BioGecko

Vol 12 Issue 03 2023
ISSN NO: 2230-5807

Efficacy of dandelion root extract to reduce copper sulfate toxic in male albino mice

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Summary

This study was designed to observe the effect of an aqueous dandelion root extract to reduce the toxicity of copper sulfate ($CuSO_4$) at a concentration (40 mg / kg of body weight) in male albino mice for 30 days, and their weight ranged between 20-23 gm. The animals were randomly divided into three groups. Each group contains 7 mice. The results of the group treated with copper sulphate showed a significant decrease in the final body weight and the weight of the organs that include the liver, kidneys, heart and testes, as well as a significant decrease in the blood parameters that include WBC, LYM, MON, RBC, HCB, HCT, MCV, MCH, MCHC. A significant increase in the values of ALT, AST, and ALP enzymes. As for the tissues of the liver and kidneys, there was congestion, inflammation, separation of the central vein and damage to the liver. As for the tissues of the kidneys, it showed degeneration of most of the cells of the urinary tubules with clear bleeding inside the tissues of the kidneys compared to the standards and tissues of the healthy control group. As for the group that was given dandelion root extract + copper sulfate, its results showed an improvement in blood parameters, liver enzymes, liver and kidney tissues compared to the healthy control group and the group exposed to copper sulfate poisoning. The study concluded that dandelion root extract has positive effects on animals that were poisoned with copper sulfate.

Key word: dandelion root,sulfate of copper,haematological, histological.

Introduction

sulfate of copper in both animal and human tissues, copper (Cu), an important metal, is found in large quantities. It is a part of several metalloenzymes, including peroxides, catalase, and cytochrome oxidase (1,2). Potential environmental problems result from the high copper content in drinking water. Plates, brickwork, tapes, and numerous alloy goods are sources of it(3). By causing the formation of reactive oxygen species (ROS) and damaging lipid peroxidation, copper is a catalyst that causes oxidative stress(4). The gastrointestinal tract, skin, and lungs all contribute to the body's absorption of copper. It is transferred to the portal circulation via binding to plasma albumin and amino acids, and its primary target organs are the liver and kidneys. This results in liver toxicity and decreased antioxidant activity (5,6,7).Contrarily, exposure to CuSO4 causes oxidative stress, which then sets off a chain of events that triggers the mitochondrial apoptosis pathway and, eventually, nephrotoxicity (8). Dandelion (Taraxacumofficinale) is a medicinal plant used in alternative medicine for its preventive and several therapeutic capabilities. Medicinal herbs have long been employed for their disease-protective benefits (9).A perennial plant that grows practically anyplace and is both edible and medicinal, dandelion is more widely distributed in temperate regions of the planet (10). The Asteraceae family, which includes this plant, was first discovered in Asia, North and South America, and Europe (11). Additionally, it is regarded as a strong medicinal plant (12). Dandelions include phenylpropanoids, flavonoids, polysaccharides, coumarins, chlorogenic acid, and hydroxycimanic acids, according to phytochemical research (13).Citamines, which include carotene, choline, and trace elements including iron, magnesium, sodium, silicon, zinc, potassium, phosphorus, and copper, are abundant in dandelion leaves (14). The roots, on the other hand, are abundant in asparagine, insulin, bitter glycosides,

sterols, volatile oils, and taraxacin(15). The powerful alkalinizing properties of dandelion root make it a superior blood cleanser that also neutralizes acids in the body. Due to its high vitamin A and C content, mineral content, potassium content, and strong antioxidant activity, it supports the health of the liver and gallbladder. It stimulates the liver to support toxin removal. Since antiquity, dandelion has been utilized for its therapeutic properties, particularly in traditional Chinese medicine, where it is used to treat bacterial infections as well as liver ailments(16).The hepatoprotective properties of dandelion stem from its anti-inflammatory and antioxidant properties(17, 18).sources of medications having anti-inflammatory and anti-cancer effects (9).Dandelion extract is a significant diuretic and antioxidant (19). The purpose of the study is to determine how male albino mice exposed to copper toxicity respond to dandelion aqueous extract in terms of blood parameters and histological changes in the liver and kidneys.

