HEPATOPROTECTIVE EFFECT OF ALHAGI MAURORUM BOISS (LEGUMINOSAE) ALCOHOLIC EXTRACT AGAINST HEPATOTOXICITY IN RATS

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**Hepatoprotective Effect of Alhagi Maurorum Boiss (Leguminosae) Alcoholic Extract Against Hepatotoxicity in Rats**

Maysa M. El Mallah*

**Abstract**

Alhagi maurorum (camel thorn plant) is a promising medicinal plant due to the presence of flavonoids and phenolic compounds as major contents of its constituents. This study was aimed to investigate the hepatoprotective effect of oral administration of alcoholic extract of Alhagi maurorum against CCl4-induced liver injury in rats after 4 weeks of treatment on serum levels of total cholesterol (TC), triglycerides (TG), lipoprotein fraction, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP); total protein (TP), albumin, total bilirubin (TBil) and oxidative stress markers such as glutathione (GSH), Malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) were determined. Histopathological examination of liver was also performed. Forty two adult male (Sprague-Dawley strain) weighting 200+5 g were divided into six equal groups as follows: group1: negative control group, group 2: positive control (CCl4) group injected subcutaneously by a single dose of CCL4 (2 ml/kg b.wt) at the last day of the experiment, group 3: rats treated with standard drug Silymarin (200 mg/kg b.wt) once daily for 4 weeks prior a single subcutaneous injection of CCL4 (2 ml/kg b.wt.) and groups (4), (5) and (6) were orally administered Alhagi maurorum alcoholic extract at doses of (200, 400 and 600) mg/kg b.wt once daily for 4 weeks prior a single subcutaneous injection of CCL4 (2 ml/kg b.wt.) to induce experimental hepatotoxicity. The results showed that oral administration of Alhagi maurorum alcoholic extract in a concentrations of 600 mg/kg b.wt to rats for 4 weeks prior inducing hepatotoxicity by CCl4 significantly improved TC, TG, lipoprotein fractions, decreased the elevated serum levels.

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of liver enzymes (AST, ALT and ALP), total bilirubin and increased serum total protein when compared to the control positive group. Oxidative stress markers (MDA, GSH, GPx, SOD and CAT) were significantly improved as compared to the control positive group. Histopathological examination of liver section of rats orally given Alhagi maurorum alcoholic extract prior inducing hepatotoxicity by CCl4 showed alleviation of histological degeneration changes in protected groups compared to control positive group. It is concluded that Alhagi maurorum alcoholic extract has high hypolipidemic, hepatoprotective effect and antioxidant effects in CCl4-intoxicated rats.

**Introduction**

Hepatotoxicity implies chemical-driven liver damage. Liver plays central role in transformation and clearance of most chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometime even when introduced within therapeutic ranges, may injure the organ. Chemicals that cause liver injury are called hepatotoxins. (Park et al., 2009) Chemicals produce a wide variety of clinical and pathological hepatic injury. Biochemical markers (i.e. alanine transferase, alkaline phosphatase and bilirubin) are often used to indicate liver damage (Singal and Kumar 2009).

Liver damage is further characterized into hepatocellular (predominantly initial Alanine transferase elevation) and cholestatic type (initial alkaline phosphatase rise). However they are not mutually exclusive and mixed type of injuries are often encountered (Sibe et al., 2005). Chemicals and drugs such as Carbon tetrachloride (CCl4) catabolised radicals induced lipid peroxidation, damage the membranes of liver cells and organelles, causing the swelling and necrosis of hepatocytes and result to the release of cytosolic enzymes in to the blood (Mumoli et al., 2006). A large number of medicinal plants have been shown hepatoprotective effects. Flavonoids and other phenolics of plant origin have been reported to have roles as scavengers and inhibitors of lipid peroxidation (Raju et al., 2008). In humans and higher animals, flavonoids have long been recognized to

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possess anti-inflammatory, antioxidant, ant-allergic and hepato-protective property (Xiao-feng et al., 2011). They are also believed to be antithrombotic, antibacterial, antifungal, antiviral, and cancer protective, and also to protect against cardiovascular disease (Ram., 2013). Alhagi maurorum (Camel thorn) is a very common woody perennial shrub, rich in phenolic and flavonoid compounds with more than twelve different isolated flavonoids have been reported which are responsible for varied pharmacological and medicinal properties (Awaad et al., 2006).

*Alhagi maurorum* is in the family (Leguminosae) in the major group Angiosperms (Flowering plants). The perennial plant grows from a massive rhizome system which may extend over six feet into the ground. New shoots can appear over 20 feet from the parent plant. Above the ground, the plant rarely reaches four feet in height. It is a heavily branched, gray-green thicket with long spines along the branches. It bears small, bright pink to maroon pea flowers and small legume pods, which are brown or reddish and constricted between the seeds. The seeds are mottled brown beans (Atta and Abo El-Sooud 2004).

*Alhagi maurorum* known locally as “Camel’s Thorn”. It is widely distributed in Asia, the Middle East, Europe, and Africa. It is used in Saudi folk medicine for the treatment of liver problems, migraine, and cataract, as tonic, digestive, antipyretic, laxative, diuretic, aphrodisiac and anti-inflammatory (Neamah., 2012). Alhagi maurorum species contains fatty acids, alkaloids, flavonoids, coumarins, tannins, sterols and ascorbic acid (Jalil et al., 2015). Seed oil is reported to contain unsaturated fatty acid 88% and a lot of microelements necessary for physiological functions (Lei., 2008).

