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(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: group 1: 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

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 % .

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 $23\pm 1^{\circ}\text{C}$, 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 of 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)** .

$$\text{LDL-Cholesterol} = \text{Total cholesterol} - (\text{HDL-C} + \text{TG}/5)$$

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)**.

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

Photochemical	Test result
Phenolics	+++
Flavonoids	+++
tannins	+++
Anthraquinon	-
Alkaloids	++
Saponin	-
Sterols	-

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

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

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 and 133.53±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

Groups	AST (U/L)	ALT(U/L)	ALP(U/L)
G1: Negative contro l (-ve)	52.22±1.36e	49.04± 0.36e	59.77±8.90e
G2:Positive control (+ve)	140.22±0.21a	120.81±0.22a	133.53±1.55a
G3:Silymarin + CCl4	53.45± 2.44e	51.01±0.13de	59.01 ± 1.34 e
G4: AMAE at (200ml/kg b.wt)+ CCl4	104.23±4.76b	100.15±0.48b	102.21±21.78b
G5: AMAE at (400ml/kg b.wt)+ CCl4	79 .58±3.43 c	69.37±0.33 c	80.45±14.91 c
G6: AMAE at (600ml/kg b.wt) + CCl4	60. 52±1.78d	55.29± 0.46d	66.64 ± 1.51d

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

Groups	Total protein (g/dL)	Albumin (g/dL)	Total bilirubin (g/dL)
G1: Negative contro l (-ve)	$8.95 \pm 1.32a$	$4.43 \pm 0.02a$	$5.53 \pm 1.78d$
G2: Positive control (+ve)	$5.31 \pm 1.2 d$	$2.99 \pm 0.12c$	$9.42 \pm 1.76a$
G3: Silymarin + CCl ₄	$8.66 \pm 1.73a$	$4.15 \pm 0.32b$	$6.17 \pm 1.94c$
G4: AMAE at (200ml/kg b.wt)+ CCl ₄	$7.12 \pm 1.15b$	$3.17 \pm 0.04b$	$7.99 \pm 0.83b$
G5: AMAE at (400ml/kg b.wt)+ CCl ₄	$7.61 \pm 1.01b$	$3.35 \pm 0.04b$	$7.32 \pm 0.74b$
G6: AMAE at (600ml/kg b.wt) + CCl ₄	$8.17 \pm 1.51a$	$3.93 \pm 0.11b$	$6.31 \pm 1.45c$

Data are presented as means \pm 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 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

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

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 macro vesicular 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).

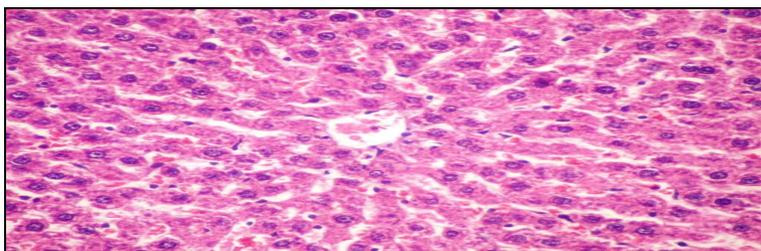


Photo. (1): Liver of the negative control rat showing the normal histological structure of hepatic cells. (H and E x 400).

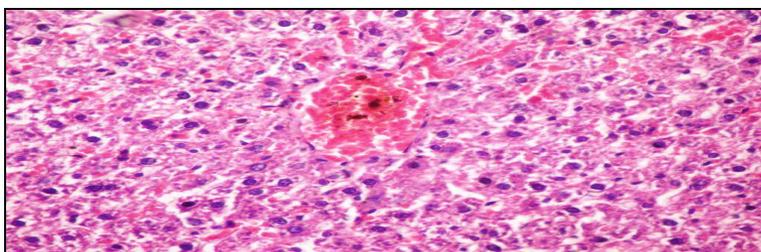


Photo. (2): Liver of intoxicated rats (non-treated) (+ve) rat showing congestion of hepatic central vein (Arrow) (H and E x 400).

