Evaluation of Hepatoprotective Effect of *Artimesia Monosperma* Against Carbon Tetrachloride-induced Hepatic Damage Rat

1S.A. El-Toumy, 2E.A. Omara, 3I. Brouard, 3J. Bermejo

1Chemistry of Tannins Department, National Research Center, El-Bohouth St., Dokki, 12622 Cairo, Egypt,

2Pathology Department, National Research Center, El-Bohouth St., Dokki, 12622 Cairo, Egypt

3Instituto de Productos Naturales y Agrobiología, Av. Astrofisico F. Sanchez 3, 38206 La Laguna, Tenerife, Spain

**Abstract:** The hepatoprotective effect of aqueous ethanol extract of *Artimesia monosperma* aerial parts was investigated against carbon tetrachloride-induced acute hepatotoxicity in rat. The hepatoprotective activity of *A. monosperma* was evaluated by determination of liver enzyme markers in the serum (aspartat amino transferase AST; serum alanine transaminase ALT and alkaline phosphatase ALP). The histopathological studies were also carried out to support the above parameters. Oral administration of *A. monosperma* (100 and 200 mg/kg) markedly reduced the elevated values of AST, ALT and ALP caused by CCl₄ treatment. Glutathione (GSH) significantly decreases in the group treated with CCl₄. *A. monosperma* (two doses) and silymarin significantly increased GSH levels when they administrated with CCl₄. However, silymarin normalized liver enzymes and increased GSH levels than *A. monosperma* (two doses), when compared with the control group. Histopathological results revealed that *A. monosperma* treatment with its two doses exhibited almost normal architecture, compared to CCl₄-treated group. Image analysis of liver revealed a marked reduction in liver damage area after treatment with *A. monosperma* (100 or 200 mg/kg) and silymarin compared with CCl₄-treated group.

A phytochemical study of *A. monosperma* resulted in the isolation of a quercetin 3-O-β-glucopyranoside; quercetin 5-O-β-glucopyranoside; isorhamnetin 3-O-β-glucopyranoside; 5, 4’ - dihydroxy 6, 7-dimethoxy flavone; 5, 3’ - dihydroxy 6, 7, 4’- trimethoxy flavone; 5, 7, 3’ - trihydroxy 3, 6, 4’ - trimethoxy flavone; quercetin and isorhamnetin. Structures of the isolated compounds were established by chromatography, UV and 1D/2D ¹H/ ¹³C spectroscopy. Hepatoprotective effect of *A. monosperma* is probably due to combined effect of flavonoids.

**Key words:** *A. monosperma*, Carbon tetrachloride, Hepatoprotective activity, Flavonoids.

**INTRODUCTION**

The liver is the key organ regulating homeostasis in the body. It is involved almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction (Ward and Daly, 1999). The liver is expected not only to perform physiological functions but also to protect against hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account of a high death rate (Pang et al., 1992). In view of server undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and evaluate scientific basis for the traditional herbal medicines which are claimed to possess hepatoprotective activity (Shahani, 1999).

In recent years, flavonoids and other phenolic compounds of plant origin have received increasing attention, especially in the field of pharmaceutical sciences and medicine, due to their potential to prevent a numerous of chronic and degenerative diseases including cancer and cardiovascular diseases (Havsteen, 2002; Ross and Kasum, 2002; Surh, 2003; Boudet, 2007). These compounds have been shown to exert a wide range of antioxidant properties in vitro, and most of their health-promoting effects have been attributed to their antioxidant effect including their ability to scavenge reactive oxygen species (ROS), chelate metal ions, hepatoprotective and terminate free radical reactions (Fang et al., 2002; Sanmugapriya and Venkataraman, 2006; Akachi et al., 2010; Liu et al., 2010). The flavonoids comprise the largest group of plant polyphenols.
found ubiquitously in significant quantities in vegetables and fruits as well as in plant-derived food products and beverages such as tea and wine. The average daily intake of total flavonoids is estimated to be a few hundred of milligrams (expressed as aglycones), which indicates a substantial exposure of humans to these phytochemicals (Hollman and Katan, 1999).