Materials and Methods

Prepare of experimental samples:

Samples collection: Dandelion roots were collected from local markets and diagnosed by plant classification and cleaning specialists.

Preparation of plant extracts: Prepare the aqueous extract of dandelion roots, according to the method used before (20).

Animals used: The Tikrit University College of Veterinary Medicine provided male white rats, which were used. Thirteen animals each from each of the three groups of twenty-one were employed and weighed, and their location was set for light and darkness (12: 12 hours)ventilation and temperature 25±2 °C. Give the test subjects the food and supplies that were used. To keep the cages clean and to make cleaning and sterilizing easier, sawdust was swept over the cage floors. Sterilized water was also given in plastic cylinders. The experiment was conducted on mice for 30 days, and the weights of the mice were assessed at the start and end of the experiment using a sensitive scale.

Starting weight - final weight = weight gain (gm).Experimentgroups:

Group1: (Control group).

Group 2: Administered copper sulfatewith 40 mg/kg b.w. by gavage.

Group 3: Administered with dandelion root aquatic extract 400 mg/kg b.w + copper sulfate 40 mg/kg b.w by gavage.

All of the animals underwent a ten-hour fast at the conclusion of the trial, although they were still given free access to water. Following weighing and chloroform anesthesia, the animals were killed by cutting the jugular vein. Then about (1.5) ml of blood was taken from each animal, and (0.5) ml of blood was put into EDTA tubes containing anticoagulants to measure most of the tests such as complete blood counts (CBCs) and other blood cell parameters, complete blood counts (CBCs) (complete blood analysis). Hematological analysis of White Blood Cell Count (WBC), Monocyte (MON), Lymphocyte Count (LYM), Granulocyte (GRAN), Red Blood Cell Count (RBC), Hemoglobin Concentration (HB), Hematocrit (Hct), Mean Corpuscular Hemoglobin Concetration (MCHC), Mean cellular volume (MCV), Mean Corpuscular Haemoglobin (MCH), and platelet count (PLT) were determined using an automated hematology analyzer (Syamex model: K-1000, Japan) (21). The updated procedures employed were also able to corroborate the majority of blood tests(22).Regarding the remaining blood, which was placed in tubes without anticoagulant and centrifuged using a centrifuge at 3000 rounds per minute for 15 minutes to obtain the serum, which was then kept at a temperature of -20°C until biochemical tests, such as Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST), were performed using (Kits) made by BIOLABO SA, France**.**

Histological study: tissue slice preparation The abdomen of each mouse was cut to open the abdominal cavity and chest (sternum) in an inverted (T) shape after chloroform anesthesia and blood collection. The organs, including the heart, spleen, liver, and kidneys, were then taken from each mouse, and the weight of each organ was recorded. After being divided into (3-2) parts with a sharp blade, the liver and kidneys were taken and placed in normal saline solution to wash and remove the attached fatty and soft tissues. After this excess water is removed by filter paper, tissue samples are placed and fixed using Formalin (10%) for (18–24) hours, and after passing this period, washed in tap water, dehydrated in ascending grades of ethanol. Then follow a series of steps as per use (23).

Statistical analysis: To identify the significant differences between the groups, the arithmetic means of the coefficients were evaluated using Duncan's polynomial test at the level of significance (0.05) and the findings were statistically analyzed using the (SAS, 2001) program and according to one-way analysis of variance. (24).

Results and discussion

The results of the experiment in table (1) showed the effect of dandelion root extract on the weights of male mice exposed to (CuSO4) poisoning. Copper sulfate poisoning led to a significant decrease P<0.05 in body weight compared to the healthy control group. In comparison to the hazardous group, the dandelion root extract + copper sulfate group likewise produced a much higher P<0.05 rise in weight. This is caused by ingesting a lot of copper sulfate through the mouth. In the case of prolonged exposure, copper induces the generation of free radicals by the Fenton-type reaction and lysosomallipid peroxidation, which results in cell death. Copper ions produce direct stomach discomfort, mimicking nausea and vomiting(25).

