The Protective Effects of Vit C on Experimentally-Exposed Mice to Copper Toxicity

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Abstract

Protective effects of Vit C against copper (Cu) toxicity have been examined in this study. The diagnosis of sub acute copper toxicity in laboratory mice was based on body weight gain, hematological and biochemical parameters and histopathology of liver and kidney .

The experimental groups were a control group, mice treated with 4 mg/kg/day Cu (Cu group) , and mice treated with 4 mg/kg/day Cu plus 60 mg/kg /day Vit C (Cu+VitC group). The groups that were given Cu and Vit C intraperitoneally (ip) injection daily for four weeks.

The results revealed significant decrease in body weight, hemoglobin, PCV, and RBCs count in Cu group, when compared with control and (Cu+VitC) group ($P< 0.05$). While WBCs count was significantly decreased in Cu group as compared with control. But (Cu + VitC) group was not statistical affect as compared with control group.

The Cu group showed a significant increase in the levels of liver enzymes (ALT, AST). However, the Cu + Vit group showed less affected when compared with control group.

Some histopathological changes were found in liver such as: infiltration of mononuclear cells at parenchymal tissues, degeneration and necrosis. The kidney of Cu intoxicated mice show sever acute damage in the glomerulus and proximal tubule, but the Cu + Vit group showed less pathological effect or normal liver and kidney tissues.

In conclusion, sub acute copper administration caused liver and kidney damage, In addition, the treatment with Vit C after the administration of Cu reduced these lesions cause by Cu.

Key words: Vitamin C, Copper toxicity, Liver and Kidney histopathology, Liver enzyme.

Introduction

Copper is one of 26 essential trace elements occurring naturally in plant and animal tissues [1] For many years, it has been accepted that copper is necessary and essential element for living organisms [2]. Copper sulfate ($CuSO_4$) is frequently used in agricultural and viticulture treatments due to its antifungal properties it is present in many fertilizers and pesticides and they can contaminate aquatic ecosystem, and is routinely used as an algaecide commercial and recreational fish ponds. It has also been used as an effective treatment for pathogenic protozoan parasites of fish [3]. Copper may be present in source of water or may enter tap water in distribution system of the individual household [4].

Copper is an essential nutrient and at the same time a toxic element at higher levels for all domestic animals, toxicities can be acute or chronic [5]. Reports of copper intoxication in human most often arise from accidental poisoning or suicide attempts [4,6]. Recently, a study A. Akintowa *et al* [7], indicates that the overload of common copper *in vivo* can induce a set of toxicological activities such as hepatocirrhosis, changes in lipid profile,

oxidative stress, renal dysfunction, and stimulation of mucous membrane of alimentary canal, ect [8].

In humans, the liver is the primary organ of copper induced toxicity [9]. In animals , adverse effects were observed mainly in organs involved in the absorption and excretion of copper (liver, kidney, rodent fore stomach) [10]. Other target organs include bone and the central nervous systems [9]. Bile is the major pathway for the excretion of copper and is vitally important in the control of liver copper levels. [11,12].

Some studies have reported that absorbed Cu especially accumulates in lysosomes of cell at this point lysosomes play the important role in metal homeostasis , storage and in the turnover of cytoplasmic proteins such as metallothioneins, excessive metal accumulation in lysosome disrupts the normal process of lysosomal biogenesis causing impairment of this essential cellular system [13,14].

Antioxidants nutrients such as ascorbic acid, tocopherol , B-carotene etc, are considered to give protection against oxidative damage induced by different toxicants and reduce the activity of free radical-induced reactions [15].

Thus, the aims of the current study were to investigate the effects of toxicity of copper on biochemical parameter (activities of liver enzymes), blood parameters and histopathology of liver and kidney and investigate possible protective effect of combination of vitamin C against copper toxicity in mice.

Materials and methods

1- Chemicals: Cupric sulfate(CuSO4-5H2O) and vitamin C (ascorbic acid) were obtained from the Department of biology , College of Education , University of Basrah

2 –Animals: Twenty one adult male Albino mice Mus musculus L., weighing between 20-25g are used throughout this study. They were housed and maintained in controlled condition animal house (25±2C°) and 10% relative humidity with 12:12 light-dark cycle, food and water were given [16].

