The effect of aqueous *Urtica dioica* extract in male rats exposed to copper sulfate poisoning.

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Abstract

This study was designed to note the preventive effect of *Urtica dioica* aqueous extract (100 mg / kg of body weight) in healthy white male rats exposed to copper sulfate poisoning (40 mg / kg bw) throughout the 30day treatment period, and the weights ranged between 240 - 260 g and their ages are between 2-3 months, and the animals were divided randomly into four groups, each group containing 7 rats. Results showed that when *Urtica dioica* extract was administered, it led to a significant decrease of P <0.05 in cholesterol concentration and a significant increase in high-density lipoprotein (HDL) and no significant difference in organ weights, glucose, uric acid, urea, creatinine, triglycerides, low-density lipoprotein (LDL) and Very low-density lipoprotein (VLDL) compared to the control group. When animals were given copper sulfate, it led to a significant decrease in body weight, and a significant increase in weights (liver, kidney and spleen), glucose, uric acid, urea, creatinine, TG and LDL triglycerides, and it did not differ significantly in cholesterol, HDL and VLDL compared with the control group. When administering (*Urtica dioica* + copper), it led to a significant decrease in kidney weight, glucose, uric acid, urea, creatinine, triglycerides and LDL, and a significant increase in body weight and HDL while it did not significantly differ in cholesterol and VLDL from compared with the group that was given Copper sulfate. It is concluded from the study that the *Urtica dioica* extract had positive effects in animals exposed to copper sulfate poisoning.

Key word: *Urtica dioica*, copper sulfate, physiological and biochemical parameters

1. Introduction

Copper (Cu) is one of the most important heavy metals, and an essential element in the enzymes that contribute to the formation of hemoglobin and for some antioxidant enzymes such as Cu / Zn superoxide dismutase and for cytochrome C enzymes that enter into cellular respiration [1, 2]. And copper is an essential influential element capable of producing toxic effects in animals and humans when consumed acutely or chronically [3]. It is found in some foods such as fish and some vegetables. Poisoning with copper salts may occur when foods containing organic acids are cooked in uncoated copper containers. Copper is also found in drinking water. It dissolves from the pipes made of it when the water remains inside it for a long time [2-6]. Copper poisoning may occur as a result of its presence in eye shadow and lipstick samples, and copper contamination is external during the industry due to its environmental presence even in the air or to use a colorant that contains copper in its composition, such as copper powder or copper-tinted colorant, which is included in preparations to obtain brown and its tones [7]. And it appeared in a study that all average concentrations of copper in all colors exceeded the limit value allowed for its presence in drinking water, according to the World Health Organization, which is 2.21 ppm [8]. Copper poisoning causes damage to various tissues and organs, including the kidneys, liver, intestines, testes and brain, where the kidneys are susceptible to toxic effects due to filtering functions [9, 10]. Nettles (*Urtica dioica*) family Urticaceae used for centuries in traditional medicine. Nettles contain a significant number of biologically-active compounds.Such as, the leaves are rich sources of terpenoids, carotenoids and fatty acids, as well as of various essential amino acids, chlorophyll, vitamins, tannins, carbohydrates, sterols, polysaccharides, isolectins, minerals and polyphenols [11]. It is located in the center and north of Iraq, the Western Desert, and the date of cultivation is from October to March [12]. It is used to treat many diseases such as rheumatoid arthritis and diabetes [13]. And urinary tract inflammation and infections, nephrolithiasis, poor circulation, allergies, spleen enlargement and other endocrine disorders [14]. It is also used in prevents the damage of liver tissue structure in rats [13], and the process of stopping bleeding [15]. The study aims to know effect of *Urtica dioica* aqueous extract in some parameters physiological and biochemical variables in white male rats exposed to copper sulfate poisoning.
2. Materials and methods

Preparation of the plant extract: The *Utrica dioica* plant was collected from the Sharqat region / Salah al-Din Governorate and it was diagnosed by researchers specialized in plant classification. It was cleaned and removed foreign materials from it and then dried in the shade with constant stirring until it was obtained dry, after which the samples were milled using a laboratory electric mill to obtain fine powder. The aqueous extract was obtained using the method [16]. 100 grams of the powder of the nettle plant were weighed in a sensitive balance, and a beaker was placed. 200 ml of distilled water were added to it and left for 24 hours in the refrigerator after which the mixture was stirred, then filtered by medical gauze and then returned. The washing process was using 100 ml of distilled water and re-filtered, then the washing process was repeated and re-filtering using 50 ml of distilled water. Display the filtrate to evaporation with a rotary evaporator at a temperature of 55 °C until a concentrated liquid is obtained. Finally, the extract is placed in weight-bound plastic containers in the freezer at -20 °C until use.

