Liver is the largest organ in the vertebrate body and the site for intense metabolism. Liver diseases remain one of the serious health problems and the Indian traditional system of medicine, especially Ayurveda, have put forward a number of medicinal plants and their formulations for liver disorders. In this modern age, it is very important to provide scientific proof to justify the various medicinal uses of herbs. Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost (1). However, a satisfactory remedy for serious liver diseases is still unknown and search for effective and safe drugs for liver disorders continues to be an area of interest. Today, there has been a global trend for the revival of interest in the traditional system of medicine. The most popular ayurvedic herbal preparation Liv.52 by Himalaya Drug Company is widely used for the treatment of various liver diseases. The scientific investigation or evaluation of medicinal plants has become more essential today. The present study has been undertaken to investigate the hepatoprotective activity of the aqueous and alcoholic extracts of *Vitis vinifera* Linn. roots against carbon tetrachloride-induced liver damage in rats.

**Vitis vinifera** Linn. (commonly known as Grape Vine) (Vitaceae), native to the Mediterranean region, central Europe, and southwestern Asia, from Morocco and Spain north to southern Germany and east to northern Iran. Traditionally, different parts of the plant are used in the treatment of various diseases like sap of young branches are used as remedy for skin diseases. Leaves are astringent and used in diarrhoea. Juice of unripe fruit is astringent and also used in throat infections. Dried fruit is demulcent, cool, sweet, laxative, stomachic and used in thirst, coughs. A malagma made from the seed is said to be a folk remedy for condylomata of the joints. The fruit, prepared in various manners, is used in the treatment of liver disorders, uterine tumors, and cancer (1, 2). The juice, prepared in various manners, is said to be a remedy of tumors of the tonsils, tumors of the neck, chronic tumors, and cancers. Common uses include preparation of wine, raisins, juice, with some cultivars adapted for the canning industry. Grape seeds contain 6–20% oil, used for edible purposes, soaps, and as a linseed substitute (3). From the preliminary phytochemical screening, a large number of phenolic compounds, flavonoids and terpenoids have been evaluated in *Vitis* species and very less number of sterols and carbohydrates are obtained.

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**NATURAL DRUGS**

HEPATOPROTECTIVE ACTIVITY OF *VITIS VINIFERA* ROOT EXTRACT AGAINST CARBON TETRACHLORIDE-INDUCED LIVER DAMAGE IN RATS

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**Abstract:** The ethanolic extract of the root of *Vitis vinifera* (Vitaceae) was evaluated for hepatoprotective activity in rats with liver damage induced by carbon tetrachloride. The extract at an oral dose of 200 mg/kg exhibited a significant protective effect by lowering the serum levels of SGPT, SGOT, alkaline phosphatase and total bilirubin. The extract at this dose also increases the level of total protein. These biochemical observations were supplemented by histopathological examination of liver sections. The activity of extract was also comparable to that of silymarin, a known hepatoprotective drug.

**Keywords:** *Vitis vinifera*, hepatoprotective, silymarin, carbon tetrachloride

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MATERIALS AND METHODS

Plant material
The root of *Vitis vinifera* collected from the local field of Hisar, Haryana (India) was identified by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR) (Ref. no. NISCAIR/RHMD/Consult/-2008-09/1020/51) India. The voucher specimen has been deposited in the herbarium section of the Pharmacognosy Division, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar for future reference.

Preparation of extract
The roots were dried at 40 ± 1°C, grounded into a granulated powder and defatted with petroleum ether. The ethanolic extract was obtained by extracting 4 kg of defatted root powder with ethanol (95%) at 50°C for 72 h in Soxhlet apparatus followed by filtration and concentrated in rotary vacuum evaporator at 50 ± 5°C to dryness. The yield was found to be 30 g/kg of the plant. The residue has brown color.

Drugs and chemicals
Carbon tetrachloride (CCl4) was obtained from E. Merck (India) Ltd. Mumbai. Silymarin was purchased from Micro Labs, India. All other chemicals used in the study were of analytical grades.

Animals
Wistar rats (200–250 g) were procured from Disease Free Small Animal House, Chaudhary Charan Singh Haryana Agriculture University, Hisar (Haryana). The rats were housed in a single cage (polycarbonate cage size: 29 × 22 × 14 cm) under laboratory conditions with alternating light and dark cycle of 12 h each. The animals had free access to food and water. The animals were kept fasted 2 h before and 2 h after drug administration. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India (Registration No. 0436).

