The Effect of *Alhagi Maurorum* (Akool) on Hepatotoxicity in Rats

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ABSTRACT

This study aimed to evaluate the effect of *Alhagi Maurorum* (Akool) on CCL4- induced hepatotoxicity in rats. Thirty-five rats were separated into two group. The 1st group, rats (n=7) was fed on a basal diet and kept as a negative control group, the 2st group: the hepatotoxic group, rats (n=28), was injected with CCl_4 at 1ml/kg b.wt. After 24 h from injection for 3 days, a rat from each group was taken tomeasure liver function to be sure that all rats had liver injury. After liver injury rats were divided as follows: subgroup 1 served as the control positive group and 3 treated rat subgroups were fed on a basal diet supplemented with the *A. Maurorum* powder concentration (2.5, 5, 10%) per kg of basal diet, respectively. Results revealed that supplementation with different levels of *A. Maurorum* powder had improved body weight and liver weight accompanied by a significant decrease in levels of liver functions (ALT, AST, ALP and total bilirubin), as well as in lipid profile, while a significant increase in a high-density lipoprotein-cholesterol (HDL-C). In addition, malondialdehyde (MDA) was significantly reduced, while GSH was significantly increased ($P<0.05$). **Conclusion** *A. Maurorum* could introduce a potential natural therapy against hepatotoxicity.

Keywords: *Alhagi Maurorum*, Hepatotoxicity**,** Liver Disease, Rats.

INTRODUCTION

Hepatotoxicity is most commonly seen in the form of malfunction or damage to the liver due to excess amounts of drugs or xenobiotics **(Bahar *et al.*, 2013)**. Hepatotoxicants are exogenous agents of clinical relevance which may include an overdose of certain medicinal compounds, industrial chemicals (alcohol, CCl4, beta-galactosamine, thioacetamide, etc., which causes liver injury **(Pandit et al., 2012)**. Chronic liver disease is a condition in which the liver slowly deteriorates and malfunctions due to chronic injury. Scar tissue replaces healthy liver tissue, partially blocking the flow of blood through the liver **(Pinter et al., 2016)**. It can lead to cirrhosis, hepatocellular carcinoma if left untreated and liver failure **(Rajathi and Jiji, 2019)**.

Plants are complementary and alternative medicine due to their ability to produce secondary metabolites such as proteins, flavonoids, alkaloids, steroids, and phenolic compounds which are used to recover health **(Moradi and Esfahani, 2016)**. *Alhagi maurorum* Medik. (from the *Fabaceae* family) is a perennial plant with a wide geographical distribution. Animal and human studies have been conducted on its effects, some of which include antioxidants, anti-inflammatory, antipyretic, diaphoretic, diuretic, expectorant and analgesic properties **(Al-Snafi, 2015)**. Its morphology, nature, and clinical uses have been explained in the Materia Medica manuscripts in the Islamic era by the sage physicians. Tarangabin, a kind of manna that is produced on some *Alhagi* species, is collected mostly in Iran and Afghanistan and exported from these areas to other countries **(Tavassoli et al., 2020)**.

Aqueous and ethanol extracts of aerial parts also significantly lowered FBG, TGs, TC, LDL-C, and VLDL-C, and increased HDL-C concentration in diabetic rats. It is also reported to protect rats from cisplatin-nephrotoxicity **(Akbar & Akbar, 2020)**. Therefore, this study aimed to evaluate the effect of *Alhagi Maurorum* (Akool) on CCL_4- induced hepatotoxicity in rats.

MATERIALS AND METHODS

MATERIALS:

1- **Plant**: *Alhagi Maurorum* (Akool) was purchased from the Ismailia desert, Egypt.

2- **Chemicals**: Casein, vitamins, minerals, cellulose, and CCl_4 were purchased from El-Gomhoria Company, Cairo, Egypt.

3- **Kits** for blood analysis were purchased from Alkan Company for Biodiagnostic Reagents, Dokki, Cairo, Egypt.

4- **Rats**: Thirty-five adult male rats **(**Sprague Dawley strain), weighing about 150±10 g b.wt. were obtained from the Laboratory Animal Colony, Helwan, Egypt. They were housed at constant conditions of room temperature and 55 ± 5% humidity under 12-hr light/12-hr dark cycles. All rats have continuous access to feed and water and will acclimate to laboratory conditions for 1 week.

