Effect preventive of aqueous extract Portulaca oleracea on haematological and histological changes of liver and kidneys in male mice exposed to copper sulphate poisoning.

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Abstract. The current study was designed to observe and test the preventive for Portulaca oleracea aqueous extract on blood parameters and histological changes of liver and kidneys in in males albino mice that exposed to copper sulphate poisoning (40 mg / kg bw) for 30 days. The animals were divided into (3) groups containing five (5) animals and weighing (20-24 gm). The results of oral administration with copper sulphate showed significant decrease in (P <0.05) in body weight and organ count, which included (kidney, heart, liver and testicles), WBCs, LYM, MON, RBCs, HCB, HCT, MCV, MCH and MCHC. And significantly increased (P <0.05) in the spleen, GRAN, PLT ALP, AST and ALT compared with the control group. The results of the oral dose of Portulaca oleracea + copper sulphate showed a positive effect for these values compared with the group given copper sulphate. The oral administration of copper sulphate causes satisfactory tissue changes in the liver and kidneys of male white rats. The tissue is Destruction (DS) around the central vein (CV) with the pool of inflammatory cells (IF) and the presence of congestion (CON) in the liver and the presence of Haemorrhage (H) Hayline cast (HYC) in some urinary tubules also shows a collection of inflammatory cells (IF) in the kidneys. And treatment with Portulaca oleracea + copper sulphate showed the return of hepatocytes (HC) and central vein (CV) to normal as can be seen Sinusoids (S). The renal Glomerulus (G) and proximal and distal urinary bulb (UT) were normal compared with the copper sulfate control group.

Key word: Portulaca oleracea, copper sulphate, haematological, histological.

Introduction  
Copper has a toxic effect. In spite of being an important biological trace element necessary for different physiological system, is essential as a trace element for metabolic process [1]. Copper is one of the most important heavy metals, importance in medicine and industries [2]. There are two forms of copper toxicity the first form is acute copper toxicity results from ingestion of high copper salts, pesticides, poultry litter and other high copper substance, The
second form is chronic copper toxicity occur when high levels of copper are ingested over period of time [3]. Copper Toxicity is becoming increasingly common these days. It is a condition in which a increase in the copper retention in the kidney occurs. Copper first start depositing in the liver and disrupts the liver’s ability to detoxify elevated copper level in the body thus adversely affect nervous system, adrenal function, connective tissue and reproductive system [4]. Copper in higher concentration is toxic and results in various organ dysfunction [5]. Portulaca oleracea (P. oleracea) belonging to the family Portulacaceae is an herbaceous plant widely distributed throughout the world [6]. used this plant in many countries for a variety of purposes, including human nutrition, and pharmaceutical processing industries [7]. Active ingredients of this plant include maleic acid, Kinamik acid, oxalic acid, caffeic acid, citric acid, coumarin, alkaloid, flavonoids, tannin, alanine, alphalinolenic acid and Glikozoid Mnitropiny [8]. and antioxidants such as A, B1, B2, C, E, beta-carotene, other essential amino acids, minerals are calcium, iron, phosphorus, copper and potassium [9; 10]. It is used to treat many diseases and pharmacological actions such as hepatotprotective, analgesic and anti-inflammatory, wound healing, neuropharmacological, bronchiodilatory, antidiabetic, antioxidant, antihypertensive and many other reported biological actions [11]. The purpose of this study was to investigate the effect of Effect preventive of Portulaca oleracea aquatic extract in male mice exposed to copper sulphate poisoning.

**Materials and methods**

**Collection and preparation of samples:**
Fresh *P. oleracea* plants were harvested from a private fields (Sharqat city, Salah Al-deen Governorate, Iraq), and was diagnosed by specialists. Large quantity of the fresh specimens of *P. oleracea* were washed free of soil and debris, and the Green leaves were manually separated from plant. The leaves were air-dried in the shade and then grinded with a national electric blender (Japan) for a fine powder.