A new flavonoid, isorhamnetin-3-O-[-alpha-l-rhamnopyranosyl-(1 - > 3)]-beta-D-glucopyranoside, along with two known flavonoids 3’-O-methylorobol (2) and quercetin 3-O-beta-d-glucopyranoside, was isolated from *Alhagi maurorum*. (Riaz et al., 2010). In addition, six main flavonoid glycosides were isolated from the ethanolic extract of A. maurorum (Marashdah, and Hazimi 2010). Moreover, A. maurorum roots contain lupeol (Beata et al., 2015) which is used as an antiangiogenic, antioxidative,
and anti-inflammatory agent. It has been used as diaphoretic, diuretic, expectorant, and ulcer treatment (Shaker et al., 2010). Oil from its leaves was used for rheumatoid treatment and as laxative (Mohammad, and Seyyed., 2007) and (Laghari et al., 2011). Water extract of the roots is used to enlarge the ureter and to remove the kidney stones, whereas the methanolic extract is used as an antidiarrheal agent. (Ghane et al., 2008). As herbal cough syrup (Hamed et al., 2012). Very little scientific validation has been done on the whole plant materials of A. maurorum as a hepatoprotective agent until now. Therefore, the present study was aimed to evaluate the phenolic and flavonoid contents as well as hepatoprotective activity of Alhagi maurorum as one of flavonoids rich plant against carbon tetrachloride induced hepatotoxicity

Materials and Methods

Materials

Alhagi maurorum Boiss (Leguminosae)

Camel thorn plant (A. maurorum) was collected from Wadi El Natrun region (Egypt) after getting the agreement of the Director of Wilderness Areas in El-Beheira. The aerial parts of A. maurorum were collected, washed three times with tap water and two times with distilled water, dried in the shade, and milled to fine powder by Wiley mill (Model 4-GMI, Germany. still used for both Determination of phenolic compounds content and for preparation of alcohol extract.

Rats and Basal Diet:

Forty two male albino rats (Sprague-Dawley strain) weighting 200+5 g were obtained from the Laboratory Animal Colony, Helwan, Egypt. The rats were kept under controlled hygienic conditions in plastic cages and fed on the basal diet for one week before starting the experiment. Basal diet constituents (Casein, cellulose, vitamin mixture, mineral mixture and choline chloride) were purchased from El-Gomhorya Company for Pharmaceutical and Chemical, Cairo, Egypt.
Carbon tetrachloride:
Carbon tetrachloride (CCl4) was purchased from El Gomhorya Co., Egypt in the form of 40% liquid dispensed in 1 L plastic bottles.

Silymarin tablets:
Silymarin tablets were purchased from Sigma Chemical Co. St. Louis, MO., USA. All chemicals were of the highest analytical grade.

Kits for biochemical analysis:
All kits were purchased from Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Methods
Preparation of plants extract:
Alhagi maurorum were powdered with a mechanical grinder to obtain a fine powder (200 gm.) the fine powder of whole flowers were packed in high quality filter paper, which was then subjected to successive extraction in a soxlet apparatus. The methanol extract was prepared by soaking 200 g of fine powder in 1 liter of 90% ethyl alcohol with daily shaking for 5 days and kept in a refrigerator. The ethanol was evaporated using a rotatory evaporator apparatus (manufactured in Russia) attached with a vacuum pump. Twenty grams of either extract (semisolid) were suspended in 100 ml distilled water with 2 ml of Tween 80 (suspending agent) to prepare a 20% alcoholic extract. (Kanchana and Nuannoi, 2012).

Preliminary phytochemical screening of Alhagi maurorum Extract:
Preliminary screening of of Alhagi maurorum extract was performed to investigate the presence or absence of the different phytochemical constituents such as phenolic, flavonoids, tannins, saponins and alkaloids using standard procedures described by (Harborne, 2007).

Preparation of the basal diet:
Basal diet was prepared according to Reeves et al., (1993). It consists of 20 % protein (casein), 10 % sucrose, 4 % corn oil, 0.2% chlorine chloride, 1% vitamin mixture, 3.5 % salt mixture , 5% fibers (cellulose) and the remainder was corn starch up to 100 %.
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**Induction of Hepatotoxicity:**

All animals, except normal control group, will be injected subcutaneously by a single dose of CCL4 (2 ml/kg b.wt) at the last day of experiment to induce acute hepatotoxicity according to the method described by Sundaresan and Subramanian (2003).

**Experiment and grouping of rats:**

All animals were housed at a controlled room temperature of 23±1°C, 55% humidity and under a 12 h light/12-h dark schedule. The animals were fed on basal diet and water was provided ad libitum for one week before starting of the experiment for acclimatization. After one week adaptation period, the rats were randomly distributed into 6 equal groups, of 7 rats each. Group I was fed on basal diet and kept as a negative control group (normal rats).

Group (2): (Hepatotoxin group) Rats injected subcutaneously by a single dose of CCL4 (2 ml/kg b.wt) at the last day of experiment to induce acute hepatotoxicity (Sundaresan and Subramanian, 2003).

