Hepatoprotective effect of hydroalcoholic extract of *Oxalis corniculata* L. On carbon tetrachloride induced hepatotoxicity in mice

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**ABSTRACT**

*Oxalis corniculata* Linn. (family: Oxalidaceae) is herbaceous plant found in Himalayan region of India. The juice of this plant is traditionally used in liver disorders by Lepcha tribal people of the Sikkim and Darjeeling region in India. No scientific evidence is reported till date to prove the role of this plant in liver disorder. The present study was carried out to prove the hepatoprotective effect of hydroalcoholic extract of *O. corniculata* (HAEOC) in experimental albino mice in CCl₄ induced hepatotoxicity model. Phytochemical screening confirmed the presence of carbohydrates, flavanoids, glycosides, tannins and vitamin C in the extract. The present study shows a significant increase in the levels of SGPT, SGOT and serum bilirubin levels and decrease in TP levels on exposure to CCl₄, indicating considerable liver damage (Table 2). Administration of HAEOC at dose levels (200 and 400 mg/kg) significantly (P<0.01, P<0.005) decreased the elevated level of the serum enzymes (SGPT & SGOT) and bilirubin level, produced by CCl₄ whereas significantly (P<0.01) increased the level of total protein and hence, caused a subsequent recovery towards normalization comparable to the control groups animals. In conclusion, the hydroalcoholic extract of *O. corniculata* has shown significant protection against CCl₄ induced hepatocellular damage which may be attributed to the presence of flavonoids, C-glycosylflavones, β-carotene and Vitamin C as well as due to its antioxidant activity which also justify its traditional claim in the treatment of liver disorder.

**Keywords:** *Oxalis corniculata*, hepatoprotective, hydroalcoholic extract, CCl₄.

**INTRODUCTION**

*Oxalis corniculata* Linn. (Oxalidaceae), commonly known as Creeping wood sorrel [English], Tinpatiya [Hindi], Changeri [Sanskrit], Khatt-i-butti [Urdu] is a sub-tropical plant and native of India [1]. It is a somewhat delicate-appearing, low-growing, herbaceous plant abundantly distributed in damp shady places, roadsides, plantations, lawns, nearly all regions throughout the warmer parts of India, especially in the Himalayas up to 8,000 feet height [2].

The entire plant is rich in vitamin C and β-carotene [3]. Preliminary phytochemical screening reported carbohydrates, fatty acids, flavanoids, glycosides, tannins, phytosterols, and volatile oil in this plant [4]. Three C-glycosylflavones viz. 6-C-glucosylluteolin (isoorientin), 6-C-glucosylapigenin (isovitexin) and isovitexin 7-methyl ether (swertisin) have been isolated from the leaves of *Oxalis corniculata* [5].

This plant is well known for its medicinal value as a good appetizer and as a remover of kapha, vata and piles. It is also known to cure dysentery, diarrhea and skin diseases [1]. The plant is traditionally used in stomach aches [6], headache [7], worm infection, scorpion sting and to stop bleeding from wounds in Margalla hills of Pakistan [8] and mouth apathae [9]. In India, whole plant juice (5-7 ml) is taken once daily for 6-7 weeks in liver disorders by Lepcha tribal people of the Sikkim and Darjeeling region [10].

The plant is reported to possess antibacterial [11], antidiarrhoeal [12], anticancer, antifungal [13], Antim-plantation and abortifacient [14], cardioprotective [15] and nematocidal [16] activities etc.

Since, no scientific study has reported the role of *O. corniculata* in liver protection till date, therefore, the present work was undertaken to prove the hepatoprotective effect of hydroalcoholic extract of this plant and to justify its traditional claim.
MATERIALS & METHODS

Plant materials

Whole plant of *Oxalis corniculata* L. was collected from the Campus of Kurukshetra University, Kurukshetra, India during September-October 2009. The plant was authenticated by Dr. H.B Singh, Raw Herbarium and Museum NISCAIR, New Delhi, India where a voucher specimen (NISCAIR/ RHMD/Consult/-2009-10/1345/147) of plant was preserved in the herbarium for further reference.