Table (1) shows how dandelion root extract affected the body weight of male mice poisoned with copper sulfate.

Groups	body weight		
	Initial body weight	final body weight	Increase in weight
control	20.853±0.381a	19.732±2.112a	0.606±0.715
copper sulfate	20.900±0.781a	14.586±0.875c	6.313±1.236
copper sulfate +dandelion root extract	21.080±0.892a	16.610±0.195b	4.470±1.083

Different letters in the same column indicate significant differences at the level of probability (p≤0.05).

Table (2) shows the effect of dandelion root extract on the weights of the internal organs in male mice exposed to copper sulfate poisoning. There was a significant decrease P<0:05, of weights (liver, kidney, heart and testes) in the treated group copper sulfate compared with the control group and the group of dandelion root extract + copper sulfate. As for the spleen weights, the results of the study showed that the copper sulfate poisoning group showed a significant increase P<0.05 compared with the two control groups and the extract group and Dandelion roots + copper sulfate. However, when high enough Cu levels are taken, chronic overexposure to Cu can lead to liver and kidney damage (26).

Table (2)shows how dandelion root extract affected the organ weight of male mice poisoned with copper sulfate.

Groups	Measured Standards (g)				
	liver	Kidneys	heart	Spleen	testes
Control	1.101 ±0.073a	0.368 ±0.032a	0.125 ±0.006a	0.441 ±0.142b	0.520 ±0.036b
copper sulfate	0.903 ±0.003b	0.246 ±0.036b	0.100 ±0.006b	1.010 ±0.005a	0.400 ±0.005c
copper sulfate + dandelion root extract	1.296 ±0.003a	0.420 ±0.005a	0.136 ±0.003a	0.163 ±0.008b	0.650 ±0.011a

Different letters in the same column indicate significant differences at the level of probability (p≤0.05).

Additionally, table (3)'s results demonstrate how dandelion root extract affected the blood's white blood cells in male mice that had been poisoned with copper sulfate.There was a decrease in the numbers of (WBC, LYM, MON) with a significant level P<0.05 for the copper sulfate group Compared with the control group and there was no significant difference in the group of dandelion root extract + copper sulfate compared with the control group. High exposure to copper can destroy red blood cells, which can lead to anemia (26).

As for the numbers of (PLT, GRA) cells in the dandelion root extract + copper sulfate group, it showed a significant decrease, P<0.05, compared with the control and copper sulfate groups.

Table (3)shows how dandelion root extract affected the quantity and make-up of blood cell structures in male mice who had been poisoned with copper sulfate.

Groups	Parameters				
	WBC 10^9/L	**LYM (%)**	**MON(%)**	**GRAN(%)**	**PLTU/L**
Control	5.2± 0.300a	79.014± 1.088a	11.666± 0.363a	9.2± 1.393b	536± 1.683a
copper sulfate	3.9± 0.577b	75.800± 0.577b	10.500± 0.115b	14.5± 0.577a	542± 1.154a
copper sulfate + dandelion root extract	5.5± 0.230a	80.400± 0.115a	11.800± 0.577a	7.8± 0.577c	535± 1.154b

Significant variations at the level of probability (p≤0.05) are indicated by different letters in the same column.

The results of the study in table (4) of the effect of dandelion root extract on the numbers and components of blood cell structures showed a decrease in HTC, HB, and RBC with a significant level P<0:01 for copper sulfate group compared with the two control groups and a significant decrease in P< 0:05 compared with the group of dandelion root extract + copper sulfate. As for MCV, MCH and MCHC, the results of the study showed a significant decrease in the level P<0:05 for the copper sulfate group compared with the two control groups and it did not show significant differences with the group Dandelion root extract + copper sulfate. The results of the study did not show significant differences for MCH for the group of dandelion root extract + copper sulfate with the control group. High exposure to copper can destroy red blood cells, which can lead to anemia [26].