2.1. Experimental animals

In the current study, white rats of the Sargue dawely strain that were brought from the College of Veterinary Medicine at the University of Tikrit. 28 rats were used, which were divided into four groups, each group of 7 white rats, and their place was prepared for 12 hours of light and 12 hours of darkness. The ventilation and the temperature of 24-25 °C were also placed in a cage to ensure the accuracy of feeding the food and the materials used in the experiment. The floors of the cages were furnished with sawdust for the purpose of keeping the cages clean, easy to clean and sterilize, and the food feed for them was prepared and consisted of 5 kg of protein, 10 kg of soybeans, 17.5 kg of wheat, 17.5 kg of yellow corn and 1 kg of powdered milk and the provision of sterile water with plastic cylinders. Weight measurement: the rats' weights were measured by a sensitive electronic scale at the beginning of the experiment and at the end of the experiment, as the weight increases were calculated for the rats by calculating the difference between their weight at the beginning of the experiment and their weights at the end of the experiment, the initial weight - the final weight = weight gain (g).

2.2. Experience design:

The animals were divided into four groups. Each group included seven animals, weighing 240-260 g.

The first group: (the control group) was given drinking water and regular food for 30 days.

The second group: (aqueous *Utrica dioica* extract) This group was treated with (100 mg / kg of body weight) of the nettle extract by oral dosage daily for 30 days.

The third group: (copper sulfate) This group was treated with (40 mg / kg of body weight) of copper sulfate by daily oral dose for 30 days.

The fourth group: (aqueous *Utrica dioica* extract + copper sulfate) were treated with (100 mg / kg of body weight) of *Utrica dioica* extract. And (40 mg / kg of body weight) of copper sulfate by oral dose daily for 30 days.

2.3. Blood samples

After the 30-day trial period ended, the animals were starved for 10 hours, then weighed and anesthetized with chloroform, then blood was drawn from the animals by cutting the iliac vein in the neck, and the blood was kept in test tubes without anticoagulant and left for a quarter of an hour Approximately in a water bath at 37 °C until it is coagulated. Then he was placed in a centrifuge for 15 minutes at 3000 revolutions / minute, and the serum was withdrawn by a micropipette and placed in new, clean plastic tubes and kept at -20 °C until chemical tests were performed that include glucose, uric acid, urea, creatinine, and cholesterol, triglycerides and HDL are used in several standard solutions (Kits) manufactured by (BIOLABO SA, France) by measuring them with a Spectrophotometer. The concentration of LDL-C and VLDL-C in serum was determined according to the following relationship[17].

VLDL concentration (mg/dl) = (Triglycerides/5)

LDL-C concentration (mg/dl) = Total cholesterol – (HDL-C) – VLDL-C.
2.4. statistical analysis

The results were analyzed statistically using the SAS program, 2001 according to one-way analysis of variance. Arithmetic averages of the parameters were tested using the Duncun multiple ranges with a significant level (0.05) to determine the significant significant differences between the groups [18].

3. Results and Discussion

Table 1 shows the effect of aqueous *Utrica dioica* extract on the weights of male rats exposed to copper sulfate poisoning and the presence of significant differences at the end of the experiment, as the weights of males treated with aqueous *Utrica dioica* extract increased significantly (P <0.05) compared to the control group. It may be the reason for this is that *Utrica dioica* leaves contain proteins, vitamins and minerals [19]. The reason for the weight gain may be attributed to the extract containing the amino acids lysine and glutamine, and small quantities of methionine, which are important in the process of growth and body building [20]. The weights of males treated with copper sulfate decreased significantly compared to the control group and the *Utrica dioica* extract group, as well as with the group (*Utrica dioica* + copper) in the increase in weight gained at the end of the experiment with a significant level (P <0.05). Maybe the reason for this is due to oral ingestion of a large amount of copper. Copper ions cause direct stomach irritation that manifests as nausea and vomiting. In the case of chronic exposure, copper causes free radicals to form via the Fenton-type reaction and the lysosomal lipid peroxidation, leading to cell death [21].