**Extract preparation:**
The aqueous extract was obtained using a method [12], 100 g of *P. oleracea* 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 returned using 100 mL of distilled water and the filtration was returned. The washing and re-filtration process was then repeated, using 50 mL distilled water. Vaporizer display for evaporation using rotary vapor evaporator at 55 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.

**Animals used in the study:** The experimental study was designed comprising of male albino mice of weighing 20-24 gm obtained from the Faculty of Veterinary Medicine - University Tikrit, randomly divided the animals into 3 groups containing each group 5 animals. It was placed in metal cages with metal covers and dimensions (19x25x21cm), with 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 to adapt to the new conditions and to make sure they were free from disease. The animals were fed to the fodder consisting of 35% wheat, 34% yellow
corn, 20% soybean, 10% animal protein, 1% powdered milk and 50g preservatives and antifungal substances ad libitum and in sufficient quantities throughout the breeding and treatment of animals[13]. The distribution of experimental totals was as follows:

1- Group control
2- administered orally copper sulphate (40 mg/kg of b.wt) by gavage daily for period 30 days.
3- administered orally (*P. oleracea* aquatic extract 40mg/100g b.wt a day + copper sulphate 40 mg/kg of b.wt) by gavage daily for period 30 days.

**The obtaining of blood samples:**

At the end of experimental period of (30) days, all the animals were fasted for (10) hours, but still allowed free access to water. The animals It was then weighted and were anesthetized by chloroform and sacrificed by severance of jugular vein. Then take approximately (1.5) ml of blood from each animal, put (0.5) ml from blood in EDTA tubes containing anticoagulant for measuring the most of complete blood counts (CBCs) and other blood cells parameters, concerning the remaining part of blood put in test tubes devoid of anticoagulant, then it lets in water bath for period (15) minutes at 37°C, after this centrifuged for (15) minutes at 3000 rpm, then separate the serum and kept temporarily in (-20) °C in clean plane tubes so that using later for determination of biochemical and physiological tests, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), using several standard solutions (Kits) manufactured by BIOLABO SA, France [12].

Complete blood counts (CBCs) (whole blood analysis).
The haematological analysis of White blood cells (WBCs) count, lymphocyte (LYM), monocyte (MON), granulocyte (GRAN), Red blood cells (RBCs) count, haemoglobin concentration (HGB), haematocrit (Hct), mean cellular volume (MCV), MCH, mean corpuscular haemoglobin (MCHC) and platelets counts (PLT) were determined by using automated haematology analyzer (Syamex model: K-1000, Japan) [14]. In addition to, the most of blood tests asserted by used modified methods [15].

**Histo-physiopathological study:**

Preparation of histological sections, after anesthetized of the animal by chloroform and take the blood from each animal, laparotomy to opened abdominal cavity and chest (sternum) in form (T) inverted shape, then, quickly extirpated the heart, spleen, tasticular, liver and kidneys from each mice, then the weighed, afterwards taken liver and kidneys, divided into (2-3) parts by sharp blade. Then, put in normal saline for washed and removed the adhering fatty and soft tissues, after this excess of water removed by filter paper, then fixed tissue samples by using formalin (10%) for period (18-24) hours. After passing this period, the (liver and kidney) were washed by flowing tap water for half-hour for ridding the parts of organs from fixative solution (formalin), then, carrying out series of processes [16; 17].

**statistical analysis:**

The results were analyzed statistically and using SAS, 2001, according to one-way analysis of variance. The mean of the coefficients were tested using the Duncun multiple rang test at a significant level (0.05) to determine the significant differences between the aggregates [18].
Results and Discussion

Results in table (1) shows the effect of oral dosage of P. oleracea aquatic extract in body weight and organs for mice exposed to copper sulphate poisoning for 30 days. Oral administration with copper sulfate resulted in a significant decrease in final body weight, kidney, heart, liver, testes and significant increase in spleen compared to control group. The oral dose P. oleracea aquatic extract +copper sulphate significantly increased the final body weight, kidney, heart, liver and testicles and significantly reduced spleen compared to the group given copper sulfate.