Extraction

The air dried plant material was extracted with hydroalcohol (50:50) using soxhlet apparatus as per reported methods [17]. The extract was concentrated to dryness using rotary evaporator (Heidolph, model number-4011, USA) and preserved in a tightly closed air tight bottle in refrigerator at 4°C. The yield of the hydroalcoholic extract of O. corniculata (HAOEC) was determined as 16.8% w/w.

Animals

Albino mice (25-30g) of either sex were procured from Choudhary Charan Singh Haryana Agriculture University, Hisar, India and used for the study. The animals were kept and maintained under controlled laboratory conditions of temperature (21°C±2°C), humidity (60% ± 1%) and 12 h light/dark cycles. Experimental protocols & procedures used in this study were approved by Institutional Animal Ethical Committee of this University and conformed to the guideline of Committee for the Purpose of Control & Supervision of Experiments on Animals (Reg No. 235/CPCSEA).

Chemicals

All the chemicals used in the study were of analytical grade. Carbon tetrachloride (CCl₄) was purchased from Loba chemicals Pvt. Ltd., Mumbai, India. Silymarin was obtained as gift sample from Advik Laboratories, Gurgaon, Haryana. Standard kits for measuring biochemical parameters were obtained from ERBA diagnostics, Mumbai.

Phytochemical analysis

Hydroalcoholic plant extract was subjected to preliminary phytochemical screening to identify the presence of various constituents as per reported methods [17].

Experimental Procedure

Slightly modified procedure for CCl₄ induced hepatotoxicity was followed [18]. Albino mice were divided into five groups each containing five mice. Group I (normal control) received only Tween 80 (2% v/v, 1 ml/kg body weight, p.o.) for 9 days. Group II (CCl₄ treated) received Tween 80 (2% v/v, 1 ml/kg body weight, p.o.) for 9 days, and CCl₄-liquid paraffin (1:1, 0.1 ml/kg body weight, i.p.) on days 7 and 9. Group III was treated with the standard drug silymarin (100 mg/kg body weight, p.o.) daily for 9 days, and also received CCl₄-liquid paraffin (1:1, 0.1 ml/kg body weight, i.p.) on days 7 and 9, 30 min after administration of silymarin. Groups IV and V (test group animals) were administered 200 and 400 mg/kg doses of HAOEC p.o., respectively, for 9 days; in addition, they received CCl₄-liquid paraffin (1:1, 0.1 ml/kg body weight, i.p.) on days 7 and 9, 30 min after administration of HAOEC. On day 10, animals were anaesthetized. The blood samples were collected separately into the sterilized dry centrifuge tubes. The separated serum was estimated for various biochemical parameters like Serum glutamic pyruvic transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT) total bilirubin (TB) and total protein (TP) levels [19-21].

Statistics

The Dunnett's test was employed for statistical comparison. p<0.05 and p<0.01 were considered significant in relation to control.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical study confirmed the presence of carbohydrates, flavanoids, glycosides, tannins and vitamin C in HAOEC as shown in Table 1.

Hepatoprotective activity

In the present study, HAOEC was evaluated for the hepatoprotective activity using CCl₄ induced hepatotoxicity in mice. CCl₄ is used extensively to investigate hepatoprotective activity on various experimental animals [22]. The hepatotoxicity induced by CCl₄ is due to the formation of its metabolite, a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage. Hepatocellular necrosis leads to rise in the levels of the serum marker enzymes, which are released from the liver into blood. The increased levels of SGPT, SGOT and serum bilirubin are conventional indicators of liver injury [23-24].

The present study shows a significant increase in the levels of SGPT, SGOT and serum bilirubin levels and decrease in TP levels on exposure to CCl₄, indicating considerable liver damage (Table 2). Administration of HAOEC at dose levels (200 and 400 mg/kg) significantly (P<0.01, P<0.005) decreased the elevated level of the serum enzymes (SGPT & SGOT) and bilirubin level, produced by CCl₄, whereas significantly (P<0.01) increased the level of total protein and hence, caused a subsequent recovery towards normalization comparable to the control groups animals. Similar results have also been reported previously where plant extracts have significantly shown the hepatoprotective effect in CCl₄ induced liver toxicity [25-27].

