

Protective effect of aqueous extract of *Alhagi maurorum* in spermatogenesis and antioxidant status of adult rats exposed to carbon tetrachloride

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Abstract

This study aimed to investigate the efficiency of aqueous extraction of *Alhagi maurorum* leaves against oxidative stress induced by carbon tetrachloride (CCl₄) on spermatogenesis and the level of glutathione, superoxide dismutase, malondialdehyde in adult rats. Plant Leaf's dried and then extracted. Experiment included 24 male rats divided into 4 groups 6 subjects in each group. Groups treated orally for 30 days as following: first was control group which administered with 1 ml of physiological saline 0.9%, second group administered once with CCl₄ 3 ml/Kg, third and fourth groups administered with aqueous extract 300 mg/kg and aqueous extract together with CCl₄ respectively. The results showed that CCl₄ caused a significant decrease in sperm count, sperm vitality, normality, glutathione (GSH) and superoxide dismutase (SOD), significant increase in sperm mortality, abnormality and malondialdehyde (MDA) compared with control group. While aqueous extract treatment caused no significant difference in compare to control group. Groups treated with aqueous extract together with CCl₄ showed a significant increase in sperm count, vitality, normality and GSH and decreasing in mortality, abnormality and MDA in compare to CCl₄ group. It could be concluded that the aqueous extract of *Alhagi maurorum* have a positive effect on male reproduction and antioxidants in rats exposed to oxidative stress.

Keywords: *Alhagi maurorum*, CCl₄, spermatogenesis, GSH, SOD, MDA

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التأثير الوقائي للمستخلص المائي لنبات العاقول في عملية تكوين النطف ومستوى مضادات الأكسدة في الجرذان البالغة المعرضة لرباعي كلوريد الكربون

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الخلاصة

هدفت الدراسة إلى التقصي عن كفاءة المستخلص المائي لنبات العاقول *Alhagi maurorum* ضد الإجهاد التأكسدي المحدث بواسطة رباعي كلوريد الكربون (CCl₄) في عملية تكوين النطف وحالة مضادات الأكسدة في ذكور الجرذان البالغة. جففت اوراق النبات ومن ثم تمت عملية الاستخلاص. تضمنت التجربة ٢٤ جرذاً من الذكور قسمت إلى ٤ مجموعات ٦ عينات في كل مجموعة. تم معاملة المجموعات لمدة ٣٠ يوماً على النحو الآتي: الأولى كانت مجموعة السيطرة التي أعطيت ١ مل المحلول الفسلي ٠,٩% والمجموعة الثانية أعطيت CCl₄ ٣ مل/كغم من وزن الجسم لمرة واحدة، المجموعة الثالثة والرابعة أعطيت المستخلص المائي لوحده ٣٠٠ ملغم/كغم من وزن الجسم والمستخلص المائي سوية مع CCl₄ على التوالي. أدت المعاملة بمركب CCl₄ انخفاضاً معنوياً في عد النطف، النطف الحية، النطف الطبيعية، مستوى الكلوتاتايون والسوبر اوكسايد ديسميوتيز، وزيادة معنوية في نسبة النطف الميتة، المشوهة ومستوى المالوندايالديهيد MDA بالمقارنة مع مجموعة السيطرة. بينما لم تظهر المعاملة بالمستخلص المائي لوحده فروقا معنوية للمتغيرات المدروسة بالمقارنة مع السيطرة. وأدت المعاملة بالمستخلص المائي للعاقول سوية مع CCl₄ لزيادة معنوية في عد النطف، النطف الحية والطبيعية ومستوى GSH

وانخفاضاً معنوياً في نسبة النطف الميئة والمشوهة ومستوى MDA بالمقارنة مع مجموعة CCl_4 . نستنتج من ذلك أن للمستخلص المائي لنبات العاقول تأثيراً إيجابياً في عملية التكاثر ومستوى مضادات الاكسدة في الجرذان المعرضة للإجهاد التأكسدي.

Introduction

Male fertility correlated with a proper function of organs and hormones, most of male infertility cases because of sperm abnormalities which categorized by whether they affect sperm count, sperm movement, or sperm shape (1). Sperm abnormalities caused by lots of factors, including, disease, lifestyle and chemical exposure, but many causes of sperm abnormalities are still unknown (2). Previous studies (3,4) approved that CCl_4 is a chemical exposure cause oxidative stress due inducing free radical production which cause oxidation of germ cells in the testes. Free radicals contribute to male infertility, damaging cells, tissues and organs (5,6). To maintain and protect normal cell function, excess of free radicals must be inactivated continuously by antioxidants in seminal plasma and block the formation of new reactive oxygen species ROS or keep removing generated ROS (3).