Group (3): orally given Silymarin in a dose of 200 mg/kg b.wt for 4 weeks followed by injection subcutaneously by a single dose of CCL4 (2 ml/kg b.wt) at the last day of the experiment.

Groups 4, 5 and 6 were orally given *Alhagi maurorum* extract (AME) in doses of 200, 400 and 600 mg/kg b.wt respectively towards the end of the experiment period rats were injected subcutaneously with CCL4(2 ml/kg b.wt). After 24hrs of CCL4 injection all animals were sacrificed, blood was collected to separate serum for biochemical analysis. Liver was excised out, washed in ice cold saline and small portion was fixed in 10% formalin for histopathological analysis and the other portion was frozen to homogenate.

**Biochemical Analysis**

Triglycerides (TG) was determined according to the method described by Trinder (1969), Total Cholesterol (TC) was determined according to the method described by Allain et al. (1974) and High Density
Lipoprotein Cholesterol (HDL-C) concentrations were determined according to the method described by Lopes-Virella et al. (1977). Low Density Lipoprotein Cholesterol (LDL-C) concentration was calculated by using formula of Friedwald et al., (1972).

\[ \text{LDL-Cholesterol} = \text{Total cholesterol} - (\text{HDL-C} + \frac{\text{TG}}{5}) \]

Activities of serum liver enzymes aspartate and alanine aminotransferases and alkaline phosphatase (AST, ALT and ALP) were chemically estimated according to Bergmeyer et al. (1978). total protein (TP), albumin, total bilirubin (TBil) were chemically determined as described by Burtis et al. (2006). Serum uric acid was determined as described by Fossati et al. (1980). Serum creatinine concentrations were colorimetrically determined by the Jaffe reaction (Husdan and Rapoport, 1968).

**Lipid Peroxidation and Antioxidant Enzymes in Liver Tissues**

Liver homogenate was used for determination of tissue lipid peroxide (MDA), enzymatic (GPx, SOD and CAT) and non-enzymatic (GSH) antioxidants. Malondialdehyde was determined according to Ohkawa et al. (1979). The reduced glutathione (GSH) content in liver homogenate was determined colorimetrically by the method modified by Vaziri et al., (2000). Activities of Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD) and Catalase (CAT) antioxidant enzymes were determined chemically according to Paglia and Valentaine(1979) ; Spitz and Oberley(1989) and Sinha (1972) respectively.

**Histopathological Examination:**

Liver of the scarified rats was taken and immersed in 10% formalin solution. The fixed specimens were then trimmed washed and dehydrated in ascending grades of alcohol. Specimens were then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Haemtoxylin and Eosin stain for histopathological examination as described by Carleton, (1979).
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Statistical Analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (SPSS Inc., Chicago, IL, USA). Collected data was presented as mean± standard deviation (SD). Analysis of Variance (ANOVA) test was used for determining the significances among different groups according to Armitage et al. (2002). All differences were consider significant if $P < 0.05$.

Results

The phytochemical screening of *Alhagi maurorum* alcoholic extract (AMAE) revealed that it contains large amounts of Phenolic acids, flavonoids, and tannins; moderate amounts of alkaloids Steroids and absence of the Anthraquinon, Saponin and Sterols as depicted in Table (1).

Table (1): phytochemical screen of *Alhagi maurorum* alcoholic extract

<table>
<thead>
<tr>
<th>Photochemical</th>
<th>Test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolics</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>tannins</td>
<td>+++</td>
</tr>
<tr>
<td>Anthraquinon</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
</tr>
<tr>
<td>Sterols</td>
<td>-</td>
</tr>
</tbody>
</table>

The following symbol indicated the intensity of active compounds: absence of the constituents (−), a small amount (+), a moderate amount (++), and large amount (+++).

As shown in Table (2), rats injected subcutaneously with CCl4 (2 ml/kg b.wt) in the last day of the experimental period had significant increases ($P < 0.05$) in serum levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and decreased level of HDL –c (183.59± 4.14, 120.35± 2.25, 126.85±0.05 and 32.67± 3.64 mg/dl, respectively) when compared to the negative control group (84.57± 4.59, 40.25± 2.31, 28.51 ±1.22 mg/dl and 48.01 ± 2.90 mg/dl, respectively) or with stander
group (86.32± 1.32, 40.98 ± 1.41, 30.13±0.02 and 47.99 ±1.01 mg/dl, respectively). Pr-treated with Oral administration of *Alhagi maurorum* alcoholic extract at (200, 400 and 600 mg/kg) to rats intoxicated with a single subcutaneous injection of CCl4 at the last day of the experimental period caused a significant (*P*< 0.05,) decrease in the elevated serum TC, TG and LDL levels and increased serum HDL when compared to CCl4-intoxicated group. Both of Silymarin and high dose of *Alhagi maurorum* alcoholic extract had the best effect near to the normal group.