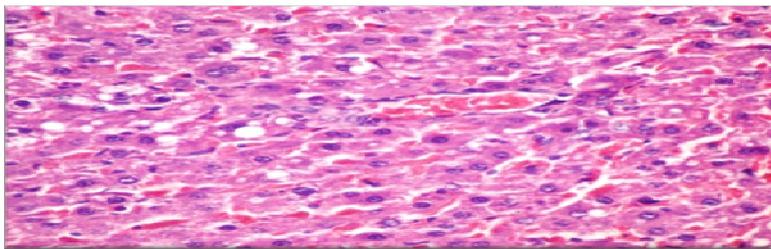


Photo. (3): Liver of intoxicated rats of Pretreated group with Silymarin showing normal hepatocytes (arrow mark) with central vein (H and E x 400).

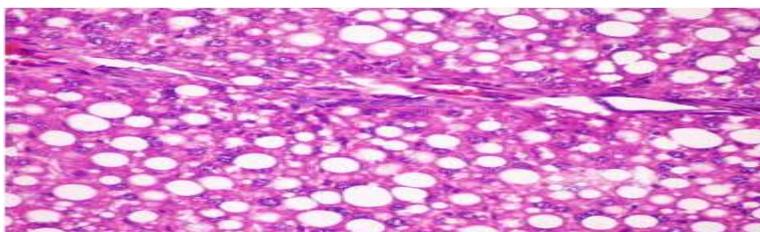


Photo. (4): Liver of intoxicated rats of pretreated group with *Alhagi maurorum* alcoholic extract in a dose of 200 mg/kg showing a moderate number of recovered hepatocytes with a small amount of necrosis, vacuolization and macrovesicular fatty changes (H and E x 400).

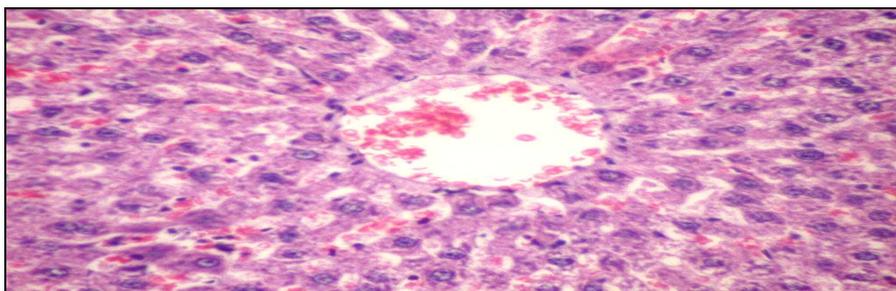


Photo. (5): Liver of intoxicated rats pretreated with *Alhagi maurorum* alcoholic extract in a dose of 400 mg/kg showing minimal inflammation and near-normal architecture possessing higher hepatoprotective activity

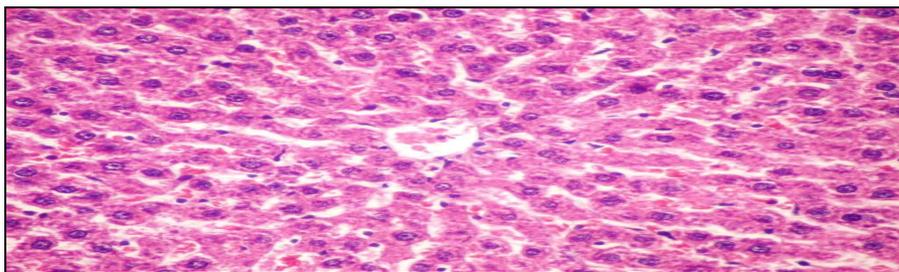


Photo. (6): Liver of intoxicated rats pretreated with *Alhagi maurorum* alcoholic extract in a dose of 600 mg/kg showing Significant protection evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration, supplementing the protective effect.

