by the aqueous extract (6.94\%) and then the chloroform extract (1.45\%).

The preliminary phytochemical analysis of the powdered material of plant revealed the presence of alkaloids, saponins, flavonoids, tannins, sterols, glycosides and cumarin. The plant was devoid of anthraquinone compounds.

There were no significant changes in the concentration of bilirubin and in AST, ALT and ALP levels in the control group I and standard drug group III.

In CCl\(_4\) tested group II, the concentration of bilirubin and the activities of AST, ALT and ALP were significantly (p<0.001) increased when compared with the standard drug silymarin group (Table 1).

Simultaneous treatment with water extract of C. decidae stems to rats in group IV (200 mg kg\(^{-1}\)) and group V (400 mg kg\(^{-1}\)) significantly (p<0.001) reduced the activities of serum AST, ALT and ALP and concentration of bilirubin especially in group IV (200 mg kg\(^{-1}\)) compared with group II (CCl\(_4\) group). There were no significant changes in these values when compared with group III (standard drug group).
Table 1: Effect of C. decidae stems aqueous and methanolic extracts administered simultaneously with CCl4 on serum constituents levels on rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bilirubin (mg dl⁻¹)</th>
<th>ALP (U L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 5</td>
</tr>
<tr>
<td>Control</td>
<td>0.21±0.04</td>
<td>0.17±0.03**</td>
</tr>
<tr>
<td>CCl4</td>
<td>0.22±0.03</td>
<td>0.81±0.03***</td>
</tr>
<tr>
<td>Standard drug</td>
<td>0.20±0.03</td>
<td>0.36±0.04***</td>
</tr>
<tr>
<td>200 mg kg⁻¹</td>
<td>0.21±0.04</td>
<td>0.33±0.05**</td>
</tr>
<tr>
<td>(water extract)</td>
<td>0.17±0.03</td>
<td>0.35±0.36***</td>
</tr>
<tr>
<td>400 mg kg⁻¹</td>
<td>0.26±0.05</td>
<td>0.57±0.06***</td>
</tr>
<tr>
<td>(methylene extract)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 mg kg⁻¹</td>
<td>0.20±0.03</td>
<td>0.49±0.07***</td>
</tr>
<tr>
<td>(methylene extract)</td>
<td></td>
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<table>
<thead>
<tr>
<th>AST (U L⁻¹)</th>
<th>ALP (U L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Control</td>
<td>35.2±4.93</td>
</tr>
<tr>
<td>CCl4</td>
<td>27.4±2.64</td>
</tr>
<tr>
<td>Standard drug</td>
<td>30.4±1.54</td>
</tr>
<tr>
<td>200 mg kg⁻¹</td>
<td>31.0±2.33</td>
</tr>
<tr>
<td>(water extract)</td>
<td>35.4±3.56</td>
</tr>
<tr>
<td>400 mg kg⁻¹</td>
<td>24.6±3.74</td>
</tr>
<tr>
<td>(methylene extract)</td>
<td></td>
</tr>
<tr>
<td>200 mg kg⁻¹</td>
<td>24.4±3.40</td>
</tr>
<tr>
<td>(methylene extract)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SD. The difference was found to be significant (p<0.05, **p<0.01, ***p<0.001) when compared with CCl4 group and significant (p<0.05, **p<0.01, ***p<0.001) when compared with standard drug group.

The increase of the serum AST, ALT, ALP and bilirubin levels at day 5 and 10 days after CCl4 administration was significantly (p<0.01-0.001) reduced by treatment with methanolic extract of C. decidae stems at 200 and 400 mg kg⁻¹ compared with group II (CCl4 group) and (p<0.05-0.01) when compared with group III (standard drug).

**DISCUSSION**

The present study is an attempt to elucidate the likely hepatoprotective properties of C. decidae stems aqueous and methanolic extracts in carbon tetrachloride induced hepatotoxicity model in rats. Inadequacy of treatment with conventional drugs and possible hazards associated with their use prompted our search for better and safer hepatoprotectives of herbal origin. Ozbek et al. (2004) have proposed similar lines of investigation. In the present study, the choice of the plant has been based on the widely held folkloric belief that these plants possess effectiveness against jaundice and they are component of many polyherbal formulations (El-Ghazali et al., 1987; El Kamali and El-Khalifa, 1999).

Determinations of serum levels of enzymes such as ALT and AST renders a reliable means of assessment of liver damage (Gupta et al., 2004).

The results using the model of CCl4-induced hepatotoxicity in rats demonstrated that both extracts, aqueous and methanolic, from C. decidae stems reduced the levels of AST, ALT, ALP and bilirubin when given prior to CCl4 intoxication. The decreased level indicates the stabilization of plasma membrane and protection of hepatocytes against CCl4 damage (Manjunatha et al., 2008). This suggests a hepatoprotective activity endowed in this plant. Further supportive evidence is rendered by the observation that the necrotic lesions were also reduced in rats given both extracts.
The present results agree with previously reported data (Gupta et al., 2004; Singab et al., 2005; Manjunatha et al., 2008). Furthermore, the present study revealed the evidence of the existence of flavonoids, cyanogenic glycosides and triterpenes which are antioxidant and may be responsible for the hepatoprotective property of the plant as suggested by previous researchers (Al-Yahya, 1986; Evans, 2002; Satyanarayana et al., 2008; Gupta et al., 2004; Pattanayak and Priyashree, 2008). In addition, Yadav et al. (1997) reported that C. decidua was found to alter superoxide dismutase and catalase enzyme levels to reduce oxidative stress. Previous studies also showed that other species of the genus Capparis such as C. spinosa have potential protective activity against liver damage (Gadgoli and Mishra, 1995).

The present results have also shown appreciably high contents, in the plant, of minerals such as ferrous and vitamins such as vitamin C which may contribute to the extracts hepatoprotective activity as suggested by Duhan et al. (1992). The role of vitamin C in protecting liver damage may be attributed to the antioxidant properties of this vitamin in biological systems (Adams, 2001). Moreover, present results showed that the aqueous extract of C. decidua had higher activity than the methanolic extract, probably related to the more polar phytoconstituents.

Further investigation may have to be done to determine the exact phytoconstituent(s) responsible for the hepatoprotective effects of C. decidua.

REFERENCES