Herbal is the oldest form of medicine, it is used widely in practiced form of medicine (7). *Alhagi*, is a genus from family Fabaceae, Its distributed in many countries of Asia, Europe and Australia. Commonly it has lots of names like Camel thorn or Aqool (8). The previous studies showed that *Alhagi maurorum* plant contained many secondary metabolites, it exerted antibacterial, anti-inflammatory, antipyretic, analgesic, antioxidant, gastrointestinal, cardiovascular, diuretic, and dermatological, and many other effects (9-11). Very rare studies demonstrated the effect of aqueous extract of *alhagi* plant in fertility of males therefore the present study was designed to explore the protective effect of *Alhagi maurorum* on testicular dysfunction-induced by CCl_4 in rats.

Materials and methods

Experiment design

Twenty-four adult male rat average weight is 200-250 g housed at the experimental animal unit, College of Veterinary Medicine, University of Tikrit. Feed and water were provided all time. Rats divided randomly into four groups, first was control group which administered with physiological saline 0.9% and the other three groups administered once with CCl_4 3 ml /kg B.W. (5,6), aqueous extract of *Alhagi maurorum* 300 mg/kg B.W (12) and aqueous extract together with CCl_4 respectively. The animals were dosed orally once daily for 30 days using gavage needle.

Preparation of plant material

Aerial parts of *Alhagi maurorum* were collected from Alalam desert, Sallahidin province in the middle of Iraq. After scientific approval by botanical experts, leaves boiled in 3 L of distilled water for 1 hour then dried by reduced pressure. Powder kept in sterile container until use (7).

Active phytochemical compounds

Active phytochemical compounds (phenolic acids and flavonoids) determined in aqueous extracts (AE) of leaves using method of high-performance liquid chromatography (HPLC). Plant extract were performed on Shimadzu HPLC system (Fig. 1 and 2). Phenolic compounds separated on a Thermo Scientific Hypersil Gold reverse phase (RP-18) column, 250 mm x 4.6 mm C18 is the stationary phase. The gradient maintained at flow rate 1-2 ml/min, the time of run was over 10 min. The binary mobile phase consisted of a solvent A (water: acetic acid; 99:1; v/v) and solvent B (acetonitrile). The gradient elution from the column was achieved with 13% of solvent B until 10 min. The phenolic acids and flavonoids were detected by a UV detector at wavelength 200-500 nm. The chromatographic peaks were identified by comparing retention time of analytics with that of reference compounds (Fig. 1) (13,14).

Blood samples

At the end of the experiment, blood samples were taken by retro-orbital vein placed in a test tube free of anticoagulant then placed in the incubator at 37 °C then centrifuged at 3000 rpm for 15 minutes. Serum were kept at -20 °C until biochemical tests were carried out.

Histological preparation

Testes tissue were immediately put in formalin 10% for 24 hours. Dehydrated by graded alcohol series then embedded in paraffin. Samples cut into 5µm sections, stained using hematoxylin and eosin (H&E). Slides examined using light microscopy under 40x magnification (15).

Sperm count assay

Male rats were killed and the cauda epididymis were excised and then placed in petri dish containing 9.8 of formalin buffer 10%. Cauda epididymis was then cut using a fine scissors to make sperm suspension. Eosin stain added to suspension, the sperm counted using hemocytometer (improved Neubauer, Deep 1/10 mm, LABART, Germany). Using following equation, total sperm number = $(N/80) \times 400 \times 1000 \times 10 \times 10$ (16).

Table 1: Detecting flavonoid in aqueous extract of *Alhagi maurorum* using HPLC method (table show retention time, peak area and flavonoid concentration)

Flavonoid	Retention time	Peak area	Concentration $\mu\text{g/ml}$
Kaempferol	1.45	11742	-
Chrysoeriol	2.73	22094	147.41
Isorhamnetin	3.85	17381	214.9
Chrysoeriol-7-oxylsoid	4.64	30215	118.49
Isorhamnetin-3-0-b-d-apiol Galactopyranoside	5.56	19538	263.79

Table 2: Detecting phenols in aqueous extract of *Alhagi maurorum* using HPLC method (table show retention time, peak area and phenol concentration)