Discussion

Hepatotoxicity implies chemicaldriven 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 in to 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 other hand are related to the function of hepatic cell (Ismet *et al.*, 2013). In the present study, the hepatotoxicity of CCl₄ in rats was confirmed by a significant elevation of AST, ALT, ALP and total bilirubin. In addition, CCl₄ 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 CCl_4 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 CCl_4 - 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 CCl_4 -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 hepatoprotective 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

- **Al-Dosari, M. S. (2010):** The Effectiveness of Ethanolic Extract of *Amaranthus Tricolor* L.: A Natural Hepatoprotective Agent, *An International Journal of Comparative Medicine East and West*, vol. 38(6).
- **Allain, C. C., Poon, L. S., Chan, C. S. G., Richmand, W. A. and Fu, P. (1974)** Enzymatic Determination of Total Serum Cholesterol, *Clinical Chemistry*, vol. 20: 470-5.
- **Anila, L. and Vijayalakshmi, N.R. (2002):**Flavonoids from *Embllica officinalis* and *Mangifera indica* - effectiveness for dyslipidaemia. *J. Ethnopharmacol.* 79, 81-87.
- **Armitage, P. G., Berry, and Matthews, J.M.S. (2002):**Statistical Methods in Medical Research. 4th Ed. Blackwell Science Ltd.
- **Atta, A.H. and Abo El-Sooud, K.(2004):** The antinociceptive effects of some Egyptian medicinal plants. *J. Ethnopharmacol.*, 95 (2-3): 235–238.
- **Awaad. A.S., Maitland, D.J. and Soliman, G.A. (2006):** Antiulcerogenic activity of *Alhagi maurorum*. *Pharmaceut. Biol* 44 (4), 292–296.
- **Baravalia, Y., Vaghasiya, Y. and Chanda, S. (2011)** Hepatoprotective Effect of *Woodfordia Fruticosa* Kurz Flowers on Diclofenac Sodium Induced Liver Toxicity in Rats, *Asian Pacific Journal of Tropical Medicine*, vol. 4(5): 342-346.

- **Beata, O., Arafa, I.H., Wieslaw, O. and Anna, S. (2015):** Comparison of biological activity of phenolic fraction from roots of *Alhagi maurorum* with properties of commercial extract and resveratrol.; [Internet]. Available from: www.tandfonline.com
- **Bergmeyer, H.U.; Schreiber, P. and Wahlefeld, A.W. (1978):** Optimization of methods for aspartate and alanine aminotransferases. *Clin. Chem.*; 24: 58-61.
- **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
- **Burtis, C. A., Ashwood, E. R., Bruns, D. E. and Tietz, N. W. (2006)** Textbook of clinical chemistry and molecular biology, St. Louis: Elsevier Saunders.
- **Candasamy, M., Vudumula, V. R., Srikakulam, V. P. and Vallampatla, A. D. (2010):** Protective Effect of Livactine against CCl₄ and Paracetamol Induced Hepatotoxicity in Adult Wistar Rats, *N. Am. J. Med. Sci.*, vol. 2(10): 491-495.
- **Carleton, H. (1979) :** *Histological Techniques*, 4th Edition, Oxford University New York, USA, Toronto.
- **Djeridane, A.; Youssef, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P. and Vidal, N. (2006):** Antioxidant activity of some Algerian medicinal plant extracts containing phenolic compounds. *Food Chem.*; 97:654-660.
- **El-Habibi, E. M., Sirag, H. M., and Edrees, G. M. (2009):** Comparative Effect Between Chitosan and Chitosan-Cu Complex on Carbon-Tetrachloride (CCl₄) Induced Liver Damage in Rats, *The Egyptian Journal of Hospital Medicine*, vol. 36: 397-405.
- **Fossati, P.; Prencipe, L. and Berti, G. (1980):** Use of 3, 5 dichloro-2-hydroxyl enzene sulfonic acid /4-amyphenazonechromogenic system in direct nzymatic assay of uric acid in serum and urine. *Clin. Chem.*; 26: 227-231.
- **Friedewald, W.T.; Levy, R.I. and Frederickson, D.S. (1972):** Estimation of plasma or serum low density lipoprotein cholesterol concentration without use of ultracentrifuge. *Clin. Chem.*; 18: 499-502.
- **Ghane., M, Badiiei., K, Mohammadi., A.H, Mallah., A.R(2008):** Diuretic effect of *Alhagi maurorum* Goat. Is tInternational Congress of Veterinary Pharmacology and Pharmaceuatical Science, Tehran-Iran.