_Artemisia monosperma_ Del. (Asteraceae) is a perennial fragrant plant which grows widely in Arabian deserts (Migahid and Hammouda, 1974; Saleh, 1984), and has been reputed in folk medicine as an antispasmodic, anthelmintic and in treatment of cases associated with blood hypertension (Sharaf et al., 1959; Khafagy et al., 1971). Isolated ingredients from _A. monosperma_ showed insect repellent (El-wan and Dib, 1970; Zaki et al., 1984), antibiotic (Saleh, 1984) and antispasmodic activities. Plants have played a major role in the introduction of new therapeutic agents. It is our opinion that instead of random search of plants, a selective search based on traditional knowledge would be more focused and productive and certainly more economic. To the best of our knowledge there is no scientific literature on hepatoprotective activity of the aerial part of _A. monosperma_. The present study deals with the isolation and identification of flavonoids from the aerial part of _A. monosperma_ and evaluation of the hepatoprotective activity of the aqueous ethanol extract in Sprague Dawley rat.

**MATERIALS AND METHODS**

_Instruments and Equipments:_

NMR experiments were performed on a Bruker AMX 400 and 500 instruments with standard pulse sequences operating at 400, 500 MHz in $^1$H-NMR and 100, 125 MHz in $^{13}$C- NMR. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard and DMSO-d6 as solvent at room temperature. HRESI-MS was taken on a Micromass Autospec (70 eV) spectrometer. UV spectral data was measured on a Shimadzu 240 spectrometer in MeOH. Paper chromatography Whatman 1, using solvent systems A (15% AcOH) and B (n-BuOH-AcOH-H$_2$O, 4:1:5, upper layer). Compounds were visualized by exposure to UV light (365 nm), before and after spraying with AlCl$_3$ and Naturestoff-polyethylene glycol reagents.

_Plant material:_

Aerial parts of _A. monosperma_ were collected in April 2008 from the road Alexandra-Egypt about 130 Km. Identification of the plants was confirmed by Prof. Dr. Ibrahim El-Garf, Department of Botany, Faculty of Science, Cairo, University, and comparison with herbarium specimens. Voucher Specimens were kept in herbarium, Department of Botany, Faculty of Science, Cairo, University and, Cairo, Egypt (Boulos, 2002).

_Extraction and isolation:_

The air-dried aerial parts of _A. monosperma_ (1 Kg) was defatted with CHCl$_3$ (3 x 1 L) and extracted with C$_6$H$_{12}$OH: H$_2$O (7:3; 5 x 3 L) at room temperature. The combined extracts were filtered, evaporated under reduced pressure and lyophilized (150 g). Twenty grams of the dry residue was used for hepatoprotective study. Weighed samples of _A. monosperma_ extract were used to prepare the solutions, which were diluted with distilled H$_2$O to the appropriate concentration for the experiment. The rest of the dry extract was redissolved in 2 L H$_2$O and extracted with EtOAc (5 x 2 L). After evaporation of solvents, the EtOAc extract and the remaining H$_2$O phase gave dark brown solids 25 and 80 g, respectively. The EtOAc extract was loaded on a polyamid 6S column chromatography (80 x 3 cm). The column was eluted with H$_2$O, and then H$_2$O-EtOH mixtures of decreasing polarity and 10 fractions (1 L, each) were collected. The major flavonoid fractions obtained were combined into four fractions after chromatographic analysis. Fraction 1 (2.5 g) was fractionated by column chromatography on Sephadex LH-20 with aqueous EtOH (0- 70%) for elution to give compounds 1 (17 mg) and 7 (25 mg). Fraction 2 (3 g) was subjected to column chromatography on cellulose and n-BuOH saturated with H$_2$O as an eluent to give two major subfractions, then each of them was separately fractionated on a Sephadex LH-20 to yield pure samples 4 (22 mg) and 5 (18 mg). Using the same procedure fraction 3 (2.8 g) gave chromatographically pure samples 2 (15 mg) and 3 (38 mg). Fraction 4 (1.5 g) was chromatography on Sephadex LH-20 using aqueous acetone (0- 25%) for elution to give pure sample 6 (20 mg) and 8 (25 mg).