| Treatment type              | Body weight (g) |          |          |          |
|----------------------------|-----------------|----------|----------|----------|
|                            | Initial weight  | Final weight | Weight gained |
| control                    | 255.00 ±5.75 a  | 286.00 ±5.18 a | 30.00 ±1.15 a |
| *Utrica dioica* extract    | 252.00 ±4.14    | 297.00 ±3.63 a | 43.00 ±1.14 b |
| Copper sulfate             | 256.10 ±4.14 a  | 232.70 ±5.01 b | 23.40 ±0.55 ab |
| *Utrica dioica* + Copper sulfate | 255.01 ±3.44 a | 244.90 ±3.19 ab | 33.90 ±0.55 a |

Different letters in a column mean significant differences at the probability level 0.05.

The results of Table 2 showed that the effect of aqueous *Utrica dioica* extract on the organ weights (liver, kidneys and spleen) of male rats exposed to copper sulfate poisoning resulted in a significant increase of P <0.05 in the weights of the organs (liver, kidneys, spleen) of the group treated with copper sulfate Comparison of the control group and the *Utrica dioica* extract treated group. Whereas, the copper sulfate group did not show significant differences in weights (liver, spleen) with the group (*Utrica dioica* + copper). The results showed a significant increase of p <0.05 in kidney weights between the copper sulfate group compared with the control group, the *Utrica dioica* extract group, and the (*Utrica dioica* + copper) group. Where the liver is the target organ for chronic copper poisoning, and hepatotoxicity with copper appears especially in people who suffer from Wilson’s disease, Wilson’s disease is a genetic disease that results from the accumulation of systemic copper [21]. Effects on the central nervous system also appear with chronic exposure to copper, in addition to the occurrence of hemolytic anemia [22]. Or because these organs contain metallothionein, which is bound to a toxic metal [23].

The results in Table (3) showed the effect of *Utrica dioica* extract on the concentrations of glucose, uric acid, urea and creatinine in male rats exposed to copper sulfate poisoning. There was no significant difference when dosed with *Utrica dioica* extract in the concentration of glucose, uric acid, urea and creatinine compared to With proper control. The oral dose of copper sulfate led to a significant increase of P <0.05 in the concentration of glucose, uric acid, urea and creatinine compared with the healthy control group, the *Utrica dioica* extract group, and the (*Utrica dioica* + copper) group. There were no significant differences between the two groups of copper sulphate and the group (*Utrica dioica* + copper) in glucose concentration. Copper toxicity may cause oxidative stress and affect the endoplasmic reticulum of the kidneys [24].The reason may be due to the protective effect of nettle leaves, as they contain antioxidants and fortifying substances that reduce the oxidative stress that copper sulfate causes on the organs [25]. Or the reason may be due to the protective effect of *Utrica dioica* on the liver, kidneys and other tissues from the protective effect by increasing the activity of araoxonase, arylesterase.
and catalase activity in the liver tissues [26, 27]. The decrease in glucose may be due to the active antioxidant substances present in the extract [28]. The exposure to copper sulphate induce adverse effects on the hepatic and renal functions associated with oxidative stress and reduce the activities of antioxidant enzymes [29].

Table 2. Effect of aqueous Utrica dioica extract on organ weights (liver, kidneys, spleen) for males exposed to poisoning with copper sulfate.

| Treatment type | Body organ weight (g) |        |        |
|----------------|-----------------------|--------|--------|
|                | Liver weight          | Kidneys weight | Spleen weight |
| control        | 12.15 ±0.01 a         | 2.22 ±0.06 a  | 1.46 ±0.01 a |
| Utrica dioica  | 11.90 ±0.21 a         | 2.36 ±0.21 a  | 1.44 ±0.02 a |
| Copper sulfate | 14.70 ±0.12 b         | 3.45 ±0.10 b  | 1.90 ±0.18 a |
| Utrica dioica  | 12.62 ±0.07 ab        | 2.57 ±0.01 ab | 1.72 ±0.21 a |

Different letters in a column mean significant differences at the probability level 0.05.