- **Hamed., A, Perrone., A, Mahalel., U, Oleszek., W, Stochmal., A, Piacente., S(2012):** Oleanane glycosides from the roots of *Alhagi maurorum*. *Phytochem Lett* 5(4):782-787.
- **Harborne, J. B.(2007):** *Phytochemical Methods: A Guide to Modern, Techniques of Plant Analysis*, Chapman & Hall, London, UK.
- **Husdan, H. and Rapoport, A. (1968):** Estimation of creatinine by Jaffe reaction method. *Clin. Chem.*; 14: 222-228.
- **Hussein, S.M. , AL Hussein, T.M. , Sedigheh, A. and Naderi, G.H. (2006) :** Hepoatoprotictive effect of silybum marianum and calendula officinalis polyphenolic extracts in rats_, *Journal of Food and Nutrition Research*, vol. 52(2): 101–106.
- **Ismet, Y., Ismail, T., Sule, G. and Zumrut, D. (2013):** The Effects of Apricot on Serum Proteins and Liver Enzymes in Rats, *Journal of Food and Nutrition Research*, vol. 52(2): 101–106.
- **Jalil ,R., Naveed A, khan Y, Khalil A, MukhtiarA,Sabira S, Hafiz MA(2015):** Phytochemical Screening and Hepatoprotective Effect of *Alhagi maurorum*Boiss (Leguminosae) Against Paracetamol-Induced Hepatotoxicity in Rabbits. *Tropical Journal of Pharmaceutical Research.*; [Internet]. Available from: www.tjpr.org/admin.
- **Khan, M.R. and Ahmed, D. (2009) :**Protective effects of *Digera muricata* (L) Mart On testis against oxidative stress of carbon tetrachloride in rat .*J Food Chem Toxicol* .47:1393-1399.
- **Kanchana, P., and Nuannoi ,C.(2012):** Comparison of the Antioxidant and Cytotoxic activities of *Phyllanthus virgatus* and *Phyllanthus amarus* Extracts. *Med. Princ.. Pract* 21:24–29.
- **Khan, R. A., Khan, M. R., Sahreen, S., Jan, S., Bokhari, J. and Rashed, U. (2011)** Prevention of CCl₄ Induced Adrenal oxidative Stress in Rat by *Sonchus asper*, *Journal of Medicinal Plants Research*, vol. 5 (15): 3347-3350.
- **Krasteva, A.Z.; Mitcheva, M.K.; Kondeva-Burdina, M.S. and Descatoire, V.A. (2007):** In vitro study of lovastatin interactions with amiodarone and with carbon tetrachloride in isolated rat hepatocytes. *World J. Gastroenterol.*, 13(15): 2198-204.