**Table (1)** Effect administered orally of P. oleracea aquatic extract in body weight and organs weight of albino mice exposed to copper sulphate poisoning.

| Type of transaction | Initial body weight | final body weight | kidneys | heart | liver | spleen | testicular |
|---------------------|---------------------|-------------------|---------|-------|-------|--------|------------|
| control             | 20.58 a             | 28.00 a           | 0.44 a  | 0.14 a| 1.30 a| 0.15 b | 0.51 b     |
| copper sulphate     | 0.52±               | 0.57±             | 0.01±   | 0.05± | 0.11± | 0.05±  | 0.05±      |
| P. oleracea+copper  | 20.90 a             | 14.58 c           | 0.24 b  | 0.10  | 0.90  | 1.01 a | 0.40 c     |
| sulphate            | 0.78±               | 0.87±             | 0.03±   | b     | b     | 0.04±  | 0.05±      |

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

Whereas the table (2) shows the effect of oral dose of P. oleracea aquatic extract in WBC, LYM, MON and GRAN for the blood of mice exposed to copper sulphate poisoning for 30 days. Led reduced significantly in WBC, LYM, MON and significantly increased in GRAN compared to control group. The oral dosage of P. oleracea aquatic extract +copper sulphate significantly increased in WBC, LYM and a significant decrease in GRAN compared to the group given copper sulphate.

**Table (2)** Effect administered orally of P. oleracea aquatic extract in WBC, LYM, MON and GRAN of albino mice exposed to copper sulphate poisoning.

| Type of transaction | Measured Standards | WBC | LYM (%) | MON(%) | GRAN(%) |
|---------------------|--------------------|-----|---------|--------|---------|
| control             | 10^9/L             | 6.20 a | 82.03 a | 12.70 a | 5.30 c  |
| copper sulphate     |                    | 0.23± | 0.57±   | 0.11±  | 1.11±   |
| P. oleracea+copper  |                    | 3.90 b | 75.00 b | 11.60 ab| 14.50 a |
| sulphate            |                    | 0.57± | 0.57±   | 0.11±  | 0.57±   |

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

Also, a results in table (3) shows the effect administered orally of P. oleracea aquatic extract in RBCs, HCB, HCT, MCV, MCH, MCHC and PLT of albino mice exposed to copper sulphate poisoning for 30 days. Led to oral administration with copper sulphate decreased significantly in RBCs, HCB, HCT, MCV, MCH and MCHC and significant increase in PLT compared With control group. The oral dosage of P. oleracea aquatic extract +copper
sulphate significantly increased in RBCs, HCB, HCT, MCH and MCHC and significant decrease in PLT and no significant MCV compared to the group given copper sulphate.

**Table (3)** Effect administered orally of *P. oleracea* aquatic extract in RBCs, HCB, HCT, MCV, MCH, MCHC and PLT of albino mice exposed to copper sulphate poisoning.

| Type of transaction | Measured Standards |
|---------------------|---------------------|
|                     | RBCs $10^9/L$ | HCB g/l | HCT % | MCV fl | MCH pg | MCHC g/Dl | PLT U/L |
| control             | 8.67 ± 0.01 a  | 15.00 a | 41.30 a | 45.13 a | 20.10 a | 42.40 a | 531.00 c  |
| copper sulphate     | 4.60 c         | 8.96 c  | 36.00 c | 41.50 b | 18.10 b | 39.70 b | 542.00 a  |
| *P. oleracea*+copper sulphate | 6.50 b  | 12.16 b | 38.50 b | 43.16 b | 20.10 a | 41.40 a | 531.00 c  |
| control             | 27.42 c        | 45.31 c | 210.1 c | 43.10 a | 1.40 ± 0.04 | 0.17 ± 0.02 | 0.35 ± 0.05 | 0.11 ± 0.02 | 0.57 ± 0.02 |
| copper sulphate     | 292.0 a        | 72.00 a | 342.0 a | 1.24 ± 0.02 | 1.02 ± 0.01 | 1.01 ± 0.01 | 34.31 b   |
| *P. oleracea*+copper sulphate | 259.1 b  | 63.11 b | 34.31 b | 1.35 ± 0.04 | 1.02 ± 0.01 | 1.04 ± 0.01 | 34.31 b   |