_Animals:_

Forty-eight male Sprague-Dawley rats weighing (120- 150 g) were purchased from the Animal House of National Research Centre, Dokki, Cairo, Egypt. They were housed at standard environmental condition and were allowed free access to tap water and standard pellet diet. Rats were divided into eight equal groups, each
of six. The aqueous ethanol extract of *A. monosperma* was dissolved in normal saline (0.9 % NaCl) prior to oral administration to experimental animals. Group I served as control, group II: rats were orally administrated 25 mg/Kg of silymarin, group III: rats were treated with 100 mg/kg of aqueous ethanol extract of *A. monosperma*, group IV: rats were treated with 200 mg/Kg aqueous ethanol extract of treated *A. monosperma*. Groups V, VI, VII and VIII were intoxicated with CCl₄ (2.5 ml/Kg) (v / v, paraffin oil) and then groups VI and VII treated orally with *A. monosperma* (100 mg/Kg, 200 mg/Kg, respectively), group VIII treated with silymarin (25 mg/Kg) for 21 following days, daily once by gastric gavage needle. This study was approved by Local Ethical Committee, National Research Centre.

**Biochemical assessment:**
At the end of the experiment, blood samples were obtained from the retro-orbital vein plexuses, under ether anaesthesia. ALT and AST activities in serum were determined according to Reitman and Frankel (1957), ALP activity was done according to the method of Belfield and Goldberg (1971), all animals were sacrificed, then livers were removed and a part from the liver was homogenated and used for determination of the reduced glutathione (GSH) according to the method of Moron et al. (1979). The second part was subjected for histopathological examination.

**Histopathological studies:**
Liver samples were excised and fixed in 10% buffered neutral formalin and paraffin sections were taken at 5 µm thickness processed in alcohol-xylene series and was stained with alum hematoxylin and eosin. The sections were examined microscopically for histopathological changes (Drury and Wallington, 1980).

**Quantitative Measurement:**
Quantitative analysis measurement was achieved by using computerized image analyzer (Leica Qwin 500 image) in Image Analyzer Unit, Pathology Department, National Research Center, and Cairo, Egypt. Image processing and analysis system was used for interactive automatic measurement of the percentage of damaged areas on slides stained with H & E by 15 random fields per slide.

**Statistical analysis:**
Data were analyzed by one-way analysis of variance (ANOVA) followed by least significant difference (LSD) using Excel 2003 Microsoft Corp (11.5612.5606), Redmond, WA software package. Results were presented as means ± standard error of the means (X±SE). P-values < 0.05 were ranged as statistically significant.

**RESULTS AND DISCUSSION**
Fractionation of the EtOAc portion resulted in the isolation and identification of eight known flavonoids 1-8 (Fig 1) (Harborne and Baxter, 1999). The structures of the isolated compounds were established through chromatography, as well as conventional chemical and spectroscopic methods of analysis (UV, HRESI-MS, 1/2D NMR), (Agrawal, 1989).

The results given in Table 1 showed that, when CCl₄ was injected the level of ALT, AST and ALP enzymes were significantly elevated compared with the normal control. Administration of 25 mg/kg of Silymarin induced a significant reduction in serum ALT, AST and ALP compared to that shown in CCl₄ group. Treatment with 100 mg/Kg of the extract of the *A. monosperma* showed significant change in serum ALT, AST and ALP level, also the higher dose 200 mg/Kg caused a marked significant protection, evidenced by the reduction in ALT, AST and ALP values (Table 1). Administration of CCl₄ exerted a significant reduce of GSH levels in the liver tissue. The treatment with *A. monosperma* (two doses) and CCl4 caused significant increase in GSH level in the liver homogenate when compared with CCl₄- treatment alone.

Histological observations basically supported the results obtained from serum enzyme assays. The control sections of the liver showed normal histological features (Fig. 2: A). The livers of CCl₄-intoxicated rats showed massive fatty changes, gross necrosis, broad infiltration of lymphocytes, Kupffer cells around the central vein and loss of cellular boundaries (Fig. 2: B). Histological examination of rat liver treated with two doses of *A. monosperma* extract (100 and 200 mg/Kg) showed normal architecture of the hepatic lobules ( Fig 2. C).