**Table (2):** Effect of oral administration of *Alhagi maurorum* alcoholic extract on total cholesterol (TC), triglyceride (TG) LDL-C and HDL- C in rats injected S/C with CCl4 in the last day of the experimental period

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>LDL(mg/dL)</th>
<th>HDL(mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1: Negative control (-ve )</td>
<td>84.57± 4.59d</td>
<td>40.25± 2.31d</td>
<td>28.51 ±1.22d</td>
<td>48.01 ± 2.90a</td>
</tr>
<tr>
<td>G2:Positive control (+ve)</td>
<td>183. 59± 4.14a</td>
<td>120.35± 2.25a</td>
<td>126.85±0.05a</td>
<td>32.67± 3.64d</td>
</tr>
<tr>
<td>G3:Silymarin + CCl4</td>
<td>86.32± 1.32d</td>
<td>40.98 ± 1.41d</td>
<td>30.13±0.02d</td>
<td>47.99 ±1.01a</td>
</tr>
<tr>
<td>G4: AMAE at (200ml/kg b.wt)+ CCl4</td>
<td>162. 82± 3.13b</td>
<td>105.64 ±1.11b</td>
<td>105.35±0.06b</td>
<td>36.34± 2.21c</td>
</tr>
<tr>
<td>G5: AMAE at (400ml/kg b.wt)+ CCl4</td>
<td>130.35± 1.17c</td>
<td>74.91 ±3.51c</td>
<td>75.16±0.06c</td>
<td>40 .21±1.10b</td>
</tr>
<tr>
<td>G6: AMAE at (600ml/kg b.wt) + CCl4</td>
<td>89.52± 1.97d</td>
<td>43. 15±2.73d</td>
<td>34.76. ±0.07d</td>
<td>46.13±2.13a</td>
</tr>
</tbody>
</table>

Data are presented as means ± standard deviation, (n = 7 for each group) .Values with different superscripts within the column are significantly different at P< 0.05. Values with similar or partially similar superscripts are non-significant.

From data recorded in Table (3) it could be noticed that rats acutely intoxicated by a single subcutaneous injection of CCl4 at the last day of the experimental period had significant (*P*< 0.05) increases (*P*< 0.05) in the serum activities of AST, ALT and ALP enzymes (140.22±0.21, 120.81±0.22 and133.53±1.55 U/L, respectively) as compared to control (-ve) group (52.22±1.36, 49.04± 0.36 and 59.77±8.90 U/L, respectively) or with stander group(53.45± 2.44, 51.01±0.13 and 59.01 ± 1.34). Administration of *Alhagi maurorum* alcoholic extract at (200, 400 and 600 mg /kg b. wt) for four weeks before subcutaneous injection of CCl4 induced significant decrease (*P*<0.05) in all the elevated serum marker levels of AST, ALT, ALP , when compared to the hepatotoxic rats (control+v group).
Intoxicated rats pre treated with the large dose (600mg/kg b. wt) of *Alhagi maurorum* alcoholic extract (AMAE) caused the highest reduction of hepatotoxicity in the elevated serum liver enzymes AST, ALT and ALP enzymes (60.52±1.78, 55.29±0.46 and 55.29±0.46 U/L, respectively) compared to CCl4-intoxicated group (+ve) group.

**Table (3): Effect of oral administration of Alhagi maurorum alcoholic extract on serum liver enzyme AST, ALT and ALP in rats injected S/C with CCl4 in the last day of the experimental period**

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/L)</th>
<th>ALT(U/L)</th>
<th>ALP(U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1: Negative control (-ve)</td>
<td>52.22±1.36e</td>
<td>49.04±0.36e</td>
<td>59.77±8.90e</td>
</tr>
<tr>
<td>G2: Positive control (+ve)</td>
<td>140.22±0.21a</td>
<td>120.81±0.22a</td>
<td>133.53±1.55a</td>
</tr>
<tr>
<td>G3: Silymarin + CCl4</td>
<td>53.45±2.44e</td>
<td>51.01±0.13de</td>
<td>59.01±1.34e</td>
</tr>
<tr>
<td>G4: AMAE at (200ml/kg b.wt)+ CCl4</td>
<td>104.23±4.76b</td>
<td>100.15±0.48b</td>
<td>102.21±21.78b</td>
</tr>
<tr>
<td>G5: AMAE at (400ml/kg b.wt)+ CCl4</td>
<td>79.58±3.43 c</td>
<td>69.37±0.33 c</td>
<td>80.45±14.91 c</td>
</tr>
<tr>
<td>G6: AMAE at (600ml/kg b.wt) + CCl4</td>
<td>60.52±1.78d</td>
<td>55.29±0.46d</td>
<td>66.64±1.51d</td>
</tr>
</tbody>
</table>

Data are presented as means ± standard deviation, (n = 7 for each group) Values with different superscripts within the column are significantly different at P<0.05. Values with similar or partially similar superscripts are non-significant.