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

**Table (4)** Effect of orally administration of *P. oleracea* aquatic extract in the activity of blood enzymes in albino mice exposed to copper sulphate poisoning.

| Type of transaction | IU/L (Measured Standards) |
|---------------------|---------------------------|
|                     | ALP | AST | ALT |
| control             | 210.1 c | 45.31 c | 27.42 c |
| copper sulphate     | 292.0 a | 72.00 a | 43.10 a |
| *P. oleracea*+copper sulphate | 259.1 b  | 63.11 b | 34.31 b |

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

**Histological study:**

Study of the effect on the tissue characteristics of some organs. In this study, several tissue changes were identified in the histological sections taken from the liver and kidneys from the male laboratory mice given the *P. oleracea* aquatic extract given to the copper sulfate for 30 days compared to the sections taken from healthy male mice. The effects varied from one tissue to another and from one region to another. The same fabric as in the following:

A microscopic examination of the tissue sections of the liver showed the animals of the control group, showing a section of the liver having a central vein (C.V) surrounded by Hepatocytes (Hc) arranged in a regular radiograph. Sinusoids (S) are naturally observed as in
(Fig.1). The histological sections of the control group animals (Fig.2) showed that glomerulus (G) and urinary tubules (UT) were intact and naturally.

A microscopic examination of the liver tissue sections of animals treated with copper sulphate showed destruction (DS) around the central vein (CV) with the pool inflammatory cells (IF) and the observation of congestion (CON) (Fig.3). And the microscopic examination of sections of kidney tissues of animals treated with copper sulfate Hemorrhage (H) hemorrhage with hyline (HYC) in some urinary tubules also shows a collection of (IF) inflammatory cells (Fig.4).

While the liver tissue of the treated group of *P. oleracea* aquatic extract+copper sulphate showed the return of hepatocytes (HC) and central vein (CV) to the normal state, as can be seen in blood sinuses (S). (Fig.5). As noted in the tissue of the kidney group treated with *P. oleracea* aquatic extract +copper sulphate showing the Glomerulus (G) and proximal and distal urinary bulb(UT) Normally (Fig.6).

Cause of body weight loss may be due to attributed to increased metabolic costs and reduced food consumption [19]. The decrease in RBC count, Hb and PCV levels observed in this study may be due to the dysfunction of hematopoietic system induced by Copper poisoning [20], or due exposure for copper to the hemolytic effect induced by the release of oxidative stress [21; 22]. copper induced stress, may also be due to blood cell injury and disrupted Hb synthesis or due to impaired intestinal absorption of iron [23].

The reason for the increased enzymes may be Copper poisoning can lead to blood by the development of liver cirrhosis with episodes of hemolysis and progressive liver damage and necrosis which leading to liberation of these enzymes or due to extensive break down of body tissue [24]. Or may also attributed to liver mitochondrial dysfunction due to oxidized state of the effect copper poisoning [25]. Or attributes to the effect of copper on the kidney, liver and heart which consequently liberating their intracellular enzyme in to blood stream due to increase the activity of free [26; 27].

Protective effects for *P. oleracea* aquatic extract against copper sulfate poisoning in albino mice may be anti-inflammatory and antioxidative properties [28]. The active substances that include both the properties antioxidant vitamins α-tocopherol, ascorbic acid and β-carotene, as well as glutathione. *P. oleracea* is considered as a rich source of many amino acids like isoleucine, leucine, cystine, methionine, lysine, phenylalanine, tyrosine, threonine and valine, Omega-3 fatty [29]. And protein , phenols, flavonoids, Radical scavenging activity, iron, manganese, calcium and potassium [30]. In conclusion this study evidences that the ingestion of *Portulaca oleracea* aqueous extract may have a protective effect against copper sulfate poisoning induced oxidative stress in mice which may be related to its Effective antioxidant ingredients.
Figure (1) Histological section of hepatic tissue of Liver control group shows Central vein (CV), Hepatocyte (HC) and Sinusoids (S). H & E 400X.