- **Laghari ,A.H., Memon,S., Nelofar,N. and. Khan,K.M.(2011):** “Alhagi maurorum: a convenient source of lupeol,” *Industrial Crops and Products*, vol. 34, no. 1, pp. 1141–1145.
- **Lei., J.I.A. (2008):** The active components in the Alhagi pseudalhagi seed oil relative to its physiological functions. *J Gansu Agric Univ*; 5: 38.
- **Liu, C. M., Zheng, G. H., Ming, Q. L., Chao, C. and Sun, J. M. (2013)** Sesamin Protects Mouse Liver against Nickel-Induced Oxidative DNA Damage and Apoptosis by the PI3K-Akt Pathway, *J. Agric. Food Chem.*, vol. 61: 1146-1154.
- **Lopes-Virella, M. F., Stone, P., Ellis S. and Colwell, J. A. (1977):** Cholesterol Determination in High-Density Lipoproteins Separated by Three Different Methods, *Clinical Chemistry*, vol. 23(5): 882-4.
- **Marashdah.,M.S and Hazimi., A.L (2010):**Pharmacological activity of ethanolic extract ofAlhagi maurorumroots. *Arabian journal of chemistry* .Jan;3: [Internet] Availablefrom:www. sciencedirect.com/science/article.P. 3-42.
- **Masuda, Y. (2006):** Learning Toxicology from Carbon Tetrachloride-induced Hepatotoxicity. *Journal of the Pharmaceutical Society of Japan.*, 126(10): 885-899.
- **Mohammad, K.G.N. and Seyyed, A. (2007):** Gastroprotective effect of Alhagi maurorumon experimental gastric ulcer in rats. *Pakistan journal of medical science*. professional medical publication; [Internet]. Available from: www.pjms.com.p.k/issue//julsep07/article
- **Monica, R ,Ioizzo., khaled., R, Ataa., S, Marco., B, Francesco., M, Rosa., T(2014):** Antiproliferative and antioxidant properties of Alhagi maurorum Boiss (Leguminosae) aerial parts. *Industrial crops and products*, 53; [Internet] Available from: www. sciencedirect.com.
- **Mumoli., N, Cei., M, and Cosimi A (2006):** Drug-related hepatotoxicity. *N. Engl. J.Med.* 354 (20): 2191-3.
- **Neamah., N.F(2012):** A Pharmacological evaluation of aqueous extract of Alhagi maurorum. *Global J Pharmacol*; 6(1):41-46.
- **Ohkawa, H.; Ohahi, N. and Jadi, K. (1979):** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem*; 95:351- 358.

- **Paglia, D.F. and Valentaine, W.N. (1979):** Studies on glutathione and glutathione characterization of erythrocytes glutathione peroxidase. J. Lab. Clin. Med., 70:158-169.
- **Park, S.W.; Lee, C.H.; Kim, Y.S.; Kang, S.S.; Jeon, S.J.; Son, K.H. and Lee, S.M. (2009):** Protective Effect of Baicalin Against Carbon Tetrachloride-Induced Acute Hepatic Injury in Mice. J. Pharmacol Sci. Jan 11.
- **Raju., R.W, Radhika., S.S, Kunal., Mahesh., T, Kalpana., S.P and Sunil., S.J.(2008):** Screening of roots of *Baliospermum montanum* for hepatoprotective activity against paracetamol-induced liver damage in albino rats. Int. J. Green Pharm. October-December, 220-223.
- **Ram., V. (2013):** Protective role of Indian medicinal plants against liver damage. The Journal of Phytopharmacology 2(3): 1-3.
- **Reeves, P.G.; Nielson, F.H., and Fahmy, G.C. (1993):** Reports of the America Institute of Nutrition, adhoc wiling committee on the reformulation of the AIN93. Rodent Diet. J. Nutri., 123, 1939-1951.
- **Riaz, S., Ahmed, N., Saleem, M., Jabbar, A., Nisar,R .and Ashraf., M. (2010):** Antioxidant flavonoids from *Alhagi maurorum*. J Asian Nat Prod Res; 12(2):138-143.
- **Rosenblat, M., Hayek, T. and Aviram, M. (2006)** Anti-Oxidative Effects of Pomegranate Juice (pg) Consumption by Diabetic Patients on Serum and on Macrophages, *Atheroscler*, vol. 187(2): 363-371.
- **Shaker, E., Mahmoud, H., Mnaa ,S. (2010):** Anti-inflammatory and anti-ulcer activity of the extract from *Alhagi maurorum*(camelthorn).food and chemical toxicology. 48.
- **Shnakumari, S., Nishanthini, A., Muthukumarasamy, S. and Mohan, V. R. (2012):** Hepatoprotective and Antioxidant Activity of *Canscora Perfoliata* Lam (Gentianaceae) against CCl₄ Induced Hepatotoxicity in Rats, International Journal of Research in Ayurveda and Pharmacy, vol. 3(6): 822-826.
- **Sibe.,I.K. and Canan., K.(2005) :** The protective effects of *Achillea L.* species native in Turkey against H₂O₂-induced oxidative damage in human erythrocytes and leucocytes. J. Ethnopharmacol., 221-227.