Results in Table (4) explained that a single subcutaneous injection of CCl4 to male rats at the last day of the experimental period (control positive group) induced a significant liver damage which observed from a significant decrease (P 0.05) in both total protein (TP), and albumin and increase in the level of serum total bilirubin (5.31± 1.2 , 2.99±0.12 and 9.42 ± 1.76 g/dL respectively) compared with control negative group(8.95 ±1.32, 4.43± 0.02 and5.53 ± 1.78 g/dL respectively) . Rats orally given *Alhagi maurorum* alcoholic extract (AMAE) (200, 400 and 600 mg /kg b. wt) for 4weeks and injected with CCl4 subcutaneously at the last day of the experiment showed elevation in serum TP, Albumin and decrease in bilirubin level. The highest protection was observed in both intoxicated rats pretreated with Silymarin (8.66 ±1.73, 4.15±0.32 and 6.17±1.94 g/dL respectively) and the group of intoxicated rats pretreated with *Alhagi maurorum* alcoholic extract (AMAE) in a dose of 600mg/kg b.wt.,( 8.17±15.51, 3.93±0.11and 6.31 ± 1.45 g/dL
respectively) compared with control positive group (+ve) (5.31±1.2, 2.99±0.12 and 9.42 ±1.76 g/dL respectively).

Table (4): Effect of oral administration of Alhagi maurorum alcoholic extract (AMAE) on total protein, total bilirubin and albumin in rats injected S/C with CCl4 in the last day of the experimental period

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein (g/dL)</th>
<th>Albumin (g/dL)</th>
<th>Total bilirubin (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1: Negative control (-ve)</td>
<td>8.95 ±1.32a</td>
<td>4.43± 0.02a</td>
<td>5.53 ± 1.78d</td>
</tr>
<tr>
<td>G2: Positive control (+ve)</td>
<td>5.31 ± 1.2 d</td>
<td>2.99±0.12c</td>
<td>9.42 ± 1.76a</td>
</tr>
<tr>
<td>G3: Silymarin + CCl4</td>
<td>8.66 ±1.73a</td>
<td>4.15±0.32b</td>
<td>6.17±1.94c</td>
</tr>
<tr>
<td>G4: AMAE at (200ml/kg b.wt)+ CC14</td>
<td>7.12±11.15b</td>
<td>3.17±0.04b</td>
<td>7.99 ± 0.83b</td>
</tr>
<tr>
<td>G5: AMAE at (400ml/kg b.wt)+ CC14</td>
<td>7.61±10.01b</td>
<td>3.35±0.04b</td>
<td>7.32± 0.74b</td>
</tr>
<tr>
<td>G6: AMAE at (600ml/kg b.wt) + CC14</td>
<td>8.17±15.51a</td>
<td>3.93±0.11b</td>
<td>6.31 ± 1.45c</td>
</tr>
</tbody>
</table>

Data are presented as means ± standard deviation, (n = 7 for each group) Values with different superscripts within the column are significantly different at P< 0.05. Values with similar or partially similar superscripts are non-significant.

Data illustrated in table (5) showed that rats subcutaneously injected with a single dose of CCl4 at the last day of the experimental period (+ve) had significant decrease in antioxidant enzymes activity (glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT)) and also in non-enzymatic (GSH) antioxidant system in liver tissue and enhanced the end product of lipid peroxidation (MDA) level in liver tissues as compared with the negative control group. Oral administration of Alhagi maurorum alcoholic extract (AMAE) at (200, 400 and 600 mg/kg) or Silymarin group for four weeks and injected with a single dose of CCl4 at the last day of the experimental period showed significant increase in both of the enzymatic (GPX), (SOD), (CAT) and non-enzymatic (GSH) antioxidant systems in liver tissue, while the elevated (MDA) levels were found to be reduced back towards the normal level in pretreated rats given the highest dose of Alhagi maurorum alcoholic extract as well as intoxicated rats pretreated with Silymarin. The level of antioxidant enzyme was significantly improved by administration of 600mg/kg b.wt of Alhagi maurorum alcoholic extract as well as intoxicated rats pretreated with Silymarin.
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maurorum extract and in Silymarin treated rats intoxicated with CCl4 at the last day of the experimental period.

Table (5): Effect of oral administration of Alhagi maurorum alcoholic extract on GPx,SOD ,CAT,GSH and MDA in rats injected S/C with CCl4 in the last day of the experimental period

<table>
<thead>
<tr>
<th>Groups</th>
<th>GPx (U/mg)</th>
<th>SOD (U/mg)</th>
<th>CAT (U/mg)</th>
<th>MDA (µmol/dl)</th>
<th>GSH (µmol/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1: Negative control (-ve)</td>
<td>53.44 ± 3.33a</td>
<td>85.22± 1.25a</td>
<td>74.35± 0.97a</td>
<td>10.55± 1.02d</td>
<td>4.72± 0.02a</td>
</tr>
<tr>
<td>G2: Positive control (+ve)</td>
<td>25.19 ± 1.33d</td>
<td>43.43± 1.81e</td>
<td>35.54± 1.76e</td>
<td>21.99± 1.01a</td>
<td>2.54± 0.01d</td>
</tr>
<tr>
<td>G3: Silymarin + CCl4</td>
<td>50.02± 2.31a</td>
<td>85.55± 1.32a</td>
<td>73.15± 1.01a</td>
<td>13.61± 1.02cd</td>
<td>4.32± 0.03a</td>
</tr>
<tr>
<td>G4: AMAE at (200ml/kg b.wt)+ CCl4</td>
<td>42.42± 1.12c</td>
<td>56.18± 1.43d</td>
<td>35.45± 2.16d</td>
<td>18.02± 1.04b</td>
<td>3.16± 0.17bc</td>
</tr>
<tr>
<td>G5: AMAE at (400ml/kg b.wt)+ CCl4</td>
<td>42.33± 1.16c</td>
<td>68.25± 1.32c</td>
<td>49.01± 1.36c</td>
<td>17.92± 1.02bc</td>
<td>3.75± 0.10b</td>
</tr>
<tr>
<td>G6: AMAE at (600ml/kg b.wt)+ CCl4</td>
<td>45.15± 4.23a</td>
<td>80.75± 2.11b</td>
<td>71.80± 2.31a</td>
<td>15.42± 1.04c</td>
<td>4.34± 0.01a</td>
</tr>
</tbody>
</table>

