Open Access
Baghdad Science Journal **Vol.16(2)2019**

DOI: http://dx.doi.org/10.21123/bsj.2019.16.2.0315

Effect of Aqueous Extract *Cyperus rotundus* Tubers as Antioxidant on Liver and Kidney Functions in Albino Males Rats Exposed to Cadmium Chloride Toxic

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Received 1/10/2018, Accepted 22/1/2019, Published 2/6/2019

Abstract:

The experiment was conducted in two stages: first stage: determination of the most effective dose of *Cyperus rotundus* tubers aquatic extract in male rats for 5 days, which was concentrated as (200 mg/kg body weight). The second stage was designed to observe and test the protective effects of *C. rotundus* tubers aquatic extract in the liver and kidney functions of male rats exposed to cadmium chloride poisoning (5 mg/kg bw) for 30 days. The animals were divided into (4) groups within each group (5) animals weighted (200-220g). The results of oral dose of cadmium chloride showed a significant increase of ($P<0.05$) in the activity of both enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), glucose, urea, uric acid, creatinine, malondialdehyde (MDA) and peroxynitrite ($ONOO^-$). And a significant decrease ($P<0.05$) in the values of total protein, albumin, globulin and glutathione (GSH) compared with the control group. The oral dosage of the *C. rotundus* tubers aquatic extract resulted in a significant increase in GSH. The values of each were not significantly different ALT, AST, ALP, Glucose, urea, uric acid, creatinine, total protein, albumin, globulin, MDA and peroxy nitrite Compare with control group. The results of the oral dosage with *(C. rotundus* tubers aquatic extract + cadmium chloride) showed a positive effect on these values compared with the control group and cadmium chloride. It is concluded that the *C. rotundus* tubers aquatic extract has protective effects and reduces the effects that cadmium chloride can cause in rats liver and kidney functions through its antioxidant activity and removal of free radicals.

Key words: Cadmium chloride, *C. rotundus*, Liver and kidney functions.

Introduction:

Cumulative effect of urbanization, industrialization and population growth increases pressure on the limited natural resources. The change in living style has aggravated the problems. Among the diverse environmental problems and increasingly serious, the discharge of heavy metals in environment through industrial, agricultural and domestic activities is of great concern (1). Cadmium (Cd) a heavy metal of considerable toxicity, is one of the most widely existed toxic environmental pollutants. Industrial production such as batteries manufacture, pigments generation and metal plating obviously enhances the risk for the contamination of Cd in the atmosphere, chemical fertilizers, water and soil (2). As a result, people are exposed to Cd from air, drinking water and so on. Especially, tobacco smoking is another important way for people exposed to Cd (3, 4). welding, and contaminated food and beverages(5).

(Cd) This metal presents the serious threat for both, humans and animals health, The environmental risk can lead to the absorption of large quantities of Cd and its toxic action on organisms, It adversely affects some organs in humans and animals, including the liver, kidneys, lungs, pancreas, and testis (6). (Cd) is one of the most common and harmful transition metals present in our environment. Unfortunately, this non-essential element is toxic at very low doses and non-biodegradable by a very long biological half-life (7).

Increasing attention is given to the study of natural products, which may counteract the detrimental effects of environmental toxic compounds and prevent multiple human diseases such as neurodegenerative diseases, ageing, rheumatoid arthritis, metabolic diseases such as atherosclerosis, diabetes, hypertension, cancer, etc.. In this line, different medicinal plants have been re-evaluated and recognized as valuable sources of nutraceuticals(8). Recently, several dietary supplements containing vitamins, polyphenols, or flavones also play a significant role in this matter.