- **Singal., A. and Kumar., V.I . (2009):** Effect of aqueous suspension of dried latex of *Calotropis procera* on hepatorenal functions in rat. *J.Ethnopharmacol.*, 122: 172-17.
- **Sinha, K.A. (1972):** Colorimetric assay of catalase enzyme. *Anal. Biochem.*; 47: 328-330.
- **Spitz, D.R. and Oberley, L.W. (1989):** An assay for superoxide dismutase activity in mammalian tissue homogenates. *Anal. Biochem.*; 179: 8-18.
- **Sudhahar,v.; S. A. Kumar, Y. Mythili, and P. Varalakshmi, (2007):** “Remedial effect of lupeol and its ester derivative on hypercholesterolemia-induced oxidative and inflammatory stresses,” *Nutrition Research.*, 27(12) 778–787
- **Sundaresan, S. and P. Subramanian (2003):** S-allylcysteine inhibits circulatory lipid peroxidation and promotes antioxidants in N-nitrosodiethylamine-induced carcinogenesis. *Pol. J. Pharmacol.*, 55: 37-42.
- **Tavga, A. A., Zheen, A. A., Kasim, M. J., Munaf, H. A. and Saad, A. H. (2009):** Study of the Protective Effects of Benfotiamine Against CCl4-Induced Hepatotoxicity in Rats, *Iraqi J. Pharm. Sci.*, vol. 18.
- **Trinder, P. (1969):** Triglycerides Estimation by GPO-PAP Method, *Annals of Clinical Biochemistry*, vol. 6: 24-7.
- **Vaziri, N. D., Wang, X. Q., Oveisi, F. and Rad, B. (2000):** Induction of Oxidative Stress by Glutathione Depletion Causes Severe Hypertension in Normal Rats, *Hypertension*, vol. 36: 142-146.
- **Xiao-feng,A., Jin,S., Jie ,R., Qian,M. and Yan-hua., L.u .(2011):** The role of hepatoprotective effect of a flavonoid-rich extract of *Salvia plebeia* R.Br. on carbon tetrachlorideinduced acute hepatic injury in mice. *J. Med. Plant. Res.*, 5(9): 1558-1563.
- **Xu, D.; Wu, Y.; Liao, Z.X. and Wang, H. (2007):** Protective effect of verapamil on multiple hepatotoxic factors-induced liver fibrosis in rats. *Pharmacol. Res.*, 55(4): 280-6.
- **Zalatnai, A., Sarosi, I., Rot, A. and Lapis, K. (1991) :**Inhibitory and Promoting Effects of Carbon Tetrachloride- Induced Liver Cirrhosis on the Diethylnitrosamine Hepatocarcinogenesis in Rats, *Cancer Lett.*, vol. 57: 67-73.