Histopathological observations of the liver of rats treated with *A. monosperma* extract and CCl₄ showed more or less normal architecture of the liver, having reversed to a large extent the hepatic lesions produced by the toxin, almost comparable to the normal control and the silymarin group (Fig. 2: D, E and F).
Table 1: Effect of aqueous ethanol extract of *A. monosperma* (100 and 200 mg/Kg; I & II, respectively) and Silymarin (25 mg/kg) on serum liver enzymes markers (ALT, AST and ALP) and on glutathione (GSH) in normal and carbon tetrachloride-treated rat.

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>ALT IU/ml</th>
<th>AST IU/ml</th>
<th>ALP (U/L)</th>
<th>GSH mol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29.82±0.69A</td>
<td>45.33±1.48A</td>
<td>75.6±1.42A</td>
<td>12.55±0.56A</td>
</tr>
<tr>
<td>Silymarin (25 mg/Kg.b.wt)</td>
<td>25.77±0.58B</td>
<td>38.33±0.92B</td>
<td>77.8±1.40AB</td>
<td>17.38±0.69B</td>
</tr>
<tr>
<td><em>A. monosperma</em> I (100 mg/Kg.b.wt.)</td>
<td>23.99±0.32C</td>
<td>40.17±1.09BF</td>
<td>82.1±1.16BF</td>
<td>13.14±0.58A</td>
</tr>
<tr>
<td><em>A. monosperma</em> II (200 mg/Kg.b.wt.)</td>
<td>24.78±0.88B</td>
<td>39.42±1.11BF</td>
<td>79.7±1.79B</td>
<td>15.07±0.47C</td>
</tr>
<tr>
<td>CCl4 (2.5 ml/Kg b.wt)</td>
<td>54.35±1.66D</td>
<td>88.50±2.26C</td>
<td>138.7±1.61C</td>
<td>5.12±0.26D</td>
</tr>
<tr>
<td>CCl4+ <em>A. monosperma</em> I</td>
<td>39.83±1.58E</td>
<td>59.00±1.41D</td>
<td>119.8±1.24D</td>
<td>8.65±0.26E</td>
</tr>
<tr>
<td>CCl4+ <em>A. monosperma</em> II</td>
<td>36.83±0.95F</td>
<td>50.17±1.17E</td>
<td>105.1±1.02E</td>
<td>9.88±0.35E</td>
</tr>
<tr>
<td>CCl4+ Silymarin</td>
<td>32.17±1.10G</td>
<td>41.33±1.26F</td>
<td>85.1±2.22F</td>
<td>6.60±1.66B</td>
</tr>
</tbody>
</table>

One- way ANOVA, P < 0.05. The different capital letters superscripts are significantly different at P < 0.05. Each value represents the mean ± SEM, n= 6 rats/group.

Fig. 1:

**Quantitative analysis of the area of damage:**

Significant increases in the percentage of damaged areas were observed in CCl4-treated rats when compared to the normal animals. Morphometric analysis of liver sections of the groups treated with CCl4 and *A. monosperma* at doses of (100 mg/Kg, 200 mg/Kg) resulted significant decrease in dose dependent manner in damaged areas as compared with CCl4 and silymarin 25 mg/kg as shown in (Fig. 3).

**Discussion:**

Carbon tetrachloride (CCl4) is one common hepatotoxin used in the experimental study of liver diseases (Obi et al., 1998; Ulicna et al., 2003; Yan-Jan et al., 2004). The hepatotoxic effects of CCl4 are due to its active metabolite, trichloromethyl radical (Johnson and Kroening, 1998). These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. This lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity induced by CCl4 (Kaplowitz et al., 1986). This is evidenced by an elevation in the serum marker enzymes namely AST, ALT and ALP. Thus, necrosis or membrane damage elevated these enzymes into the circulation. High levels of AST indicate liver damage, such as that caused by viral hepatitis, AST catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver tissues (Drotman and Lawhan, 1978). Serum ALP level on other hand is related to the function of hepatic cell. The increase in ALP serum
level is due to increased its synthesis, in the presence of increasing biliary pressure (Muriel and Garcipiana, 1992).