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Phenolic compounds are very important plant constituents because they exhibit an antioxidant activity by inactivating lipid free radicals or preventing decomposition. *C. rotundus* belonging to family-Cyperaceae is widely used in traditional medicine around the world for the treatment of various diseases and used in antiinflammatory, antidiabetic, antidiarrhoeal and antipyretic (9). hypolipidemic, hepatoprotective and antimicrobial properties (10). Anti-oxidant activity (11). *C. rotundus* contains some effective compounds that include flavonoids, tannins, glycosides, monoterpenes, sesquiterpenes, sitosterol, alkaloids saponins, terpenoids, essential oils, starch, carbohydrates, protein and amino acids (10). The study aims to know the protective of *C. rotundus* tubers aquatic extract antioxidant in liver and kidney functions in males albino rats exposed to cadmium chloride.

Materials and Methods:

Collection and preparation of samples:

C. rotundus was obtained from local markets and was diagnosed by specialists in University of Tikrit - College of Agriculture - Department of Horticulture and was fresh and dry. It was cleaned from foreign materials and then grinded with a national electric blender (Japan) for a fine powder.

Extract preparation:

The aqueous extract was obtained using method (12), 100 g of *C. rotundus* powder was weighed in an analytical balance. In a flask add 200 ml of distilled water and leave for 24 hours in the refrigerator after stirring, The treatment was then mediated by the medical gauze. The washing process was then using 100 mL of distilled water and the filtration was repeated. The washing and re-filtration process was then repeated, using 50 mL distilled water. Vaporizer display for evaporation using rotary vapor evaporator At 70 C° until a concentrated liquid is obtained. Finally, the extract is placed in plastic containers that are known as freezing at -20 C° until use.

Detection of active compounds in *C. rotundus* extract:

Each of the following active compounds was detected using their respective method, Resins (12). Flavones (13). Phenoles, Saponines (14). tannins (15). Alkaloids (16, 13). Coumarins (17).

Animals used in the study:

Rattus norvegicus of the (Sprague dawely) (200-220 g), obtained from the National Center for Control and Drug Research in Baghdad. It was placed in metal cages with metal covers and dimensions (19x25x21 cm), by a floor covered with sawdust. The cages cleaning and sterilization were taken care of with crosswise switch every two days. The animals were subjected to laboratory conditions from a light cycle divided into 12 light hours and 12 hours of darkness. The temperature was set at 22±2 C°. The animals were left for two weeks for adaptation. The animals were fed with fodder consisting of 35% wheat, 34% yellow corn, 20% soybean, 10% animal protein, 1% powdered milk, 50 g preservatives and antifungal substances (18). They were standard food, water ad libitum in adequate amounts all through for the experimental period. The experiment was conducted in two stages.

The first stage: **Determination of the effective dose**.

This is a pilot study to determine the most effective and optimal dose of *C. rotundus* aqueous tubers extract reduced for glucose and cholesterol in the blood. Healthy animals were randomly divided into (5) groups containing each group (3) animals, distributed as follows:

Group (1): (Group control): it was given distilled water.

Group (2): (100 mg/kg body weight) was from the *C. rotundus* extract.

Group (3): (200 mg/kg body weight) was of *C. rotundus* extract.

Group (4): (400 mg/kg body weight) was of *C. rotundus* extract.

Group (5): (800 mg/kg body weight) was of *C. rotundus* extract.

They were administered daily and over five days, after which blood samples were withdrawn by the venous vein which may blood collection (19). And the measurement of concentrations of glucose and cholesterol in them and was selected the most effective dose of the extract was (200 mg/kg body weight).

The second stage:

The animals were divided into (4) groups that included five (5) animals and close weights as follows:

Group 1: Control group.

Group 2: administered orally cadmium chloride (5 mg/kg of b.wt) by gavage daily for period (30) days (20).

Group 3: administered orally *C. rotundus* extract (200 mg/kg of b.wt) by gavage daily for period 30 days.

Group 4: This was given (*C. rotundus* extract 200 mg/kg + cadmium chloride 5 mg/kg) daily for 30 days.