It has been reported that presence of flavanoids (28,29), vitamin C [30] in several other plant extracts are found to be responsible for hepatoprotective effect in the previous studies. The in vitro antioxidant activity of aqueous extract of *O. corniculata* at 200 mg/kg dose has been already reported [15]. The observed hepatoprotective effect of this plant extract may be attributed to the presence of flavonoids, C-glycosylflavones, ß- carotene and Vitamin C as well as due to its antioxidant activity which also justify its traditional claim in the treatment of liver disorder.
CONCLUSIONS

The results of the study reveals that the hydroalcoholic extract of whole plant of *Oxalis corniculata* Linn. shows protective effect against CCl₄ induced hepatotoxicity in experimental albino mice. Therefore, the study justifies the usage of this plant in traditional medicine for treatment of liver disorders. However, further studies are required to isolate & identify the active hepatoprotective principles in the plant as well as elucidation of their mechanism of action.

Table 1. Phytochemical screening of HAEOC

<table>
<thead>
<tr>
<th>Category of phytoconstituents</th>
<th>Tests/Reagents</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff’s/Wagner’s reagent</td>
<td>-ve</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molisch reagent test</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lead Acetate/ Sodium Hydroxide/ Shinoda test</td>
<td>+ve</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Keller-Kilani/Modified Borntrager test</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>-ve</td>
</tr>
<tr>
<td>Steroids</td>
<td>Salkowski/Liebermann-Burchard reaction</td>
<td>-ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride/ Bromine solution</td>
<td>+ve</td>
</tr>
</tbody>
</table>

+ve indicates presence whereas -ve indicates the presence of phytoconstituents

Table 2. Effects of HAEOC on biochemical parameters in CCl₄ induced hepatotoxicity in mice.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment Groups and doses</th>
<th>SGPT (U/L)</th>
<th>SGOT (U/L)</th>
<th>Total Protein (g%)</th>
<th>Direct Bilirubin (mg/dl)</th>
<th>Total Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control (2% v/v Tween 80; 1ml/kg)</td>
<td>118.60 ± 18.57**</td>
<td>100.73±1.10**</td>
<td>11.30± 1.11*</td>
<td>2.25± 0.13*</td>
<td>0.83± 0.06**</td>
</tr>
<tr>
<td>2</td>
<td>CCl₄ Control (in olive oil in 1:1 ratio, 1 ml)</td>
<td>411.58 ± 16.79</td>
<td>260.30± 29.77</td>
<td>8.16± 1.17</td>
<td>4.794± 0.29</td>
<td>2.18± 0.20</td>
</tr>
<tr>
<td>3</td>
<td>Standard (Silymarin treated)</td>
<td>284.77 ± 17.90**</td>
<td>146.36±13.6**</td>
<td>12.26± 1.35</td>
<td>1.88± 0.24**</td>
<td>1.18± 0.08**</td>
</tr>
<tr>
<td>4</td>
<td>HAEOC (200 mg/kg, p.o.)</td>
<td>189.24 ± 10.25**</td>
<td>206.14± 8.41*</td>
<td>9.02± 0.85*</td>
<td>2.17± 0.60*</td>
<td>1.09± 0.15**</td>
</tr>
<tr>
<td>5</td>
<td>HAEOC (400 mg/kg, p.o.)</td>
<td>189.60 ± 3.13**</td>
<td>154.04±37.29*</td>
<td>9.42± 0.12*</td>
<td>1.70± 0.09**</td>
<td>1.33± 0.14**</td>
</tr>
</tbody>
</table>

n = 5 mice in each group. Values expressed as mean±S.E.M

**P < 0.01(Compared with CCl₄ Control)

*P < 0.05(Compared with CCl₄ Control)

REFERENCES

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