Effect of *Viscum album* on acute hepatic damage caused by carbon tetrachloride in rats

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**Aim:** To investigate the effect of *Viscum album*, a plant used for the treatment of hepatocellular carcinoma that has immune-modulating properties, on acute hepatic injury in rats.

**Materials and methods:** Hepatotoxicity was induced by CCl₄ orally (0.28 mL/kg). Rats received either *Viscum album* at 1 of 2 dose levels (0.1 or 0.2 mL/kg) once weekly subcutaneously alone or with silymarin (25 mg/kg, orally), or silymarin (25 mg/kg) once daily orally for 1 month, starting at the time of administration of CCl₄. Liver damage was assessed by determining liver serum enzyme activities and by hepatic histopathology.

**Results:** *Viscum album* administration decreased the increases in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), and also prevented the development of hepatic necrosis caused by CCl₄. The effect of *Viscum album* was dose-dependent. *Viscum album* administered at 0.1 or 0.2 mL/kg caused significant reduction in the elevated plasma ALT by 51.2% and 65.6%, AST by 52.6% and 61.1%, and ALP by 27.7% and 57.6%, respectively. In comparison, the elevated serum ALT, AST, and ALP levels decreased to 48.9%, 51.8%, and 30.8% of the controls, respectively, with 25 mg/kg of silymarin. *Viscum album* (0.2 mL/kg) administered together with silymarin resulted in 73.1%, 67.6%, and 65.8% decreases in serum ALT, AST, and ALP levels, respectively. Histopathologic examination of the livers of rats treated with CCl₄ and administered *Viscum album* at 0.2 mL/kg showed marked restoration of the normal architecture of the liver tissue with minimal fibrosis.

**Conclusion:** Results of the present study indicate that the administration of *Viscum album* in a model of liver injury induced by CCl₄ in rats results in less liver damage.

**Key words:** *Viscum album*, hepatic injury, rat, CCl₄

**Introduction**

Currently, the standard therapy for patients with hepatitis C virus infection involves the combined administration of interferon and ribavirin (1,2). With the use of interferon and ribavirin, sustained response is obtained in approximately 55% of patients (3,4). Despite the fact that this combination therapy can eliminate the virus and prevent the progression of the disease, frequent side effects and relapses preclude the use of this form of therapy in a substantial proportion of patients (4,5). Thus, it is clear that there is still a need for the identification of compounds that are capable of modulating chronic inflammation in the liver of such patients because hepatic inflammation appears to be the key pathological substrate that drives fibrogenesis (6,7).

Apart from interferon-ribavirin therapy, silymarin, a standardized extract of milk thistle (*Silybum marianum*), is widely prescribed for chronic liver disease (8). Silymarin possesses antioxidant properties attributable to its flavonoid components, silibinin, silicristin, and silidianin (9). In animal models of hepatic injury, the herb has been shown to ameliorate liver injury and to
exert antifibrotic effects (10-13). Studies in patients with chronic hepatitis C and alcoholic liver disease have shown that silymarin increases superoxide dismutase activity of lymphocytes and erythrocytes (14), and increases glutathione and decreases lipid peroxidation in peripheral blood cells (15,16). Silymarin, however, does not appear to affect viral load or liver histology in hepatitis B or C (17).

Recently, patients with chronic hepatitis C treated with *Viscum album* preparations had a significant improvement of both AST and ALT (18). *Viscum album* preparations consist of aqueous extracts of *Viscum album* L., the European mistletoe (family Loranthaceae). The latter is a semiparasitic plant that is normally found growing on a variety of trees in temperate regions worldwide. European mistletoe preparations are aqueous extracts obtained from the whole plant. They have cytotoxic and immunomodulatory properties and are among the most widely used unconventional cancer therapies in Germany and Central Europe (19,20). In Germany, mistletoe extract is listed in certain directories as a commercially available drug for cancer treatment (21).

Since there is a need to explore alternative therapies for chronic liver disease, the present study aimed to investigate the effect of *Viscum album* on the development of experimental liver damage induced by the administration of the hepatotoxin CCl₄ in rats. The effect of *Viscum album* was evaluated both on biochemical markers as well as by histological techniques and compared with that of silymarin, in view of the wide use of this plant extract as a hepatoprotective agent in patients with chronic hepatitis C.

### Materials and methods

#### Animals

Adult Sprague-Dawley rats of either sex, weighing 120-130 g, were used throughout the experiments and fed with standard laboratory chow and water ad libitum. All animal procedures were performed according to approved protocols and in accordance with the recommendations for the proper care and use of laboratory animals.