Blood samples:

After 30 days, animals were starved for 10 hours, They were then weighted and numbed with chloroform. The blood samples were then removed by cutting were jugular vein in the neck, collecting about 6-8 ml of blood. Test tubes free of anticoagulant left for about a quarter of an hour in a water bath at 37 C° until coagulation and then placed in the centrifuge for 15 minutes at 3000 cycles / minute, and the serum was withdrawn by micro-pipette and placed in new plastic tubes and cleaned (Plane tubes) and kept at -20 C° until the conduct of special biochemical tests, which include both glucose, urea, uric acid, creatinine, total protein Albumin, ALT, ALP, AST and using several standard solutions (Kits) manufactured by BIOLABO SA, France (12).

The concentration of malondialdehyde (MDA) in the serum was estimated using method (21).

glutathione (GSH) was estimated in the serum using the method used by (22, 23).

The concentration of the Peroxynitrite radical was estimated using the method (24).

The Determination globulin in blood serum according to the following equation (25).

Concentration of globulin (g/dl) = Total protein Conc. – Albumin Conc.

Statistical analysis:

The results were analyzed statistically and using SAS, 2001, according to one-way analysis of variance. The mean of the coefficients was tested using the Duncun multiple rang test at a significant level (0.05) to determine the significant differences between the aggregates (23).

Results:

Detection of active compounds in the extract:

Table (1) indicates that the *C. rotundus* aqueous extract contains active substances that include Resins, Saponins, Tannins, Alkaloids, Coumarin, Flavonoids and Phenols. volatile oils, terpenes, steroids. These results agreed with those of (26) *C. rotundus* contained flavonoids, tannins, glycosides, monoterpenes, sitosterol, alkaloids, saponins, terpenoids, essential oils, starch, carbohydrates and proteins.

Table 1. Type of active compounds found in the *C. rotundus* aqueous extract.

Type of sample	Resins	Saponins	Tannins	Alkaloids	Coumarin	Flavonoids	Phenols
C. rotundus extract	+	+	+	+	+	+	+

Effective dose determination:

Table (2) shows the effect of *C. rotundus* aqueous extract on reducing the concentration of glucose and cholesterol in healthy male white rats in order to determine the most effective dose. This dose was found to be 200 mg/kg bw,it was adopted as a used dose for the substances under study.

Table 2. Determination of effective dose of *C. rotundus* aqueous extract in glucose and cholesterol parameters in male white rats.

group	Concentration (mg/kg body weight)	Glucose (mg/dl)	Cholesterol (mg/dl)
control	0.0	110 AB ±2.30	95.33 AB ±1.45
	100	109 AB ±2.30	93.66 BC ±2.40
C. rotundus extract	200	106 B ±1.73	88.00 C ±2.30
	400	113 AB ±2.30	90.00 BC ±1.73
	800	114 A ±1.73	97.33 A ±0.88

The figures followed by vertically different letters mean that there are significant differences at the probability level ($P \leq 0.05$).

Table (3) effect orally feeding of *C. rotundus* aqueous extract in the activity of blood serum enzymes of male rats exposed to cadmium chloride poisoning. The oral dose of cadmium chloride at a concentration of 5 mg/kg body weight for 30 days significantly increased ($P<0.05$) in the efficacy values of ALP, AST and ALT. The oral dosage of *C. rotundus* aqueous extract at 200 mg/kg body weight for 30 days in male rats resulted in no significant difference in the efficacy of ALP, AST and ALT compared with control group. Treatment with *(C. rotundus*+cadmium chloride) significantly reduced ($P<0.05$) in enzyme activity compared to control with cadmium chloride.

Table 3. Effect of orally administration of *C. rotundus* aqueous extract in the activity of blood enzymes in male rats exposed to cadmium chloride poisoning.

Type of transaction	Measured Standards (IU/L)		
	ALP	AST	ALT
control	211.0 C ±0.70	43.52 C ±0.30	29.62 B ±0.33
Cadmium chloride	295.0 A ±1.84	69.00 A ±1.16	40.00 A ±1.53
C. rotundus extract	209.0 C ±0.74	43.00 C ±0.35	28.90 B ±0.32
C. rotundus+ cadmium chloride	263.0 B ±1.39	60.20 B ±1.01	36.40 A ±1.14

The figures followed by vertically different letters mean that there are significant differences at the probability level (P≤0.05).