Table (4): Effect of dandelion root extract on the numbers and hematopoietic components in the blood of male mice infected with copper sulfate poisoning.

Groups	Parameters					
	RBCs 10^{12}/L	HB g/l	HCT %	MCV fl	MCH pg	MCHC g/Dl
control	6.491 ± 0.599a	11.622± 0.622a	38.433± 0.808a	42.877± 0.647a	19.066± 0.66a	40.9± 0.461a
copper sulfate	4.600 ± 0.230c	8.966± 0.317c	36.000± 0.577c	41.5± 0.577b	18.10±0 .577b	39.9± 0.057b
copper sulfate + dandelion root extract	6.200 ±0.230ab	10.900± 0.208b	38.000± 0.577b	42± 0.577b	19.000± 0.577ab	40.6± 0.057b

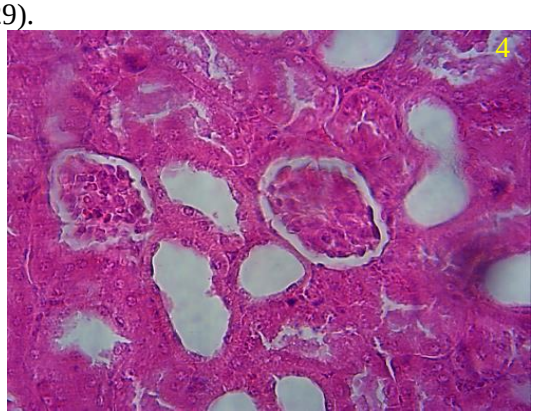
Different letters in the same column indicate significant differences at the level of probability (p≤0.05).

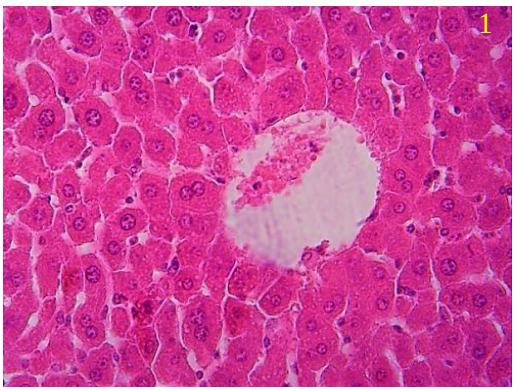
The table displays how dandelion root extract affected the liver enzymes of male mice that had been poisoned with copper sulfate.When copper sulfate was given, a significant increase the level P<0:05in ALT, AST, and ALP values occurred compared to the healthy control group. And when copper sulfate + dandelion root extract was given, a significant decrease in the values of each of ALT and AST ALP occurred compared to the group exposed to copper sulfate poisoning.The concentration of these enzymes has almost returned to the normal concentration level. The decrease in the activity of hepatic enzymes in dandelion root extract after treatment supports the therapeutic effects of the presence of antioxidants in it and repairing the damage and scavenging the resulting free radicals caused by copper sulfate. And Significantly increased enzymes are common indicators of liver injury.