METHODS:

Preparation of plant Powder:

The aerial parts of *A. Maurorum* (Akool) were washed separately with water and dried in the shade. They dried it in the shade and milled it to a fine powder.

Induction of hepatotoxicity in rats:

Carbon tetrachloride (CCl_4) – induced acute hepatotoxity in rats **(Jayasekhar *et al*., 1997)**. Intraperitoneal injection of male albino rats with CCl_4 (1 mL/kg), a 1:1 mixture with corn oil for 3 days increased serum alanine transaminase, aspartate transaminase, and alkaline phosphatase activity as well as total bilirubin, triglycerides and total cholesterol levels. This is in addition to the disrupted histology.

Experimental Design

The experimental animals were done using (n=35) male rats, with a body weight of 150 ±10 g. The rats were housed in cages under hygienic conditions in a temperature-controlled room at 25°C. Basal diet was semi-synthetic and nutritionally adequate (AIN-93 M), vitamins mixture and minerals mixture to meet recommended nutrient levels for rats were prepared as described by **(Reeves *et al.*, 1993).** After a period of adaptation on a basal diet (one week), the animals were randomly divided into two main groups as follows:

• **First group:** Negative control group, rats (n=7) were fed on basal diet only during the experimental period.

• **Second group:** Hepatotoxic group, rats (n=28), were injected with CCl_4 at 1ml/kg b.wt **(Jayasekhar *et al*., 1997)**. After 24 h from injection for 3 days, rat from each group was taken to measure liver function to be sure that all rats had liver injury. After liver injury rats were divided as follows:

Subgroup (1): Hepatotoxic rats (positive control group) were fed on a basal diet only.

Subgroup (2): Hepatotoxic rats were fed on a basal diet supplemented with the *A. Maurorum* powder concentration of 2.5% per kg of basal diet.

Subgroup (3): Hepatotoxic rats were fed on a basal diet supplemented with the *A. Maurorum* powder concentration of 5% per kg of basal diet.

Subgroup (4): Hepatotoxic rats were fed on a basal diet supplemented with the *A. Maurorum* powder concentration of 10% per kg of basal diet.

Nutritional evaluation:

The biological evaluation of the diet was carried out determination of feed intake, body weight gain percent (BWG %) and feed efficiency ratio (FER) according to **Chapman, (1959)** using the following equation:

$$\textbf{BWG } \% = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

FER = Weight gain (g) / Feed intake (g)

At the end of the experimental period (4 weeks), rats were fasted overnight, then the blood was collected under slight ether anesthesia. Serum was separated by centrifugation at 3000 rpm for 15 min. The obtained serum was used immediately for routine laboratory investigation.

Biochemical Analysis:

Liver Function:

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to (**Bergmeyer *et al*., 1978)**, Alkaline phosphates (ALP) was determined according to **Belfield and Goldberg (1971)**. Serum Bilirubin were measured by **Weissman *et al*., (1950).**

Serum Lipid Profile:

Serum total cholesterol (TC) **(Richmond, 1973)**, triglycerides (TG) **(Wahlefeld, 1974)**, and high-density lipoprotein (HDL) **(Albers *et al*., 1983)** were determined. Meanwhile, low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were calculated according to **Fridewald *et al*., (1972)**.

$$\text{LDL-c} = \text{TC}-[\text{HDL-c} + (\text{TG}/5)] \quad -- \quad \text{VLDL-c} = \text{TG}/5$$

Antioxidant Enzymes

The plasma level of malondialdehyde (MDA) was calculated to measure lipid peroxidation and was determined according to **Draper and Hadley (1990).** Glutathione (GSH) was measured by the methods of **Moin, (1986).**

Statistical analysis:

Statistical analysis will be performed using the SPSS computer program (Graph pad software Inc, San Diego, CA, USA). One-way analysis of variance (ANOVA) followed by Duncan's multiple tests was done $P \leq 0.05$ was significant **(Armitage and Berry, 1987)**.

RESULTS AND DISCUSSION

Recorded results in **Table (1)** interpreted the effect of different levels of Akool powder on body weight gain (BWG), feed intake (FI), food efficiency ratio (FER) and liver weight of hepatotoxicity rats. It shows that the injected rats with CCl_4 (+ve group) had a significant reduction ($P<0.05$) in BWG%, FI , FER, and liver weight compared to the normal rats (-Ve group). In contrast, rats that were fed different levels of Akool powder (2.5, 5, and 10%), had a significant increase ($P<0.05$) in BWG%, FI, FER, and liver weight as compared to the positive control group. The best mean values of BWG% and FER increase were observed in the group fed 10% Akool powder. The obtained results were also in line with **Saber et al., (2022)** showed that concomitant treatment with the *A. maurorum* extract markedly attenuated the decrease in body weight gain.