#### Drugs and chemicals

Carbon tetrachloride (BDH Chemicals, England), *Viscum album* (viscum fraxini-2, ATOS Pharma Co., Cairo), and silymarin (Sedico Pharmaceutical Co., Cairo) were used in the experiments. The mistletoe preparation for the study was an aqueous injectable solution. It contained 1 mL of viscum fraxini in dilution stage 2 (15 mg extract of 20 mg mistletoe herb from ash tree, diluted in Na₂HPO₄, ascorbic acid, and water), which is equivalent to 10,000 ng/mL injection ampoules. Further dilution with distilled water was done to obtain the necessary doses. The doses employed were based upon the human dose after conversion to rat doses according to Paget and Barnes’ (22) conversion tables.

**The carbon tetrachloride model of hepatic damage**

The rats were divided into 6 equal groups of 6 rats each. Groups 1-5 received CCl₄ in olive oil (1:1, vol/vol) orally at a dose of 2.8 mL/kg. Rats were administered half the initial dose of CCl₄ (0.14 mL/kg) once weekly after the first administration of CCl₄ so as to maintain hepatic damage. Starting on the first day of CCl₄ administration, rats were treated with saline (CCl₄ control, or group 1), *Viscum album* (at 1 of 2 dose levels, 0.1 or 0.2 mL/kg) once weekly subcutaneously (sc) (groups 2 and 3, respectively), silymarin only (25 mg/kg) orally once daily (group 4), or *Viscum album* (0.2 mL/kg, sc) plus silymarin (25 mg/kg, orally) once weekly (group 5) for 30 days. In addition, another group of rats (n = 6) received saline but no CCl₄ daily for 30 days (group 6). Rats had free access to food and drinking water during the study.

#### Biochemical assessment

At the end of the experiments, blood samples were obtained from the retro-orbital vein plexuses under ether anesthesia. ALT and AST activities in serum were measured according to the Reitman-Frankel colorimetric transaminase procedure (23), whereas colorimetric determination of ALP activity was done according to the method of Belfield and Goldberg (24), using commercially available kits (bioMérieux, France).

#### Histopathological studies

After the end of the treatment period, rats were killed under ether anesthesia, and livers were excised.
and fixed in 10% formalin saline. Sections were prepared and stained with hematoxylin and eosin (H&E) for histological investigation.

Statistical analysis

All results are expressed as means ± SE. Comparison of the values before and after CCl₄ was made by paired Student’s t-test. Multiple group comparisons were performed by ANOVA followed by Duncan’s test. P < 0.05 was considered statistically significant.

Results

Biochemical results

The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in plasma were significantly higher in CCl₄-treated rats than in the saline control group, indicating the severity of hepatic injury and cholestasis caused by CCl₄. Thus, compared with the saline control group, ALT, AST, and ALP were raised by 222%, 173.4%, and 186.5%, respectively (Table). *Viscum album* administered at 0.1 mL/kg significantly reduced the elevated plasma ALT by 51.2%, AST by 52.6%, and ALP by 27.7%. *Viscum album* at the higher dose of 0.2 mL/kg significantly decreased the raised plasma ALT by 65.6%, AST by 61.1%, and ALP by 57.6%. In comparison, the elevated serum ALT, AST, and ALP levels were decreased to 48.9%, 51.8%, and 30.8% of the controls, respectively, with 25 mg/kg of silymarin. *Viscum album* (0.2 mL/kg) combined with silymarin resulted in 73.1%, 67.6%, and 65.8% decreases in serum ALT, AST, and ALP levels, respectively (Table).

Histopathological results

The livers of saline control rats revealed the characteristic hepatic architecture (Figure 1). The liver of rats subjected to CCl₄ showed loss of liver tissue architecture, severe dilatation and congestion of blood vessels (either central veins or portal tract vessels), marked lymphocytic infiltration, and fibrosis extending between the portal areas (Figure 2). Sections of liver tissue from rats treated with *Viscum album* at 0.1 mL/kg showed slight distortion of the normal architecture of liver tissue with marked dilatation and congestion of the portal veins in portal areas (Figure 3). Sections of liver tissue from rats treated with *Viscum album* at 0.2 mL/kg showed preserved liver architecture with marked dilatation of blood sinusoids, denoting edema with congestion and dilatation of blood vessels (Figure 4).

Discussion

In the present study, acute hepatic injury was induced by injection of CCl₄ in rats, leading to high levels of serum aminotransferases and considerable perivenular necrosis. Hepatic injury was reduced by once weekly administration of *Viscum album* with a reduction in plasma levels of hepatocellular enzymes.