Fig. 2:

A. Normal photomicrograph of a section of rat liver showing the architecture of the hepatic lobule, central vein (CV), hepatocytes (H), nucleus and blood sinusoids (S) (H&E 400).

B. Photomicrograph of a section of rat liver treated with CCl₄ showing distorted hepatic architecture fatty change (F), necrosis, cellular infiltration (IN) and activated Kupffer cells (K) (H&E 200).

C. Photomicrograph of a section of rat liver received extract of *A. monosperma* (200 mg/Kg) showing normal architecture of the hepatic lobules (H&E 400).

D. Photomicrograph of a section of rat liver treated with CCl₄ & silymarin (25 mg/kg) showing nearly the same control with congested of central vein. Binucleated and activated Kupffer cells were observed (H&E 400).

E. Photomicrograph of a section of rat liver treated with CCl₄ and extract of *A. monosperma* at dose (100 mg/kg) showing cords of hepatocytes well preserved with dilated and congested of central vein (H&E 400).

F. Photomicrograph of a section of rat liver treated with CCl₄ and extract of *A. monosperma* at dose (200 mg/kg) showing cords of hepatocytes well preserved nearly the same of control group (H&E 400).

Administration of CCl₄ caused significant (P<0.05) elevation of the studied enzyme levels (AST, ALT and ALP) when compared to control. There were significant (P<0.05) restoration of these enzymes level by the combined administration silymarin or *A. monosperma* extract in a dose dependent manner. The reversal of increased serum enzymes in CCl₄-induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew and Joice, 1987). It was evidenced that *A. monosperma* and silymarin significantly decreased ALP values which indicated that there were early improvement in the secretary mechanism of the hepatic cells.

Hepatic-GSH level showed significant reduction in the group treated with of CCl₄ as compared to the control rats. Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the hepatoocytes. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains
membrane protein thiols (Prakash, et al, 2001). The decreased level of GSH is associated with an enhanced lipid peroxidation in CCl4 treated rats. Treatment with *A. monosperma* significantly (P<0.05) increased the level of GSH in a dose dependent manner.

In the present study, histopathological examination revealed that CCl4 treatment, showed massive fatty changes, gross necrosis and broad infiltration of lymphocytes and Kupffer cells around the central vein and loss of cellular boundaries. In addition serum levels of ALT, AST and ALP were elevated. This is in agreement with data report by Rienke *et al*, (1988), Obi *et al*, (2003) and Yan-Jun *et al*, (2004).

CCl4 treatment generates free radicals that trigger a cascade of events resulting in hepatic fibrosis. Free radical mediated process has been implicated in pathogenesis of most of the diseases. The protective effect of *A. monosperma* against CCl4 induced hepatotoxicity in rats appears to be related to inhibition of lipid peroxidation and enhancement of antioxidant enzyme levels in addition to free radicals scavenging activity. The phytochemical studies reveal the presence of flavanoids in ethanolic extract of *A. monosperma*. The observed antioxidant and hepatoprotective activity of *A. monosperma* may be due to the presence of flavanoids. Flavonoids are reported as hepatoprotective and antioxidant (Ramanthan *et al*, 1989, Wegner and Fintelmann, 1999) and they are effective scavengers of superoxide anions (Robak and Gykgwski, 1988).

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. Both silymarin and *A. monosperma* extract decreased CCl4-elevated enzymes level in the tested groups. This indicating that *A. monosperma* has protective effect restore structural integrity of hepatocytes cell membrane and regeneration of damaged liver cells.

In conclusion, the present study demonstrated that the *A. monosperma* has hepatoprotective activity in dose dependent manner, whereas the higher dose 200 mg / kg more effective than the lower dose 100 mg / kg. In addition, the hepatoprotective effect property may be attributed to the presence of active principles in the plant extract especially flavonoids and other polyphenolic compounds.

REFERENCES