Table 3. The effect of aqueous Utrica dioica extract on the concentrations of glucose, uric acid, urea and creatine in male rats exposed to copper sulfate poisoning.

| Treatment type                  | Measured Standards |        |        |
|--------------------------------|--------------------|--------|--------|
|                                | glucose mg/dl       | uric acid mg/dl | urea mg/dl | Creatine mg/dl |
| Control                        | 95.65 ±2.88 a       | 39.00 ±0.55 a  | 3.37 ±0.24 a | 0.68 ±0.01 a  |
| Utrica dioica                  | 90.30 ±1.13 a       | 33.00 ±1.50 a  | 3.48 ±0.27 a | 0.41 ±0.10 a  |
| Copper sulfate                 | 128.02 ±1.71 b      | 46.00 ±0.26 b  | 4.98 ±0.13 b | 1.13 ±0.01 c  |
| Utrica dioica +Copper sulfate  | 111.01 ±1.6 ab      | 43.50 ±3.78 a  | 3.90 ±0.23 a | 0.81 ±0.02 b  |

Different letters in a column mean significant differences at the probability level 0.05.

The results also showed in Table (4) the effect of Utrica dioica extract on the blood lipid parameters of male rats exposed to copper sulfate poisoning. It was found that there was a significant decrease of P <0.05 in concentration of cholesterol and triglycerides and a significant increase in HDL-C and did not differ significantly VLDL-C and LDL-C for the Utrica dioica group. Compared with the healthy control group, and when administering copper sulfate, it led to a significant increase in cholesterol concentrations, VLDL-C and LDL-C triglycerides, and a significant decrease in HDL-C compared with the Utrica dioica extract group. In the (Utrica dioica + copper) group, there was a significant decrease in HDL-C. Improvement in blood lipid parameters compared to the copper sulfate group. The reason for the high lipid profile may be attributed to the effect of copper sulfate, which is similar to the effects as a result of changes in the gene expression of cadmium chloride effect of some liver enzymes such as Hydroxyl-methyl CoA reductase (HMG-CoA), which in turn lowers the receptor for the gene expression of LDL, which led to a decrease in the concentrations of cadmium chloride. Bad cholesterol, LDL-C, inside the cell, and high in the blood [27]. Copper caused lipids damage and so lipid peroxidation generation with the significant decrease in the hepatic and renal catalase, superoxide dismutase and glutathione in the copper sulfate treated group [30]. The reason for the decrease in the concentration of cholesterol may be through the Utrica dioica leaves containing sterols that are similar in composition to cholesterol, which replace the cholesterol in the mixture of micelles, which are hydrophobic of cholesterol during absorption in the intestine [31]. The active substances in Utrica dioica leaf may have a direct role in lipoprotein synthesis and metabolism [32]. Or, the reason for the low concentration of LDL-C may be due to the presence of polyphenols in the leaves of nettle that prevent LDL-C oxidation by binding these phenols to the LDL-C complex in the blood serum, thus preventing its oxidation and becoming resistant to oxidation, and thus its concentration in Serum [33].
may be attributed to the nettle leaf containing phenols and sterols [34]. Aqueous U. dioica extract as an antioxidant agent may for decrease risk factors of cardiovascular incidence [28].

**Table 4.** The effect of aqueous *Urtica dioica* extract on blood lipid parameters of male rats exposed to copper sulfate poisoning.

| Treatment type              | Measured Standards | VLDL-C mg/dl | HDL-C mg/dl | LDL-C mg/dl | Triglycerides mg/dl |
|-----------------------------|--------------------|---------------|-------------|-------------|---------------------|
| control                     | 94.60 ± 1.09 b     | 75.11         | 26.81       | 53.26       | ±1.32 a             |
| *Urtica dioica*             | ±7.94 a            | ±5.56 b       | ±6.18 a     | ±1.09 ab    | ±0.96 a             |
| Copper sulfate              | 76.56              | 59.02         | 36.65       | 28.95       | 11.10               |
| *Urtica dioica* + Copper sulfate | ±3.90 a          | ±4.91 a       | ±0.36 b     | ±4.15 a     | ±0.96 a             |
|                             | ±4.60 b            | ±5.81 c       | ±1.95 a     | ±1.76 b     | ±1.18 b             |

Different letters in a column mean significant differences at the probability level 0.05.

**Conclusion**

The results obtained from the present study indicated that *Urtica dioica* aqueous extract exhibited a wide range of antioxidant activity. The overall antioxidant activity of this plant mightbe attributed to its which plays act as a pro-tective agent in the treatment of Copper sulfate toxicity in male albino rat.

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