Tables (4 and 5) indicate the effect of the oral dosage of the *C. rotundus* aqueous extract in some physiological and biochemical parameters in the serum of male white rats exposed to cadmium chloride. The oral dose cadmium chloride at a concentration of 5 mg / kg body weight for 30 days resulted in a significant decrease (P<0.05) in total protein, albumin, and globulin, and significant increase in glucose, uricacid, urea and creatinine compared with the control group.The oral dosage of the *C. rotundus* aqueous extract of 200 mg / kg body weight for 30 days in male rats resulted in significant differences in total protein values, albumin, globulin, glucose, uric acid, urea and creatinine compared with control group.Treatment with *(C. rotundus* + cadmium chloride) resulted in an improvement in these values compared to control with cadmium chloride.

Table 4. Effect of orally feeding of *C. rotundus* aqueous extract in protein concentrations in blood serum male rats exposed to cadmium chloride poisoning.

Type of transaction	Measured Standards (g/dl)		
	Total protein	Albumin	Globulin
control	6.90 A ±0.33	3.90 A ±0.34	3.00 A ±0.42
Cadmium chloride	4.50 B ±0.14	2.84 B ±0.17	1.46 B ±0.12
C. rotundus extract	7.60 A ±0.33	4.30 A ±0.33	3.30 A ±0.66
C. rotundus+ cadmium chloride	5.12 B ±0.15	2.60 B ±0.40	2.52 AB ±0.42

The figures followed by vertically different letters mean that there are significant differences with the probability level (P≤0.05).

Table 5. Effect orally feeding of *C. rotundus* aqueous extract in the Urea, uric acid, creatinine, glucose of male rats exposed to cadmium chloride poisoning.

Type of transaction	Measured Standards (mg/dl)			
	Urea	uric acid	Creatinine	Glucose
control	45.40 B ±0.34	4.1 B ±0.23	0.81 B ±0.07	115.5 B ±0.70
Cadmium chloride	53.00 A ±2.22	6.4 A ±0.33	1.90 A ±0.32	131.3 A ±1.17
C. rotundus extract	45.00 B ±0.35	4.6 B ±0.43	0.87 B ±0.32	110.6 B ±1.84
C. rotundus+ cadmium chloride	49.90 B ±0.33	5.6 A ±0.11	1.00 B ±0.11	126.0 A ±1.14

The figures followed by vertically different letters mean that there are significant differences at the probability level (P≤ 0.05).

Table (6) shows the effect of *C. rotundus* aqueous extract in some antioxidants and parameters in the blood of male rats exposed to cadmium chloride.

As shown by the Table, the effect of cadmium chloride resulted in a significant decrease(P<0.05) in GSH and a significant increase (P<0.05) in MDA and peroxynitrite compared to control group.The oral dose of a 200 mg / kg body *C. rotundus* aqueous extract in male rats for 30 days resulted in a significant increase (P<0.05) in GSH and no significant difference in MDA and peroxynitrite compared to the control group. The treatment of (*C. rotundus* + cadmium chloride) improved the values of GSH, MDA and peroxynitrite compared to control with cadmium chloride.

Table 6. Effect of orally feeding of *C. rotundus* aqueous extract in Glutathione of male rats exposed to cadmium chloride poisoning.

Type of transaction	Measured Standards (Mmol/L)		
	Glutathione	Malondialdehyde	Peroxynitrite
the control	460.1 B	1.52 C	59.2 C
	3.53±	0.17±	1.00±
Cadmium chloride	303.0 D	3.90 A	75.3 A
	1.58±	0.07±	1.14±
C. rotundus extract	490.0 A	1.61 C	60.2 C
	3.53±	0.17±	0.51±
C. rotundus+ cadmium	399.0 C	3.00 B	70.1 B
chloride	4.25±	0.18±	0.66±

The figures followed by vertically different letters mean that there are significant differences at the probability level (P≤0.05).