Table (1): Effect of different levels of Akool powder on body weight gain (BWG), food intake (FI), food efficiency ratio (FER) and liver weight of hepatotoxicity rats

Parameters / Groups	BWG %	FI g/d/rat	FER	Liver weight g
Control (-Ve)	33.10±0.93a	21	0.085±0.001a	7.91±0.01a
Control (+Ve) CCl_4	14.91±0.46d	15	0.053±0.001c	4.81±0.04d
2.5% Akool powder	20.98±0.31c	17	0.067±0.001b	4.84±0.05d
5% Akool powder	25.71±0.47b	20	0.071±0.001b	5.77±0.05c
10% Akool powder	31.80±0.48a	21	0.082±0.001a	6.82±0.07b

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at ($P<0.05$).

The tabulated results in **Table (2)** explained that treated rats with liver toxicity from CCl_4 injection had a significant increase ($p<0.05$) in the serum activity of AST, ALT, ALP and total bilirubin enzymes compared to normal

rates negative control group. In assessing CCl_4 -induced hepatotoxicity, serum AST, ALT, and ALP activity levels were used as indices. CCl_4-treated animals showed a significant increase in serum AST, ALT, and ALP activity levels compared to the standard group. CCl_4 is a toxic compound that exerts toxic effects on the liver. The analysis shows that CCl_4 exposure disrupts regular physiological features by disturbing the standard values of ALP, AST, ALT, and bilirubin. The chemical industry commonly uses CCl_4 as an organic solvent, and it is also well-documented as an experimental inducer of hepatotoxicity. In this study, mice treated with CCl_4 suffered damage to the hepatocyte membrane, releasing hepatocyte cytosolic enzymes. This was evidenced by significant increases in serum marker enzymes (AST, ALT, and ALP) associated with acute liver damage. This finding was consistent with a study by **Negm and Aljarari, (2023); Munir & Khan, (2023)**. Liver enzyme levels and serum biomarker concentrations are helpful indicators for monitoring liver disease. Higher AST and ALT values suggest liver damage, whereas lower AST and ALT values indicate a reasonably healthy liver status **(Lala *et al.*, 2024)**.

While the treatment of liver toxicity in rats by feeding on Akool powder at the three different levels (2.5, 5, 10%) significantly ($P<0.05$) reduced serum activity of AST, ALT, and ALP enzymes compared to the (+ve group). The rats administered with the highest ratio (10%) of Akool powder showed the greatest improvement in results. The hepatoprotective efficacy of the *A. maurorum* extract could be owing to the stabilization of hepatocellular membranes via the reduction of lipid peroxidation as a consequence of its antioxidant activity, as well as suppression of free radical production by the flavonoids and phenolic content **(Al-Saleem et al., 2019)**. In addition, 3-methyl-2-(2-oxopropyl) furan is the primary component of *A.*

maurorum extract, according to GC-MS analysis, and has been documented for its hepatoprotective activity **(Borthakur et al., 2020)**.

Our data agreed with previous reports demonstrating that an *A. maurorum* extract conferred significant protection against the liver injury caused by carbon tetrachloride **(Al-Saleem et al., 2019)**. Similarly, **Saber et al., (2022)** showed that treatment with the *A. maurorum* extract mitigated the lead-induced liver damage, as evidenced by an obvious decrease in the serum ALT activity and the restoration of the serum AST activity to the normal value.

Table (2): Effect of different levels of Akool powder on liver function of hepatotoxicity rats

Parameters / Groups	AST (µ/L)	ALT (µ/L)	ALP mg/dl	Total Bilirubin µmol/L
Control (-Ve)	28.02±0.42e	46.17±0.61e	120.44±1.89d	16.18±0.18e
Control (+Ve) CCl_4	51.18±0.49a	92.06±0.35a	170.98±1.80a	33.81±0.47a
2.5% Akool powder	44.58±0.48b	82.73±0.59b	167.18±1.54ab	28.71±0.32b
5% Akool powder	39.98±0.53c	73.57±0.37c	162.98±1.09b	23.62±0.37c
10% Akool powder	34.74±0.60d	64.93±0.37d	153.18±1.69c	19.77±0.24d

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at ($P<0.05$).