Phenols	Retention time	Peak area	Concentration $\mu\text{g/ml}$
Caffeic acid	1.32	16216	80.33
Vanillic acid	2.23	9797	46.47
Naringin	2.44	14657	223.67
Syringic	2.93	22519	175.42
Chlorogenic	3.22	5980	219.26
Coumaric	3.57	61599	186.41
Ferulic acid	4.65	47859	167.56
Sinapic acid	5.19	10479	201.61
Isoquercetin	5.55	62223	45.42
Rutin	6.48	54058	174.56
Iutolin	7.05	9170	0.178

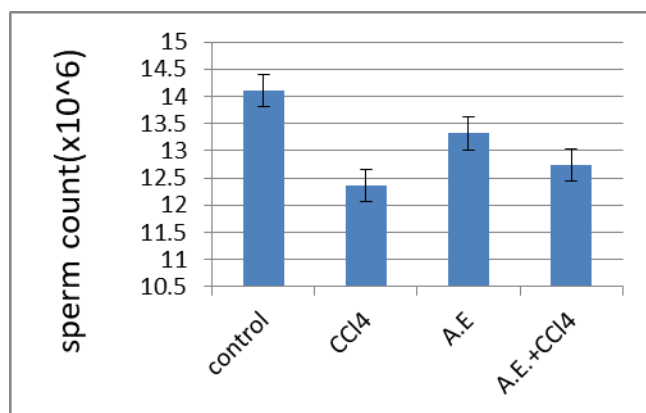


Figure 4: Effect of aqueous extract of *Alhagi maurorum* on sperm count in rats exposed to CCl_4 . Value were expressed as means \pm SE from 6 rats per group. Significant value $P \leq 0.01$.

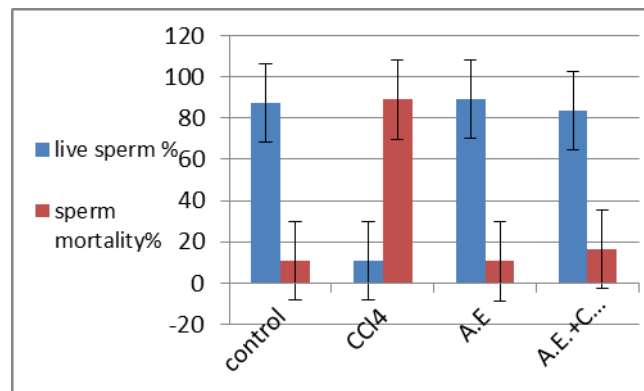


Figure 5: Effect of aqueous extract of *Alhagi maurorum* on ratio of live/dead sperm in rats exposed to CCl_4 . Value were expressed as means \pm SE from 6 rats per group. Significant value $P \leq 0.01$.

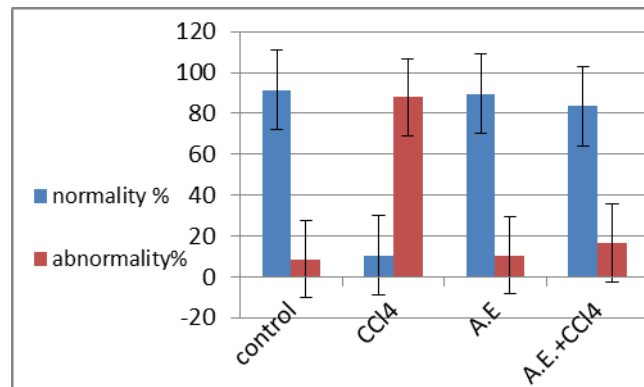


Figure 6: Effect of aqueous extract of *Alhagi maurorum* on sperm morphology in rats exposed to CCl_4 . Value were expressed as means \pm SE from 6 rats per group. Significant value $P \leq 0.01$.

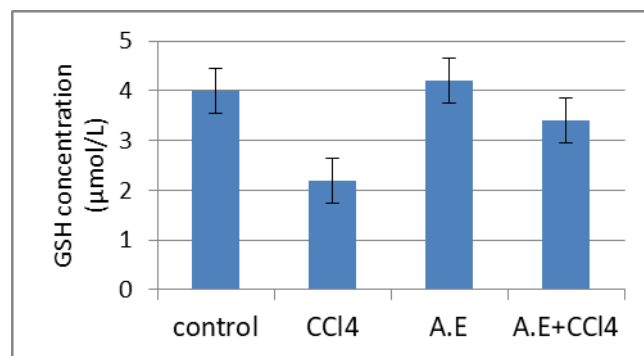


Figure 7: Effect of aqueous extract of *Alhagi maurorum* on GSH level in serum in rats exposed to CCl_4 . Value were expressed as means \pm SE from 6 rats per group. Significant value $P \leq 0.01$.