Discussion:

The reason for the rise in the level of these enzymes indicates liver damage from the effect of cadmium chloride (27). This can lead to the necrosis of the liver which causes increasing permeability of the cell membrane leading to secretion or transmission of transaminases enzymes in the bloodstream, and that increasing in alkaline phosphatase represents general hepatic toxicity (28). Alkaline phosphates are usually the response of the liver to any form of bile duct obstruction (29) The liver is one of the critical target members of cadmium toxicity that causes liver necrosis and can cause abnormal release of ALT and AST in the blood (30).

The reason for the increase in urea may be due to increased concentrations of free radicals in the body and the occurrence of oxidative stress and cause oxidation of proteins and amino acids and thus increase urea as a secondary product (31). The increase can be due to the increase of pyurines, which are the basic materials for the formation of uric acid, which may result from the destruction of nucleic acids, and that cadmium destroys DNA (32). The reason for the significant increase in serum creatinine concentration may be due to non-filtration of creatinine from the blood through renal glomeruli due to damage and damage caused by glomeruli due to oxidative damage to cadmium chloride, allowing the release of creatinine in the blood (33). The decrease of proteins in the cadmium chloride group can cause effect-free radicals resulting from oxidative stress leading to diabetic nephropathy and increasing the amount of blood-to-urine protein (albumin) through the glomerular glands (34, 35).

The damage of cadmium chloride in the liver and kidneys, induced the over-generated ROS and diminished antioxidant enzymes lead to uncontrolled oxidative stress. Oxidative stress stimulates the damages of DNA, lipids, proteins and other cellular biomolecules, as well as promoting the interruption of cellular redox homeostasis, the cellular apoptosis and the abnormal activation of signaling pathways (36, 37). The reason for the increase in the concentration of MDA in animals exposed to oxidtive stress is the result of treatment with cadmium chloride, which may result in the formation of free radicals that attack the fat and its compounds in the body, especially in the cellular membranes and work on oxidation and damage. This process is called lipid peroxdation, and the result of lipid peroxdation is MDA (Malondialdehyde) (38, 39) MDA is a lipid peroxidation product from polyunsaturated fatty acids and commonly used to be an indicator of oxidative damages in cellular and organic functions (40, 41). In addition, the concentration of peroxynitrite (ONOO-) in the serum, which may be attributed to increased production of freeradicals, especially the superoxide oxide (O_2^{-}), was observed directly, which in turn reacted with nitricoxide to produce ONOO·, which is more oxidized of both (42, 43). This is due to the increase in the formation of free radicals, especially ROS, and the occurrence of oxidative stress, which cause oxidation of glutathione as a result of its antioxidant effect. Thus converting it to the oxidative form Glutathione disulfide (GSSG), which is toxic and stimulates the production of new varieties of free radicals (44, 45).The reason for the decrease in the enzymes activity when treated with a *C. rotundus* extract may be due to the hepatoprotective effect of could be attributed to the improvement of antioxidant status of the animals of the presence of free radical scavenging substances such as flavonoid (46). May be due to the that flavonoids can repair damaged liver cells through removing free radicals, causing the decrease in the level of enzymes activity (47). Or possibly due to liver activation can be attributed to the ability of cell membrane stability to prevent the enzyme from leaking out (48). The significant increase in the total proteins of the blood serum treated with *C. rotundus* extract may be due to the effectiveness of the antioxidant components, which play an important role in reducing oxidative stress.