As shown in **Table (3)** the effect of different levels of Akool powder on TC, TG, HDL-C, LDL-C, and VLDL-C in rats with liver toxicity. The positive control group showed significant increases ($P<0.05$) in mean levels of TC, TG, VLDL-C, and LDL-C, while HDL-C declined significantly compared to the negative control group. Abnormalities in lipid and lipoprotein levels are commonly linked with liver diseases. Because abnormalities in lipid profiles have been linked to an increased risk of coronary heart disease, ideal liver disease treatment should also improve lipid profiles **(Taha et al., 2022; Negm and El-Soadaa, 2020).**

Induced group rats that had Akool powder at different levels showed a significant decrease at levels ($P<0.05$) in the mean levels (2.5%, 5%, 10%) of lipid profile compared to the control positive group, while the HDL-C was significantly increased. Moreover, there was a significant difference ($P<0.05$) in the mean values of TC, TG, VLDL-C, and HDL-C for those feeds supplemented with different levels of Akool powder. The group that consumed cake fortified with Akool powder at 10% showed the greatest improvement in lipid profile. **Othman et al., (2022)** observed that the levels of cholesterol, TG, LDL, and HDL in rats that received a diet rich in lipids were significantly decreased by *Alhagj marorum* extract (AME), indicating the antihyperlipidemic activity of both plants. **Shahrivar et al., (2017)** showed that *A. maurorum* extract improves the negative effects of streptozotocinon triglycerides and LDL cholesterol and boosts the serum level of HDL cholesterol. Although Alhagi ethanolic extract decreases the levels of total cholesterol, triglycerides and LDL by about 22.6%, serum concentration of HDL is increased **(Salama, 2016)**. **Sheweita et al. (2016); Peluso et al., (2016)** reported that *Alhagi maurorum* extract improves lipid profiles and lipid functions in the diabetic rats.

Table (3): Effect of different levels of Akool powder on lipid profile of hepatotoxicity rats

Parameters / Groups	TC mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control (-Ve)	110.15±1.90d	60.60±0.47d	40.47±0.54a	57.29±0.41e	12.12±0.09d
Control (+Ve) CCl_4	141.14±1.98a	92.16±1.44a	24.52±0.29d	98.18±0.82a	18.43±0.08a
2.5% Akool powder	133.12±1.89b	73.11±0.90b	27.33±0.55d	91.16±0.50b	14.62±0.18b
5% Akool powder	132.98±1.26b	70.31±0.75b	32.24±0.72c	86.67±0.87c	14.06±0.05b
10% Akool powder	127.22±1.53c	64.64±0.83c	35.41±0.84b	78.87±1.07d	12.93±0.16c

Results are expressed as mean ± SE.

Values in each column which have different letters are significantly different at ($P<0.05$).

Table (4) shows lipid peroxidation as measured by serum MDA levels and GSH activity in normal rats injected with CCl_4 and supplemented with different levels of Akool powder. Injection of CCl_4 results in a considerable rise ($P<0.05$) in serum MDA levels and decreased activity of GSH enzymes compared to normal rats. Oxidative stress-mediated tissue damage also leads to the activation of fibroblast cells and inflammation through inflammatory cell infiltration in the liver **(Chen et al., 2018)**. Free fatty acid supply to the liver is also associated with the oxidative stress-mediated tissue damage and inflammation in the liver **(Masarone et al., 2018)**.

On the other hand, rats were fed a meal enriched with varying levels of Akool powder and injected with CCl4, serum MDA levels and GSH enzyme activity improved significantly when compared to the positive control group. The treated group demonstrated a superior result in serum MDA concentration and antioxidant enzyme activity due to higher levels (10%) of Akool powder. These results agree with **Saber et al., (2022)** showed that treatment with concomitant treatment with the *A. maurorum* extract significantly mediated oxidative stress by reversing the antioxidant enzyme activities and GSH level and decreasing MDA concentration. Moreover, 100 mg/kg *Alhagi maurorum* extract can reveal hepatic enzyme protection, the nature of MDA and GSH oxidation as well as the ratio of lipids **(Shahrivar et al., 2017; Al-Snafi, 2015)**. Similarly, the *A. maurorum* extract alleviated the hepatic oxidative damage triggered by carbon tetrachloride **(Al-Saleem et al., 2019),** norfloxacin **(Khalifa et al., 2020)** and Lead Acetate (**Negm and Aljarari, 2023)**.