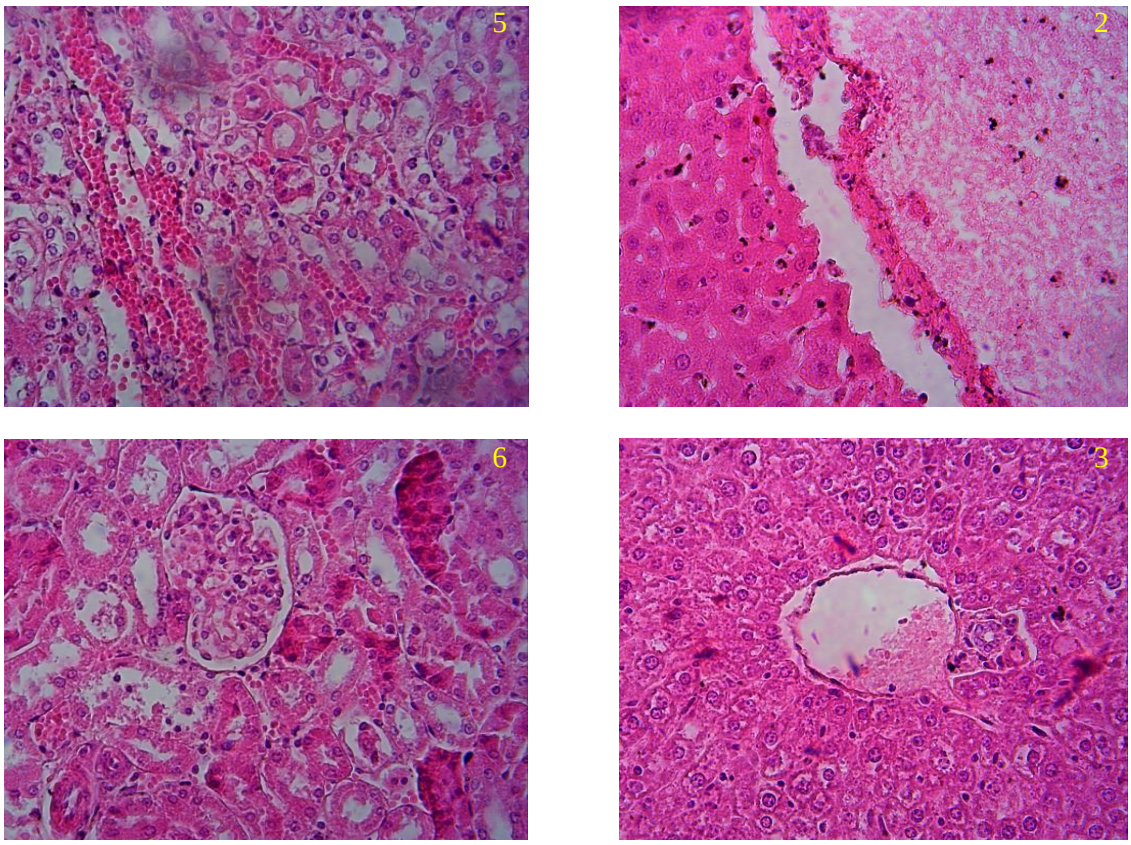
Table (5): Effect of dandelion root extract on liver enzymes of male mice exposed to copper sulfate poisoning

Groups	Parameters (U/L)		
	ALT	AST	ALP
control	20.30± 0.30 a	51.80± 1.88 a	225.97± 2.27 a
copper sulfate	27.03± 1.00 b	79.03± 0.60 b	300.63± 5.51b
copper sulfate +dandelion root extract	35.03± 0.52 ab	61.13± 2.85 ab	260.63±4.37 ab