Data are presented as means ± standard deviation, (n = 7 for each group) Values with different superscripts within the column are significantly different at P< 0.05. Values with similar or partially similar superscripts are non-significant.

Histopathological studies

Histopathological examination showed no histological change in the liver structure of normal control rats (Photo.1). Hepatic intense centrilobular, necrosis, vacuolization and macrovesicular fatty changes were observed in the liver sections of rats subcutaneously injected with a single dose of CCl4 at the last day of the experimental period (control positive group) (Photo.2). Examined liver sections of rats orally given standard drug, silymarin and intoxicated with CCl4 at the last day of the
experimental period showed normal hepatocyte (Photo.3). The intoxicated animals pretreated with different doses of *Alhagi maurorum* alcoholic extract showed recovering of hepatocyte, as follow. The pretreated with *Alhagi maurorum* alcoholic extract at 200 mg/kg b.wt, shows a moderate number of recovered hepatocyte with a small amount of necrosis, vacuolization and macro vesicular fatty changes (Photo.4). While the 400 mg/kg b.wt -treated group, showed minimal inflammation and near-normal architecture possessing higher hepatoprotective activity (Photo.5). The liver sections of the intoxicated animals pretreated with *Alhagi maurorum* alcoholic extract at 600 mg/kg b.wt., exhibited significant liver protection against CCl₄ as evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration, supplementing the protective effect (Photo.6).

**Photo. (1):** Liver of the negative control rat showing the normal histological structure of hepatic cells. (H and E x 400).

**Photo. (2):** Liver of intoxicated rats (non-treated) (+ve) rat showing congestion of hepatic central vein (Arrow) (H and E x 400).
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Photo. (3): Liver of intoxicated rats of Pretreated group with Silymarin showing normal hepatocytes (arrow mark) with central vein (H and E x 400).

Photo. (4): Liver of intoxicated rats of pretreated group with Alhagi maurorum alcoholic extract in a dose of 200 mg/kg showing a moderate number of recovered hepatocytes with a small amount of necrosis, vacuolization and macrovesicular fatty changes (H and E x 400).

Photo. (5): Liver of intoxicated rats pretreated with Alhagi maurorum alcoholic extract in a dose of 400 mg/kg showing minimal inflammation and near-normal architecture possessing higher hepatoprotective activity.
Photo. (6): Liver of intoxicated rats pretreated with Alhagi maurorum alcoholic extract in a dose of 600 mg/kg showing Significant protection evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration, supplementing the protective effect.

Discussion

Hepatotoxicity implies chemical driven liver damage. Liver plays central role in transformation and clearance of most chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometime even when introduced within therapeutic ranges, may injure the organ. Chemicals that cause liver injury are called hepatotoxins (Tavga et al., 2009).

Chemicals and drugs such as Carbon tetrachloride (CCl4) established radicals induced lipid peroxidation, damage the membranes of liver cells and organelles, causing the swelling and necrosis of hepatocytes and result to the release of cytosolic enzymes into the blood (Khan et al., 2011). A large number of medicinal plants have been shown hepatoprotective. Flavonoids and other phenolics of plant origin have been reported to have roles as scavengers and inhibitors of lipid peroxidation (Bhattacharyya, et al., 2003). In this study, the phytochemical screening of Alhagi maurorum alcoholic extract showed that it contain Phenolic acids, flavonoids, alkaloids, and, these results were in accordance with those previously reported.

Liver injury induced by CCl4 is the best characterized system of xenobiotic -induced hepatotoxicity and is commonly used models for the screening of anti-hepatotoxic and/or hepatoprotective activities of drugs. The changes associated with CCl4 induced liver damage are similar to that
of acute viral hepatitis (Masuda, 2006). Carbon tetrachloride accumulates in hepatic parenchymal cells and metabolized by cytochrome P-450 enzyme and its metabolic product; trichloromethyl free radicals (CCl3) These free radicals are highly reactive, alkylate 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. Lipid peroxidation will initiate pathological changes such as depression of protein synthesis Park et al., (2009).

In the current study the results revealed that intoxicated rats with CCl4 resulted in significant increase in serum level of total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-c) accompanied with a significant decrease in high density lipoprotein cholesterol (HDL-c) level as compared to the negative control group. Our results in agreement with (El-Habibi et al., 2009) and (Al-Dosari, 2010) who reported that there was an increase in the levels of cholesterol, triglycerides, and free fatty acids in plasma and tissues of rats intoxicated with CCl4 these results might be due to an increase in the synthesis of fatty acids and triglycerides from acetate which is responsible for the transport of acetate into the liver cell, resulting in increased substrate (acetate) availability or it could be due to increase the synthesis of cholesterol.