Figure (2) Histological section of renal tissue of a mice group control group shows the Glomerulus (G) and proximal and distal urinary bulb (UT). H & E 400X.
Figure (3) Histological section of hepatic tissue of a mice treated copper group shows tissue destruction (DS) around the central vein (CV) with a pool of inflammatory cells (IF) and the presence of congestion (CON) H & E 400X.

Figure (4) Histological section of renal tissue of a mice treated with copper show the presence of Haemorrhage (H) with the observation of the hemorrhage with hyline (HYC) in some urinary tubules as shown in the collection of inflammatory cells (IF). H & E 400X.
Figure (5) Histological section of hepatic tissue of a mice treated group of *P. oleracea* aquatic extract +copper sulphate showed the return of hepatocytes (HC) and central vein (CV) to the normal state, as can be seen in blood sinuses (S). H & E 400X.

Figure (6) Histological section of renal tissue of a mice treated with *P. oleracea* aquatic extract +copper sulphate shows the Glomerulus (G) and proximal and distal urinary bulb(UT) Normally. H & E 400X.
References:

[1] ACGIH- (A merican Conference of Governmental in dustrial Hygienists). (1986). Copper. In: Documentation of the threshold Limit Values and Biological Exposure indices, 5th ed. ACGIH, Cincinnati, OH, Pp: 146.

[2] Babaknejad,N; Moshtaghie,A.A. and Shahanipour,K. (2015). The Toxicity of Copper on Serum Parameters Related to Renal Functions in Male Wistar Rats. J Res Med Sci, 15, Pp:29-31.

[3] Meredyth, J. D. and Deon, V.M. (2008). Copper toxicity in sheep is on the rise in Kansas and Nebraska. Comparative Toxicololgy, Kansas. State Veterinary Diagnostiz laboratory.

[4] Ashish,B; Neeti,K and Himanshu, K. (2013). Copper Toxicity: A Comprehensive Study. Research Journal of Recent Sciences, 2, Pp: 58-67.

[5] Kumar,V; Kalita,J; Misra,U.K. and Bora,K.H.(2015). A study of dose response and organ susce ptibility of copper toxicity in a rat model. Journal of Trace Elements in Medicine and Biology, 29, Pp:269-274.

[6] Shehata, M. and Soltan,S. (2012). The Effects of Purslane and Celery on Hypercholesterolemic Mice, World Journal of Dairy & Food Sciences, 7(2), Pp: 212-221.

[7] Rashed, A.N; Afifi, F.U. and Disi,A.M. (2003). Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L. (growing in Jordan) in Mus musculus JVI-1. Jethnopharmacol, 88(2-3), Pp: 131-6.

[8] Mizutani,M; Hashidoko,Y. and Tahara,S. (1988). Factors responsible for inhibiting the motility of zoospores of the phytopathogenic fungus Aphanomyces cochlioides isolated from the non-host plant Portula oleracea. FEBSLett, 438(3), Pp: 236-4.

[9] Simopoulos,A.P; Norman,H.A; Gillaspy,J,E. and Duke,J.A. (1992). Common purslane: a source of omega-3 fatty acids and antioxidants. J Am Coll Nutr, 11(4), Pp: 374-82.

[10] de Lorgeril,M. and Salen,P. (2004). Alpha-linolenic acid and coronary heart disease. Nutr Metab Cardiovasc Dis, 14(3), Pp: 162-9.

[11] Masoodi,M.H; Bahar Ahmad,B; Mir,S.R; Zargar,B.A and, Tabasum,N. (2011). Portulaca oleracea L. A Review. Journal of Pharmacy Research, 4(9), Pp:3044-3048.

[12] Harborne,J.B. (1984). Phytochemical Methods, A guide to modern techniques of plant analysis. 2nd. ed. Chapman and Hail Ltd. , London.