This is reflected in the inhibition of cortisone secretion of the adrenal cortex, which plays role in the formation of glucose from non-carbohydrate sources, thus preserving the amino acids Gluconeogenesis also maintains or elevates the level of plasma proteins (49). This may be due to the role of the antioxidants in this extract in the induction of the process of making proteins in different locations in the body of the organism where the presence of flavonoids in the *C. rotundus* tubers used in the study anti-oxidation effectively plays an important role in reducing peroxide and increase catalysts Catalase and prevent oxidation in animals (50). The reason for the low blood glucose level is that the *C. rotundus* aqueous extract contains active substances with insulin-like effects (51). Previous studies have showed that *C. rotundus* compounds inhibit the free radical generation, act as antioxidant, free radical scavengers and it has also been demonstrated that treatment with *C. rotundus* inhibits the generation of superoxide radicals (52, 53, 54). The reason for the increase in glutathione may be due to the content of *C. rotundus* on antioxidants, including multiple phenolic compounds and flavonoids, which may inhibit the reactions of the formation of free radicals and remove the radicals resulting from the impact of cadmium chloride, and activates the liver and stimulate the enzymes of liver antioxidants such as glutathione Peroxidase (Gpx) Catalase and superoxide dismutase (SOD) superoxide dismutase, all of which inhibit oxidation and lipid peroxidation and inhibit the production of melonadealdehyde (55, 56). The overall antioxidant activity of *C. rotundus* extract might be attributed to its polyphenolic content and other phytochemicals constituents which exhibit and reduce free radical scavenging (56). In conclusion our study indicates that *C. rotundus* aqueous extract have Liver and kidney protection effect against cadmium chloride induced oxidative stress in rats which may be related to its antioxidant effect.

Conflicts of Interest: None.

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تاثير المستخلص المائي لدرنات نبات السعد *Cyperus rotundus* كمضاد للاكسدة في وظائف الكبد والكلى لذكور الجرذان البيض المعرضة للتسمم بكلوريد الكادميوم

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الخلاصة:

اجريت التجربة بمرحلتين: المرحلة الاولى تعيين الجرعة الاكثر تاثيراً لمستخلص درنات نبات السعد المائي في ذكور الجرذان لمدة (5) ايام والتي كانت بتركيز (200 ملغم/كغم من ون الجسم). والمرحلة الثانية صممت لملاحظة واختبار التاثيرات الوقائية لمستخلص درنات نبات السعد المائي في وظائف الكبد والكلى لذكور الجرذان المعرضة للتسمم بكلوريد الكادميوم بجرعة (5 ملغم/كغم من وزن الجسم) لمدة 30 يوما، وزعت الحيوانات الى (4) مجاميع ضمن كل مجموعة (5) حيوانات التي تزن (220-200)غم، اظهرت نتائج التجريع الفموي بكلوريد الكادميوم الى حصول ارتفاع معنوي ($P<0.05$) في فعالية انزيمات كل من ALP, AST, ALT، الكلوكوز، اليوريا، حامض اليوريك، الكرياتنين، المالونداياليديهايد والبيروكسي نتريت. وانخفاض معنوي ($P<0.05$) في قيم كل من البروتين الكلي، الالبومين، الكلوبيولين والكلوتاثيون مقارنة مع مجموعة السيطرة. وقد ادى التجريع الفموي بمستخلص درنات السعد المائي الى ارتفاع معنوي في الكوتاثيون. ولم يختلف معنوياً قيم كل من ALP, AST, ALT، الكلوكوز، اليوريا، حامض اليوريك، الكرياتنين، البروتين الكلي، الالبومين، البروتين الكلي، المالونداياليديهايد والبيروكسي نتريت مقارنة مع مجموعة السيطرة. كما اظهرت نتائج التجريع الفموي بمستخلص السعد المائي+كلوريد الكادميوم تاثير ايجابي على هذه القيم مقارنة مع مجموعة السيطرة السليمة والمصابة بكلوريد الكادميوم. استنتج من ذلك ان المستخلص المائي لنبات السعد له تاثيرات وقائية ويقلل من التاثيرات التي يمكن ان يسببها كلوريد الكادميوم في وظائف كبد وكلى الجرذان من خلال فعاليته المضادة للاكسدة وازالة الجذور الحرة.

الكلمات مفتاحية: مستخلص السعد المائي، كلوريد الكادميوم، وظائف الكبد والكلى.