Table. Effect of *Viscum album*, silymarin, or *Viscum album* plus silymarin on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in CCl₄-treated rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (IU/L)</th>
</tr>
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<tbody>
<tr>
<td>Saline control</td>
<td>71.0 ± 3.1</td>
<td>80.4 ± 2.5</td>
<td>50.9 ± 1.9</td>
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<tr>
<td>CCl₄ control</td>
<td>228.6 ± 10.0</td>
<td>219.8 ± 3.7</td>
<td>146.1 ± 6.0</td>
</tr>
<tr>
<td>CCl₄ + viscum album 0.1 mL/kg</td>
<td>111.5 ± 3.5</td>
<td>104.1 ± 3.4</td>
<td>105.7 ± 4.1</td>
</tr>
<tr>
<td>CCl₄ + viscum album 0.2 mL/kg</td>
<td>78.7 ± 3.1**</td>
<td>85.5 ± 5.2**</td>
<td>62.0 ± 5.2**</td>
</tr>
<tr>
<td>CCl₄ + silymarin 25 mg/kg</td>
<td>116.8 ± 6.7</td>
<td>106.0 ± 8.1</td>
<td>101.0 ± 7.1</td>
</tr>
<tr>
<td>CCl₄ + silymarin + viscum album 0.2 mL/kg</td>
<td>61.6 ± 3.4**</td>
<td>71.2 ± 2.5**</td>
<td>50.0 ± 2.6**</td>
</tr>
</tbody>
</table>

Results were means ± S.E. Data were analyzed by one-way ANOVA and the means of different groups were compared with Duncan’s multiple range test. *: P < 0.05 compared with the CCl₄ control group. ++: P < 0.05 compared with groups treated with *Viscum album* 0.2 mL/kg or silymarin alone.
ALT and AST as well as the cell membrane enzyme ALP and with marked improvement in liver morphology. These results pointed to a hepatic protective effect of *Viscum album*. The study also indicated the usefulness of combining both *Viscum album* and silymarin.

Little information is available in the literature on the effect of *Viscum album* on hepatic injury. The plant, however, has been widely investigated for its anticancer properties (19,20). Two recent studies done in patients with chronic hepatitis C, treated with a mistletoe preparation as monotherapy for 1 year,
reported a significant improvement in elevated transaminases (25). The mechanism(s) by which *Viscum album* modulates hepatic inflammation remains, however, unclear. *Viscum album* extracts contain mistletoe lectins, cytotoxic glycoproteins also known as viscumin or agglutinin, which are members of the family of type 2 ribosome-inactivating proteins, and viscotoxin, which is a 46-amino acid peptide that damages cell membranes (26). Other constituents include polysaccharides (galacturonan and arabinogalactan) and alkaloids. *Viscum album* extracts have both immunomodulatory (induces TNF-α and IL-12) and apoptosis-inducing properties, which are likely to be dose-dependent (27). The perioperative administration of *Viscum album* attenuated the immuno-suppressive effects of surgery, increasing the number of NK cells, the T and B cells, complement, and IgA, IgG, and IgM values (28). Studies also suggested that European mistletoe possesses insulin-secreting (29), antihyperglycemic, antioxidant activity (30), and cholinomimetic activities (31) for *Viscum album* L.

Findings in the present study suggest that the use of silymarin with *Viscum album* can have an additive beneficial effect in lessening liver inflammation and necrosis caused by CCl₄. The release of aminotransferases into the plasma was markedly reduced, indicating a reduction in the severity of liver damage by the combination. Aminotransferases are sensitive indicators of liver-cell injury and are released into the blood in increasing amounts whenever the liver cell membrane is damaged (32). Silymarin, a standardized plant extract, has been used for many years as a hepatoprotective agent (8,9). In patients with liver disease, silymarin increased superoxide dismutase activity of lymphocytes and erythrocytes (14), and increased glutathione and decreased lipid peroxidation in peripheral blood cells (15,16). In in vitro models of hepatotoxicity, silymarin decreased lactate dehydrogenase leakage, increased oxygen consumption, reduced the formation of lipid peroxides (malondialdehyde), and reduced the increased Ca²⁺ in hepatocytes (33). Silymarin also possesses important antiinflammatory properties, inhibiting the migration of neutrophils into the inflamed site (34), which are likely to be of relevance to its hepatoprotective effects.

Mistletoe preparations are among the most widely used unconventional cancer therapies in many countries (19,20). The results of the present study suggest that mistletoe preparations may be a useful therapeutic intervention for patients with chronic liver disease. Clearly, further studies are required to elucidate the mechanism(s) by which mistletoe preparations exert their hepatoprotective effects seen in the present study.

References

Viscum album and hepatic injury