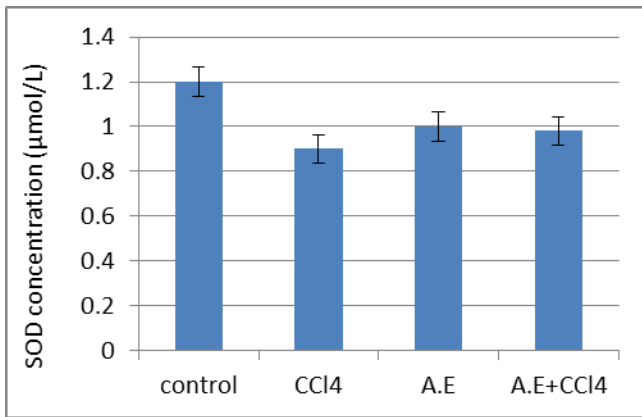


Figure 8: Effect of aqueous extract of *Alhagi maurorum* on Super oxide dismutase level in serum n in rats exposed to CCl₄. Value were expressed as means ± SE from 6 rats per group. Significant value P≤0.01.

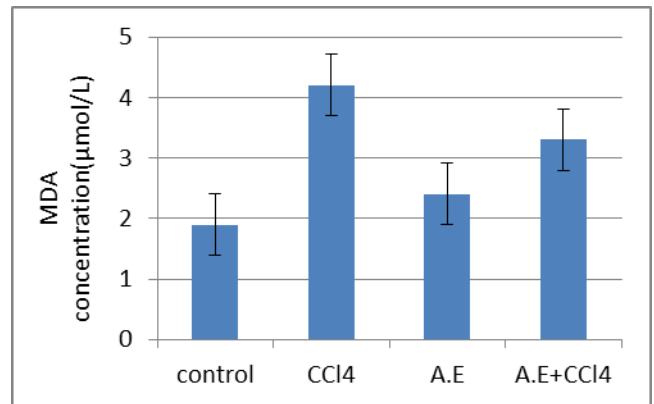


Figure 9: Effect of aqueous extract of *Alhagi maurorum* on MDA level in serum n in rats exposed to CCl₄. Value were expressed as means ± SE from 6 rats per group. Significant value P≤0.01.