التأثير الوقائي للمستخلص الكحولي لنبات العاقول ضد التسمم الكبدي في الفئران

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الملخص العربي

يعد نبات العاقول من النباتات الطبية وذلك لاحتوائه على العديد من المركبات الفينولية كمركبات اساسية فيه . قد أجريت هذه الدراسة على المستخلص الكحولي لنبات العاقول للتحقيق من تأثيره الوقائي على الكبد وذلك بإعطاءه عن طريق الفم لمدة أربعة أسابيع ثم إحداث التسمم الكبدي عن طريق حقن الفئران بـ CCl_4 في نهاية التجربة . ثم دراسة ذلك التأثير على مستويات الكوليسترول الكلي (TC)، الدهون الثلاثية (TG)، الليبيدات الدم، انزيمات الكبد، البروتين الكلي (TP)، الاليومين، البيليروبين الكلي (TBil) و مؤشرات الإجهاد التأكسدي مثل الجلوتاثيون المختزل (GSH) والمالونالدهيد (MDA) والجلوتاثيون بيروكسيداز (GPx) والسوبر اوكسيد ديسميوتيز (SOD) و الكتاليز (CAT) في مصف الفئران . كما تم إجراء الفحص الهستوباثولوجي لأنسجة الكبد . تم إجراء التجربة على عدد ٤٢ فأر قسمت إلى ٦ مجموعات كل منها ٧ فئران ، تركت إحداها مجموعة ضابطة سالبة تغذت على الغذاء الاساسى فقط والمجموعه الثانية تركت كمجموعه ضابطة موجبة حيث تم حقنها تحت الجلد برابع كلوريد الكربون بجرعة ٢ مل / كيلو جرام من وزن الجسم اخر يوم من التجربة لاحداث التسمم الكبدي وتغذت على الغذاء الاساسى فقط ايضا (مجموعه مصابة وغير معالجه) والمجموعه الثالثه تم حقنها فمويا بدواء السليمارين المعالج للتسمم الكبدي لمدة اربع اسابيع ثم حقنت أسفل الجلد برابع كلوريد الكربون بجرعة ٢مل / كيلو جرام من وزن الجسم اخر يوم من التجربة لاحداث التسمم الكبدي وتركت (مجموعه مصابة معالجه بدواء ضابط) وتم إعطاء فئران المجموعات الرابعة والخامسة والسادسة عن طريق الفم المستخلص الكحولي لنبات العاقول بجرعات ٢٠٠ و ٤٠٠ و ٦٠٠ ملجم / كجم من وزن الجسم على التوالى يوميا لمدة أربعة أسابيع ثم حقنت أسفل الجلد برابع كلوريد الكربون بجرعة ٢مل / كيلو جرام من وزن الجسم اخر يوم من التجربة لاحداث التسمم الكبدي وفى نهاية فترة التجربة تم سحب عينات دم لإجراء التحاليل البيوكيميائية فى المصل وأخذ الكبد للفحص الهستوباثولوجى . أظهرت النتائج أن تناول المستخلص الكحولي لنبات العاقول عن طريق الفم بتركيز ٦٠٠ ملجم / كجم من وزن الجسم للفئران لمدة ٤ أسابيع قبل إحداث تسمم الكبد عن طريق CCl_4 أدى إلى تحسن ملحوظ فى الكوليسترول الكلي (TC)، الدهون الثلاثية (TG)، وليبيدات الدم ، وانخفاض مستويات انزيمات الكبد (AST، ALT و ALP)، البيليروبين الكلي والبروتين الكلي في الدم وكذلك تحسنت مؤشرات الإجهاد التأكسدي مثل الجلوتاثيون المختزل (GSH) والمالونالدهيد (MDA) والجلوتاثيون بيروكسيداز (GPx) والسوبر اوكسيد ديسميوتيز (SOD) و الكتاليز (CAT) بالمقارنة مع المجموعة الضابطة الموجبة . كما أظهر فحص أنسجة الكبد تحسن ملحوظ فى التغيرات النسيجية مقارنة بالمجموعة الضابطة الموجبة . وتوصى الدراسة بأن المستخلص الكحولي لنبات العاقول له تأثير خافض للدهون وله دور وقائي للكبد وكذلك له دور فعال كمضاد للاكسدة ضد التسمم الكبدي المحدث برابع كلوريد الكربون .