Induction of hepatic injury
Hepatic injury in rats was induced separately by administration of equal mixture of CCl4, and olive oil (50% v/v, 1 ml/kg, i.p.). Liver damage was monitored by raised biochemical marker enzymes (SGOT, SGPT, and alkaline phosphatase) and bilirubin (2).

Acute toxicity studies
Acute toxicity studies were performed according to OECD-423 guidelines (3). Wistar rats selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only, Ethanolic extract of *Vitis vinifera* (AVV) was administered orally at a dose of 5 mg/kg initially and mortality, if any, was observed for 3 days. The dose administered was considered as toxic dose if mortality was observed in two out of three animals. If the mortality was in only one animal out of three animals, then the same dose was repeated again to confirm the toxic effect. The higher (50, 300, 2000 mg/kg) doses of AVV were employed for further toxicity studies if no mortality was noticed.

Experimental protocols
The animals were divided into 5 groups of 6 animals each. Group I, which served as normal control, received distilled water (1 ml/kg, p.o.); Group II received equal mixture of CCl4, and olive oil (50% v/v, 0.5 ml/kg, i.p.) once daily for 7 days (4). Group III received an equal mixture of CCl4, and olive oil and AVV (100 mg/kg, p.o.) simultaneously once daily for 7 days. Group IV received equal mixture of CCl4, and olive oil and AVV (200 mg/kg, p.o.) simultaneously once daily for 7 days. Group V served as standard group and received equal mixture of CCl4, and olive oil and silymarin (25 mg/kg, p.o.) simultaneously once daily for 7 days (5).

On the eighth day, the fasted animals were sacrificed by cervical decapitation. Trunk blood was collected in sample tubes and was allowed to clot and the serum was separated by centrifugation at 2500 rpm at 37°C and used for the assay of biochemical marker enzymes (SGOT, SGPT, and alkaline phosphatase), bilirubin and total protein. Immediately after collecting blood, the animals were sacrificed and livers dissected out for histological studies. SGOT, SGPT, serum alkaline phosphatase (ALP) and bilirubin were determined by using commercially available kits (Span Diagnostic Ltd., Surat, India). Serum total protein was measured according to the method of Lowry (6). The results were expressed as units/L (U/L).

Histopathology
The liver of the rats of all groups was excised from the animals and washed with the normal saline. The materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h and processed for paraffin embedding. Sections of 5 m thickness were taken using a microtome,
Hepatoprotective activity of *Vitis vinifera* root extract...

processed in alcohol-xylene series and were stained with alum-hematoxylin and eosin and subjected to histopathological examination.

**Biochemical assay**

The fasted animals were sacrificed by cervical decapitation on 8th day of first treatment. Trunk

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal control</th>
<th>CCl4 control</th>
<th>AVV (100 mg/kg)</th>
<th>AVV (200 mg/kg)</th>
<th>Silymarin (25 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.90 ± 0.24</td>
<td>2.61 ± 0.388</td>
<td>1.58 ± 0.25</td>
<td>1.08 ± 0.46**</td>
<td>1.05 ± 0.22**</td>
</tr>
<tr>
<td>SGOT (U/L)</td>
<td>61.13 ± 10.23</td>
<td>198.33 ± 14.87</td>
<td>154.00 ± 12.73</td>
<td>109.01 ± 12.94**</td>
<td>95.28 ± 10.69***</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>48.26 ± 8.58</td>
<td>102.10 ± 10.91</td>
<td>82.04 ± 7.99</td>
<td>67.67 ± 7.82***</td>
<td>57.08 ± 6.59***</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>129.18 ± 8.62</td>
<td>382.94 ± 12.92</td>
<td>235.51 ± 14.88</td>
<td>186.52 ± 12.62***</td>
<td>163.25 ± 10.88***</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.02 ± 0.60</td>
<td>5.41 ± 0.72a</td>
<td>6.93 ± 0.88</td>
<td>6.98 ± 0.92*</td>
<td>7.0 ± 0.85**</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. Number of rats = 6. CCl4 control group compared with normal control group *p < 0.001. Experimental groups compared with CCl4 control group **p < 0.01, ***p < 0.001. AVV = ethanolic extract of *Vitis vinifera* root.

**Figure 1. Histopathology**

Normal liver cells

Hepatotoxic cells

Liver cells (Silymarin)

*Vitis vinifera* (200 mg/kg)
blood was collected in tubes and the serum obtained by centrifugation at 2500 rpm for 5 min. was used for the determination of serum biochemical marker enzymes (SGOT, SGPT and alkaline phosphatase), bilirubin and total protein.

**Statistical analysis**

The values are expressed as the mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett's t-test; p values < 0.05 were considered to be significant.