The effect of flavonoids and flavonoid rich extracts on reducing lipid levels effectively has been reported in several studies (Anila and Vijayalakshmi, 2002). Silymarin, a flavonolignan has been widely used from ancient times to treat liver disorders, including acute and chronic viral hepatitis, toxin/drug-induced hepatitis and cirrhosis/alcoholic liver diseases (Hussein et al.,2006 ). In the present study intoxicated rats pretreated with silymarin significantly reduced serum total cholesterol, LDL-cholesterol and triglycerides with elevation of HDL-C.

In the current study Pretreatment of rats with Alhagi maurorum alcoholic extract resulted in significant improvement in the tested lipid profile parameters, that could be attributed to an increase in the inhibition of intestinal absorption of cholesterol, interference with lipoprotein production
increased expression of hepatic LDL receptor and their protection, leading to an increased removal of LDL-C from the blood and its increased degradation and catabolism of cholesterol from the body. All these events either individually or in combination lead to decrease in serum LDL-C levels, which reduced serum total cholesterol level during the pretreatment (Laghari et al., 2011). This improvement might be due to the presence of lupeol, a component of A. maurorum extract, which plays an important role in normalization of lipid profile (Sudhahar et al., 2007).

Assessment of liver function can be performed by determining the activity of serum enzymes AST, ALT and ALP, originally present in high concentrations in the cytoplasm. When there is hepatic injury, these enzymes leak into the blood stream in conformity with the extent of liver damage (Krasteva et al., 2007 and Xu et al., 2007). Total bilirubin (TBil) and total protein (TP) levels on the other hand are related to the function of hepatic cell (Ismet et al., 2013). In the present study, the hepatotoxicity of CC14 in rats was confirmed by a significant elevation of AST, ALT, ALP and total bilirubin. In addition, CC14 intoxication produced a significant reduction in plasma total protein level. This may be due to release of these enzymes from the cytoplasm into the blood rapidly after cellular damage and a reduction in hepatic protein synthesis. Liu et al., (2013) reported that elevation of AST, ALT and ALP in response to CCl4 could be attributed to hepatic structural damage because these enzymes are normally localized to the cytoplasm and are released into the circulation after cellular damage has occurred.

The results of the present study showed that, intoxicated rats pretreated with Alhagi maurorum alcoholic extract had an effective improvement in liver function and afforded a protection against CCl4 induced hepatocyte toxicity. This was manifested by decreasing in elevated enzymes leakage of (ALT, AST, and ALP) and total bilirubin level and increasing total protein and albumin as compared with, CCl4-intoxicated group. The hepatoprotective effect of Alhagi maurorum alcoholic extract may be attributed to a significant free radical scavenging and antioxidant activity as mentioned by (Shaker et al., 2010) and (Jalil et al., 2015) who mentioned that Alhagi maurorum alcoholic extract is rich in a variety of
bioactive metabolites including flavonoids and terpenoids. These bioactive ingredients have potent activities for scavenger the Superoxide radicals (O
\(2\) (\(^{\cdot}\)) and hydroxyl radicals (HO \((^{\cdot}\)) resulted from CCl4 metabolites (Riaz et al., 2010). As well as the reference drug Silymarin significantly reduced the elevation in the activities of these enzymes (Hussein et al., 2006). Effects of silymarin might be due to its antioxidant and free radical scavenging properties.

Histopathological observations of the liver of CCl4- administered rats showed revealed presence of hepatic intense centrilobular, necrosis, vacuolization and macro vesicular fatty changes. These results were in harmony with the previous data reported by (Zalatnai et al., 1991 and Candasamy 2010). A. maurorum possesses flavonoids and tannins which were confirmed by phytochemical analysis of the extract and these both groups are well recognized for their hepatoprotective action. Saponins, alkaloids, flavonoids and triterpenoids are phytochemical constituents of A. maurorum possessing antioxidant, free radical scavenging ability and inhibition of lipid peroxidation, by hydroxyl groups present in these compounds (Djeridane et al., 2006).

In the present study, a significant elevation of malondialdehyde (MDA) (lipid peroxidation product) accompanied by lower activities of the antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) had been observed in the liver tissue of CCl4-treated group compared to control negative group. The decreased activities of the antioxidative enzymes probably occurred as a result of free radical production and the excessive use of these enzymes. These results were in accordance with the result previously reported by (Baravalia et al., 2011). Evidence of lipid peroxidation by increased MDA level is one of the primary means of associated oxidative processes with an overall decrease in cellular function (Rosenblat et al., 2006). The increase in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals (shnakumari et al., 2012).
With regard to the hepatprotective effect of *Alhagi maurorum* alcoholic extract when orally administered to rats at (200, 400 and 600 mg/kg b. wt) for 4 weeks before a single subcutaneous injection of CCl4 at the last day of the experimental period on MDA, GSH, CAT, SOD and GPx, the results revealed that pretreatment with *Alhagi maurorum* alcoholic extract significantly reduced MDA and increased levels of GSH, accompanied by increase activities of the antioxidant enzymes, CAT, SOD, GPx compared to control positive group. These findings might be due to antioxidant activity of *Alhagi maurorum* alcoholic extract These result was confirmed by (Monica et al., 2014), (Beata et al., 2015) and (Lei et al., 2008) who mentioned that, raised levels of GSH have been reported to elicit a protective response against the toxic manifestations of chemicals, particularly those involving oxidative stress. (Riaz et al., 2010) reported that, oral administration of *Alhagi maurorum* alcoholic extract inhibited superoxide generation in macrophages in rats. *Alhagi maurorum* alcoholic extract significantly increased both of catalase (CTA) and Glutathione reductase levels in blood and liver, whereas glutathione peroxidase was found to be decreased (Ram., 2013).