Significant variations at the level of probability ($p \leq 0.05$)are indicated by different letters in the same column. The analysis of tissue samples taken from the study mice's organs produced the following findings: Fig. 1 A cross-section of a mouse's liver from the control group, displaying the central vein and hepatocytes grouped in the shape of hepatic cords around it, and the blood pockets are clearly observed. Fig. 2 a liver section of the copper-treated group showed congestion and cholecystitis of the liver (COV) and separation of the wall of the central vein of the liver and damage to it (DW).Cu can induce hepatic oxidative damage by suppressing the ability to scavenge free radical and reducing the mRNA levels and activities of antioxidant enzymes, which in turn leads to hepatic lesions, resulting in the activation of the mitochondrial apoptotic pathway and hepatic apoptosis (27).As for Fig. 3 a section of a liver of a group treated with copper and dandelion roots, showing sloughing of the central vein wall (DES), less infiltration of inflammatory cells (IF) and degeneration of some inflammatory cells (D). As for Fig. 4, it shows a section of the kidneys of the control group showing the renal glomerulus (G), the proximal convoluted tubules (PCT) and the distal tubules (DCT). Fig. 5 a section of the kidney of the copper-treated group shows the degeneration of most of the cells of the urinary tubules (D) with a clear hemorrhage (H) within the kidney tissue. The results of the study matched the findings(28).Histological examination of the proliferative parts of the kidneys showed changes and necrosis of the proximal convoluted tubes' epithelial lining, which include epithelial and vitreous molds. These changes are more severe in the higher dose group and may be due to intravascular hemolysis and Cu release which may lead to necrosis of the epithelial lining of the tubules. CuSO4 exposure induces oxidative stress and cascades to trigger the mitochondrial apoptotic pathway, which finally leads to nephrotoxicity in mice (8). Fig. 6 a section of the kidney of the group treated with copper and dandelion roots, showing the renal glomeruli (G) almost naturally with clear hemorrhage (H) within the kidney tissue with necrosis of some cells of the urinary tubules (N) and the inability to distinguish some urinary tubules (UT). The hepatoprotective effects of dandelion root extract are related to its antioxidant activities. Dandelion root extract increased the antioxidant enzymes and ameliorated the liver enzymes, therefore protecting the liver and kidneys against oxidative stress induced by copper sulfate and effective reducing power free radical scavenging effects. Effects of the extract of chicory root to protect the liver and kidneys from copper poisoning may be due to the extract's high antioxidant content (18).Additionally, dandelion roots dissolve gallstones, remove toxins from the liver and kidneys, boost appetite, and stimulate bile flow(29).







Conclusion: Our study showed that exposure to copper sulfate led to changes and negative effects in the weight, blood parameters, liver enzymes, liver and kidney tissues of male rats.The aqueous extract of dandelion roots had positive effects when used as a result of containing a high percentage of antioxidants.

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فعاليةمستخلصجذورالهندباءلتقليلالتسممبكبريتاتالنحاسفيذكورالفئرانالبيض.

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الخلاصة
صممتهذهالدراسةلملاحظةتأثيرمستخلصجذورالهندباءالمائيلتقليلمنسميةكبريتاتالنحاس (بتركيز 40 ملغم/ كغم منوزنالجسم) فيذكورالفئرانالبيضاءلمدة٣٠ يوماً،وتراوحتأوزانهامابين 20 - 23 جم،قسمتالحيواناتعشوائياًإلىثلاثمجموعات،كلمجموعةتحتويعلى 7 فئران. أظهرتنتائجالمجموعةالمعاملةبكبريتاتالنحاس الى

حصولانخفاضًامعنوياًفيوزنالجسمالنهائيووزنالأعضاءالتيتشملكلمنالكبدوالكلىوالقلبوالخصيتين،وكذلكانخفاضكبيرفيمعاييرالدمالتيتشمل WBC ، LYM، MON، RBC، HCB، HCT، MCV، MCH، MCHCوحصولارتفاعمعنويفيقيمانزيماتكلمن ALT, AST, ALP، أمابالنسبةلأنسجةالكبدوالكلى
فقدحدثاحتقانوالتهابوانفصالالوريدالمركزيوتلفهللكبد،أمابالنسبةلأنسجةالكلىفقدأظهرتتنكسمعظمخلاياالأنابيبالبوليةمعنزيفواضحداخلأنسجةالكلىمقارنةبمعاييروأنسجةمجموعةالسيطرةالسليمة. أماالمجموعةالتيأعطيتمستخلضجذرالهندباء + كبريتاتالنحاس،فقدأظهرتنتائجهاتحسنًافيمقاييسالدموانزيماتالكبدوأنسجةالكبدوالكلىمقارنةبمجموعةالتحكمالصحيةوالمجموعةالمعرضةلتسممكبريتاتالنحاس. وخلصتالدراسةإلىأنمستخلصجذرالهندباءلهتاثيراتإيجابيةعلىالحيواناتالتيتعرضتللتسممبكبريتاتالنحاس.