This alleviative effect may be attributable to the antioxidant activity of the *A. maurorum* extract. Such activity might be ascribed to its high content of several bioactive components. For instance, beta-D-glucopyranose, 1,6-

anhydro-(levoglucosan) (28.91%) had been found in Barleria noctiflora leaves extract and reported to exhibit antioxidant and free radical scavenging activities **(Alagar Yadav et al., 2016)**; 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, the major bioactive component detected in the *A. maurorum* extract, had been documented to have strong antioxidant properties **(Shukla et al., 2018)**; 3-methyl-2-(2-methyl-2-butenyl)-furan (rosefuran) is the main active compound identified in the *A. maurorum* extract and had been shown to have hydroperoxyl radical scavenging activity **(Boulebd, 2021)**.

Table (4): Effect of different levels of Akool powder on antioxidant enzymes of hepatotoxicity rats

Parameters / Groups	MDA ng/mL	GSH nmol/mg
Control (-Ve)	118.38±1.33e	4.23±0.04a
Control (+Ve) CCl_4	391.39±2.15a	2.03±0.03d
2.5% Akool powder	375.81±1.50b	2.66±0.03c
5% Akool powder	332.41±1.55c	2.98±0.16c
10% Akool powder	287.34±1.77d	3.74±0.60b

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at ($P<0.05$).

Conclusion:

The present study sheds light on the effect of *Alhagi Maurorum* (Akool) on hepatotoxicity by CCl_4 in rats. The CCl_4 toxicity produced radical oxygen species prompting oxidative stress. Our finding suggested that the Akool powder ameliorated the level of serum liver enzymes near to normal value, enhanced the antioxidant level, and restored the liver functioning in exposed rats. More studies are needed to know the underlying mechanism of hepatoprotective effect of Akool powder because it is a mixture of bioactive compounds presen and there may be more than one mechanism involved in it.

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تأثير العاقول (شوك الجمل) على تسمم الكبد في الفئران

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[1]قسم التغذية وعلوم الاطعمة ، كلية الاقتصاد المنزلى ، جامعة حلوان ، مصر.

الملخص العربي

هدفت هذه الدراسة إلى تقييم تأثير العاقول (شوك الجمل) على السمية الكبدية التي يسببها CCL4 في الفئران. تم تقسيم خمسة وثلاثين فأرًا إلى مجموعتين. تم تغذية المجموعة الأولى وعددها ٧ فئران على نظام غذائي أساسي وتم الاحتفاظ بها كمجموعة ضابطة سلبية، المجموعة الثانية: مجموعة تسمم الكبد، تم حقن الفئران (عددها = ٢٨) بـ CCl4 بمعدل ١ مل / كجم من وزن الجسم. بعد ٢٤ ساعة من الحقن لمدة ٣ أيام، تم أخذ الفئران من كل مجموعة لقياس وظائف الكبد للتأكد من أن جميع الفئران تعاني من إصابة الكبد. بعد التأكد من إصابة الكبد تم تقسيم الفئران على النحو التالي: المجموعة الفرعية ١ بمثابة المجموعة الضابطة الموجبة وتم تغذية ٣ المجموعات الفرعية الأخرى من الفئران المعالجة على نظام غذائي أساسي مكمل من مسحوق العاقول (شوك الجمل) بتركيزات (٢.٥، ٥، ١٠ %) لكل كجم من النظام الغذائي الأساسي على التوالى. أظهرت النتائج أن التدعيم بمكملات من مسحوق العاقول (شوك الجمل) بمستويات مختلفة قد أدى إلى تحسن في وزن الجسم ووزن الكبد، مصحوبًا بانخفاض ملحوظ في مستويات وظائف الكبد (ALT، AST، ALP، والبيليروبين الكلي)، وكذلك في مستوى الدهون، في حين سُجلت زيادة ملحوظة في كوليسترول البروتين الدهني عالي الكثافة (HDL–C). بالإضافة إلى ذلك، انخفض بشكل ملحوظ مستوى مالونديالديهايد (MDA)، بينما ارتفع مستوى GSH بشكل ملحوظ ($P < 0.05$). الخلاصة: يمكن أن يعتبر مسحوق العاقول (شوك الجمل) علاجًا طبيعيًا محتملًا لتسمم الكبد.

الكلمات المفتاحية: العقول، السمية الكبدية، أمراض الكبد، الفئران.