The biochemical results of our study were confirmed by histopathological findings, which seen in liver sections. The histological findings of liver of pretreated rats with *Alhagi maurorum* alcoholic extract showed almost completely normal structure with mild fibroblastic proliferation and sporadic cell necrosis. Oval cell hyperplasia in the portal area was very clear and necrosis was more reduced than CCl4-intoxicated rats fed on basal diet, thus may be explained by antioxidant activity of *Alhagi maurorum* alcoholic extract that may be attributed to its constituents of phytochemical. These histological findings agreed with the study of (Khan and Ahmed 2009) who reported that the oxidative damage to tissue and their cellular component can be prevented by certain antioxidant metabolites present in plants.
CONCLUSION

*Alhagi maurorum* alcoholic extract effectively improved liver functions and protected against liver tissues damage induced by toxic substances. *Alhagi maurorum* has protective effect against the loss of antioxidant activities as result of oxidative process caused by CCl4 injection due to its phytochemicals compounds (phenolic and flavonoids). This protective activity of *Alhagi maurorum* alcoholic extract suggests that regular consumption of it or food containing phenolic and flavonoids may protect against liver disease and imbalanced antioxidant. Thus, the possibility that *Alhagi maurorum* reduce the risk of liver disease and oxidation process remains open and further longitudinal studies are needed to confirm the importance of it in the prevention of liver disease.

REFERENCES


• Bhattacharyya, D; Mukherjee, R; Pandit ,S; Das, N. and Sur TK (2003): Hepatoprotective effect of Himoliv, a polyherbal formulation. Indian J Pharmacol., 47: 435 - 440


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Hepatoprotective Effect Of Alhagi maurorum Boiss (Leguminosae) Alcoholic Extract

التأثير الوقائي للمستخلص الكحولي لنبات العاقل ضد التسمم الكبدى في الفئران

اللغة العربية

بعد نبات العاقل من النباتات الطبية وذَلك لاحتوائها على العديد من المواد الفينولية، تمكّن كهربات اساسيّة فيه. قد أجِريت هذه الدراسة على المستخلص الكحولي لنبات العاقل للتحقق من تأثيره الوقائي على الكبد، وذلك بإعطاءه عن طريق الفم لردة أربعة أسابيع ثم إحداث التسمم الكبدى باستخدام الكيلى عن طريق حقنة الفئران باستخدام CCl4. ثم دراسة ذلك التأثير على مستويات الكولسترول الكلى (TC)، الدهون الثلاثية (TG)، الLDL، الLDL، الكرياتين الكلى و مؤشرات الإجهاد التاسمسي مثل الجلوتاتيون (TBIl)، الـTG، السيليرين الكلى (ALT) والـAST. في النتائج، تم اجراء التحصين على عدد 24 فئراًقسمت إلى 6 مجموعات مكملة منها 17 فئراً، تحققت أهداف مجموعة ضوابط موجبة حيث تم حقنها تحت الجلد ببراع صلوريد الكربون بجرعة 2 مل/كليلو جرام من وزن الجسم، غير برواب التسمم الكبدى، وظف أو التكرر على مجموعة مبادئها فقط، وعديدة النتائج تمكّن. أيضاً ( مجموعة مصابة وغير مفعّلة والمجموعة الثالثة) تم حقنها فموياً بدواء السليمانين المالم للدماغ الكبدى. لذا أربع أسابيع ثم حقن أطفال الجلد ببراع صلوريد الكربون بجرعة 4 مل/كليلو جرام من وزن الجسم. أخير يوم من التجربة لاحتد التسمم الكبدى، وفي نهاية فترة التجربة تم صب عينات دم لإجراء التحاليل اليوبيكيميائية في النمو، كاً من الكبد، للفحص المستقبلي. تناول المستخلص الكحولي لنبات العاقل عن طريق الفم بجرعة 500 مل/كليلو جرام من وزن الجسم للmalıdırية لمدة 4 أسابيع قبل إحداث التسمم الكبدى عن طريق CCl4. في النتيجة، لم تظهر نتائج أو تأثير خاص للدهون والكولسترول الكلى (TC)، الدهون الثلاثية (TG)، الـLDL، الطباع، والـALT. الأوسميد (MDA) والـGSH، والـCAT، والـSOD، والـGPx، والتاتتميسيد (TP) والمثبط (GSH) والمثبطات المكبلة (MDA) والـCAT، والـSOD، والـGPx، والتاتتميسيد (TP) والمثبط (GSH) والمثبطات المكبلة (MDA) والـCAT، والـSOD، والـGPx، والتاتتميسيد (TP) والمثبط (GSH) والمثبطات المكبلة (MDA)