**RESULTS**

**Acute toxicity studies**

All the doses (5, 50, 300 and 2000 mg/kg) of AVV employed for acute oral toxicity studies were found to be non-toxic. AVV did not produce any mortality even at the highest dose (2000 mg/kg) employed. Two sub maximal doses (100 and 200 mg/kg), which were found to be safe, were employed for further pharmacological investigations.

**Biochemical estimation**

The results of hepatoprotective activity of AVV on CCl₄-treated rats are shown in Table 1. The AVV treatments (100 mg/kg) have minor effect on the levels of hepatic enzymes when compared to CCl₄-treated animals. The AVV treatments (200 mg/kg) significantly (p < 0.01) reversed the levels of hepatic enzymes when compared to CCl₄-treated animals. Silymarin (25 mg/kg)-treated animals also showed significant (p < 0.01) inverted levels of hepatic enzymes when compared to CCl₄-treated animals. There was a significant decrease (p < 0.001) in the serum of biochemical marker enzymes (SGOT, SGPT and alkaline phosphatase) and bilirubin. The total protein levels in CCl₄-treated groups when compared to the control group, was significantly (p < 0.01 and p < 0.05) reversed with the treatment of AVV (200 mg/kg) and AVV (100 mg/kg), respectively.

**DISCUSSION**

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver disease (7). The assessment of liver function can be made by estimating the activities of serum enzymes such as SGOT, SGPT and ALP. During hepatic damage, there may be an increase in these enzyme levels in serum with the extent of liver damage. The altered levels of these enzymes in CCl₄-treated rats in the present study corresponded to the extensive liver damage induced by the toxin.

The present study has demonstrated that AVV at 200 mg/kg exhibited significant hepatoprotective activity against liver injury induced by CCl₄. Carbon tetrachloride induces hepatotoxicity by metabolic activation; therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Carbon tetrachloride is metabolically activated by the cytochrome P450 in the endoplasmic reticulum to form a trichloromethyl free radical (CCl₃•), which combines with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation, which leads to change in the structures of endoplasmic reticulum and other membrane, loss of metabolic enzymes activation, reduction of protein synthesis and elevation of serum transaminases leading to liver damage (8). Amino transferases constitute a group of enzymes that catalyze the interconversion of amino acids and α-ketocids by the transfer of amino groups. These are liver specific enzymes and are considered to be very sensitive and reliable indices for necessary hepatotoxic as well as hepatoprotective or curative effect of various compounds. Both AST and ALT levels increase due to toxic compounds affecting the integrity of liver cells (9). Alkaline phosphatase is a membrane bound glycoprotein enzyme with a high concentration in sinusoids and endothelium. This enzyme reaches the liver mainly from the bone. It is excreted into the bile; therefore, its elevation in serum occurs in hepatobiliary diseases. The results of the present study indicate that AVV probably stabilizes the hepatic plasma membrane from CCl₄-induced damage.

The liver is known to play a significant role in the serum protein synthesis, being the source of plasma albumin and fibrinogen and also the other important components like α- and β-globulin. The liver is also concerned with the synthesis of α₂-globulin. The serum albumin level is low in hepatic diseases. The result reveals that in the animals pretreated with hepatoprotective agents prior to the challenge with CCl₄, the liver biosynthesis of protein continues to be unaffected. The metabolic transformation of amino acid in liver by synthesis, transamination, etc., may be impaired due to the escape of both-non-proteins and protein nitrogenous substances from injured liver cells as mediated by raise in the serum enzyme levels of SGOT, SGPT and ALP. The protective activity of the extracts may be attributed to the membrane stabilizing agents present in the AVV, which may avert enzyme leakages in tissues in response to CCl₄ poisoning leading to
enhanced metabolic transformation of amino acids in liver through synthesis and transformation (10). AVV enhanced the synthesis of TP and albumin, which accelerates the regeneration process and the protection of liver cells. Therefore, the increased level of total protein in serum indicates the hepatoprotective activity of extract. The effects of AVV (200 mg/kg) were comparable with the effects of silymarin-treated groups.

CONCLUSION

It can be concluded that ethanolic extract of *Vitis vinifera* roots possesses hepatoprotective activity against CCl₄-induced liver damage in rats. According to the results obtained in this study, it may be inferred that, in general, AVV reverses the hepatic damage induced by CCl₄. To the best of our knowledge, this is the first report about *in vivo* activity of *Vitis vinifera* root and seems to raise some concern about the traditional indications of this species as a medicine for liver diseases. Certainly, further studies need to be carried out with other hepatotoxic compounds to prove the hepatoprotective efficacy of *Vitis vinifera*.

REFERENCES


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