[13] Balducci,R.E: Silviro,K; Gorge,M. and Ganazaga,H. (2001). Effect of isotretior on tooth germ of palate development in mouse embryos. Braz. Dent. J, 12(2), Pp: 115–119.

[14] Haen,P.J. (1995). Principles of hematology. Wm. C. Brown publishers. USA.

[15] WHO. (1989). Preventing and controlling iron deficiency anemia through primary health care.
[16] Reddy, S.B; Reddy, K.K; Naidu, V.G; Madhusudhana, K; Agwane, B.S; Ramakrishna, S. and Diwan, P.V. (2007). Evaluation of antimicrobial, antioxidant and wound healing potential of Holoptelea integrifolia. J Ethnopharmacol, 115, Pp: 249-256.

[17] Suvik, A. and Effendy, A.W. (2012). The use of modified masson’s trichrome staining in collagen evaluation in wound healing study. Mala J Vet Res, 3(1), Pp: 39-47.

[18] Duncan, D.B. (1955). Multiple range and F test. Biometric; Vol 11, Pp: 42.

[19] James, R; Sampath, K; Rani Jeya Mary, S. and Selvamani, P. (2004). Effect of zeolite (sodium aluminosilicate) on the removal of copper from water and fish and an improvement of RNA: DNA ratio in Oreochromis mossambicus, Ecohydrol Hydrobiol, 4, Pp: 57.

[20] Gaharwar, U.S. and Paulraj, R. (2015). "Iron oxide nanoparticles induced oxidative damage in peripheral blood cells of rat," J. Biomedical Science and Engineering, 8, Pp: 274-286.

[21] T. Mocan, T. (2013). "Hemolysis as expression of nanoparticles induced cytotoxicity in red blood cells, “Biotechnology, Molecular Biology and Nano medicine, 1(1), pp: 7-12.

[22] Zook, J.M; Mac CUSPIE, R.I; Locascio, L.E; Halter, M.D. and J. T. Elliott, J.T. (2011). "Stable nanoparticle aggregates/agglomerates of different sizes and the effect of their size on hemolytic cytotoxicity," Nanotoxicology, 5(4), pp. 517-530.

[23] Azarin, H; Reza Imanpour, M and Rajabpour, M. (2012). Effect of Sublethal Levels of Copper Sulfate on Some Hematological Parameters of Rutilus frisii kutum Fingerlings. Global Veterinaria 9 (4), Pp: 479-485.

[24] Linder, M.C. and Hazegh-Azam, M. (1996). Copper biochemistry and molecular biology. Am, Clin Nutr, 63, Pp: 797-811.

[25] Ashish, B; Neeti, K. and Himanshu, K.H. (2012). Copper toxicity: A comprehensive study. Research Journal of Recent Science, 2, Pp: 58-67.

[26] Attia, H.A; Salah, F. and Goha, R.M. (2009). Prolonged administration of high doses of copper nicotinate to rats: effect on biochemical and cellular constituents of blood and on copper level in serum, liver and muscle, inter. J. Med. Sci, 1(5), Pp: 178-183.

[27] Geyvan, P.M. (1991). The effect of heavy metals at different PH on liver enzymes and blood coagulation in Tilapia sparanani (Cichlidae), M.Sc. thesis, R and Afrikaans University. South Africa.

[28] Guoyin, A; Hao, P; Min, L; Wei, G; Zhe, C and Changquan, L. (2017). Antihepatocarcinoma Effect of Portulaca oleracea L. in Mice by PI3K/Akt/mTOR and Nrf2/HO-1/NF-κB Pathway. Evidence-Based Complementary and Alternative Medicine, 11.

[29] Ahangarpour, A; Lamoochi, Z; FathiMoghaddam, H. and Mansouri, S.M. (2016). Int. J. Reprod. Biomed. (Yazd). 14, Pp: 205.

[30] Asma, A. and El Gindy, A.A. (2017). Chemical, technological and biochemical studies of purslane leaves. Curr. Sci. Int, 6(3), Pp: 540-551.