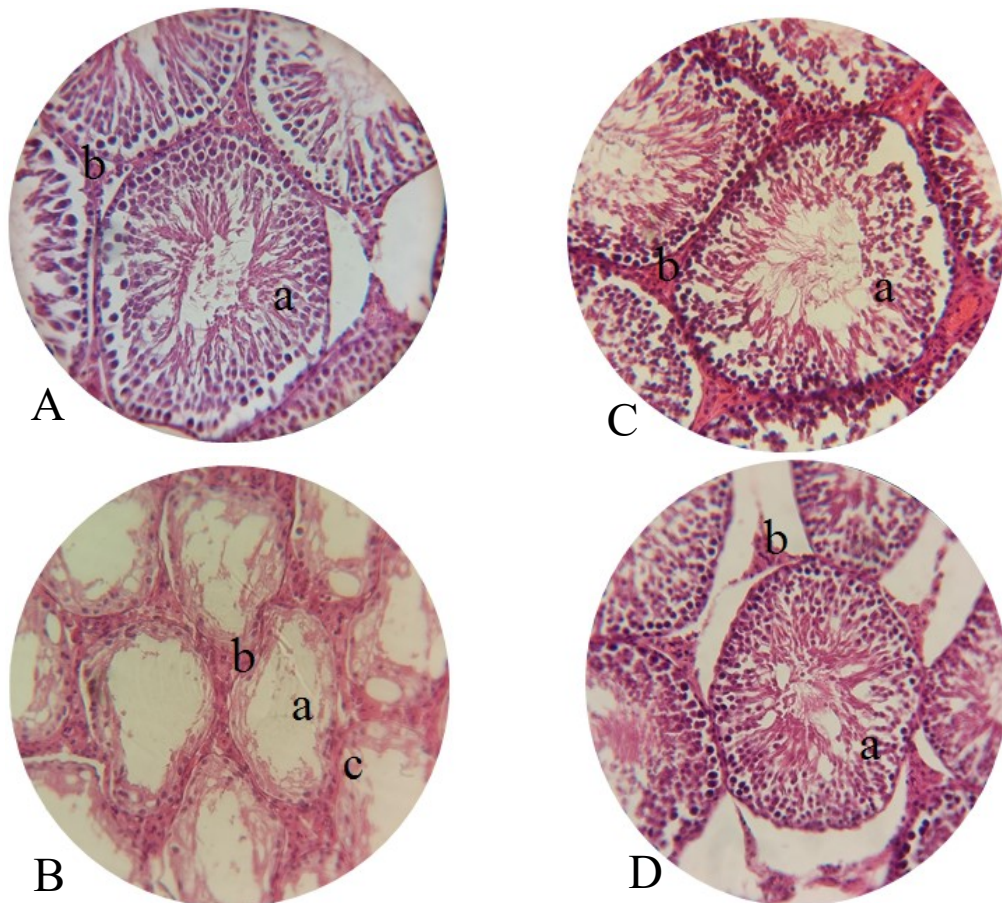


Figure 10: (A) control group, (a) normal spermatogenesis, (b) normal Leydig cell. (B) CCl₄ group, (a) Necrosis, (b) spermatogonia, (c) Leydig cells. (C) Aqueous extract of *Alhagi maurorum* group, (a) normal spermatogenesis, (b) normal Leydig cell. (D) CCl₄ + aqueous extract of *Alhagi maurorum* group, (a) normal spermatogenesis, (b) Leydig cell. H&E stain, 40x.

Discussion

Male germ cell through stages of differentiation generate reactive oxygen species ROS in low levels to regulate sperm differentiation. ROS level maintained by antioxidant (21). Sperm plasma membrane contain unsaturated fatty acid and these attacked by ROS leads to chemical reaction that called lipid peroxidation (22), and that reduces membrane enzymes activity, ion channels and the membrane fluidity, resulting inhibition in required mechanisms for production and fertilization of sperm (23) (Fig. 10-B). Excess ROS overwhelm antioxidant defense called oxidative stress (22,23). Treatment using CCl₄ cause oxidative stress of male reproductive system (Fig. 5, 6 and 7) which linked negatively with sperm production, Normality and sperm vitality. (Fig. 4, 5 and 6) in compare to control (Fig. 10-A) and that combined with (3,4). ROS promote apoptosis leading to decreased sperm concentration and increased level of caspases and proteases which increase apoptosis in mature spermatozoa (24). Researchers mentioned that the sperm is more susceptible to oxidative stress than other cells because of the limited amount of cytoplasm and the concentration of ROS suppressing antioxidants in the mature sperm in addition to high levels of unsaturated fatty acids in the sperm structure (11). The health and fertility of sperm dependent on the antioxidants to protect plasma membrane surrounding acrosome and tail (25). The leaves extract of *Alhagi maurorum* contain polyphenols (Table 2) (Fig. 3) and flavonoids (Table 1) that has antioxidant potential activity (26, 27), most flavonoids and phenols are metabolized in vivo, which can effectuate antioxidant capacity. The main phytochemical classes that comprised are phenolic acids and flavonoids. Flavonoids include several subclasses (24) (Table 1). Flavonoid-rich foods, correlated with a lower risk of many diseases (28). the aqueous extract increased glutathione GSH, SOD levels and decreased MDA level in serum in subject's exposure to oxidative stress (Fig. 7, 8 and 9). MDA is produced due to the degradation of the peroxides of unsaturated fatty acids. It is considered biomarker to determine the rate of oxidative damage (24). Testicular tissue is susceptible to oxidative stress (29), so testes protect itself from damage using antioxidants and free radical scavengers to maintain that steroidogenic and spermatogenic functions are not impacted by oxidative stress in healthy rats (25) (Fig. 8, 9 and (Fig. 10-A). Histological changes had been noticed in subject's exposure to oxidative stress induced by CCl₄ (Fig. 7, 8, 9 and 10-B) while maintain antioxidant and free radical scavengers in subject's exposure to oxidative stress give a positive result (Fig. 7, 8, 9 and 10-D). antioxidants have the ability to avoid damage by counteracting free radicals or preventing their formation in the testicular cells and prevent oxidation chain by finding primer free radicals, preventing various

diseases (30,31). It could be concluded that the aqueous extract of *Alhagi maurorum* have a positive effect on male reproduction and antioxidants in rats exposed to oxidative stress. (Fig. 10-C).

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