3-Treatments: Mice were randomly divided into three experimental groups of seven, and treated as follows: group(1) received physiological saline and considered as a control group, group(2) received copper sulfate 4mg/kg/ 0.1ml (in normal salin) (Cu group), group(3) received copper sulfate 4mg/kg/plus Vit C 6omg/kg/0.1ml (in normal salin) as (Cu+Vit C group). Groups were daily treated intraperitoneally (ip) for one month [17].

4-Biochemical parameters and measurement : At the end of the experimental period, the mice were scarified by cervical decapitation under ether anesthesia and blood samples were collected by direct heart puncture. In addition, livers and kidney were removed for histopathological examination. The blood samples were divided into two portions (1) The blood were collected in clean tubes with few drops of EDTA for complete blood parameters. (2)The blood samples were centrifuged, serum was recovered, and diagnostic Kits which were purchased from Randox were used to determine the serum activity of the Aspartat transaminas (AST), Alanine transaminase (ALT).(Randox kit No,146,147, respectively).

5-Histopathological examinations: Five µm thick paraffin sections of Bouin solution fixed liver and kidney samples

were stained with hematoxylin-eosin (HE) for photo microscopic observations of the liver and kidney histological architecture of the control and treated mice[18].

6-Statistical analysis :The results are expressed as mean ± standard deviation (SD). Differences between control and treated groups were tested for significance using a one-way analysis of variance (ANOVA) P value of <0.05 was considered significant[19].

Results and Discussion

The results of the present study showed that the nutritional status of (Cu group) was poor as compared with control and (Cu+ Vit group),no data recorded. The symptoms of in appetence and apathy were appeared, the body weight gain of mice found in this study are presented in (Table 1), the body weight gain in (Cu group) was significantly decreased as compared with control and (Cu+ Vit C group), no mortality was recorded in the control and (Cu + Vit group), but two mice died in (Cu group).

When (Cu) was given concurrently with Vit C, the decrease in the body weight was significantly less in (Cu +Vit C group) as compared with (Cu group).

Decrease in body weight seen in (Cu group) might be due to the toxic effect of copper which leads to the impairment of total metabolism in the exposed animals due to disturbances in the functional abilities of the liver [20 , 21].

Decreased body weight gain been reported in several studies in rats ingested ≥ 100 mg/ kg/day, in pigs ingested 14.6 mg/ kg/day and in mice consumed 4.2 mg/ kg/day of copper sulfate [4]. Also the growth of rats was retarded when given dietary doses of 25 mg/ kg/ day of copper sulfate, while dietary doses of 200 mg/ kg/day caused loss of appetite and death[20].The present results are in agreement with those found, in these studies.

The hematological and serum biochemical found in this study are presented in (Table 2). Hemoglobin, PCV and RBCs count significantly decreased in the (Cu group), and (Cu +Vit C group) as compared with control, in addition (Cu group) as compared with (Cu +Vit C group)

When (Cu) was given concurrently with Vit C, the caused significant decrease in hemoglobin, PCV, and RBC count was significantly less in(Cu + Vit C group) as compared with (Cu group). While WBCs count significantly decreased in (Cu group) as compared with control. (Cu +Vit C group) showed no statistically change as compared with control.

The evaluation of hematological indices showed that hemoglobin PCV, and RBCs count decreased sharply in (Cu group) as compared with control and (Cu + Vit C groups), suggesting the anemia. These finding are in agreement with those described by [22, 23] in sheep. Anemia can occur in chronic copper poisoning followed hemolytic crisis [24].

Serum biochemical analysis in this study is presented in (Table 2). The (ALT) were significantly increased in the (Cu group) and (Cu +Vit C group) as compared with control. While in (Cu group) were significantly increased as compared with (Cu + Vit Cgroup). However (AST) was not affected significantly in (Cu + Vit C group) as compared with control but was significantly increased in (Cu group) as

compared with control and (Cu + Vit group). AST and ALT activity were increased significantly in (Cu group) due to liver damage.

Ortolani *et al* [4] concluded that increasing (ALT) followed by (AST) are the best enzymes to assess copper load in sheep during the prehemolytic phase. While severe liver damage in chronic copper poisoning could leads to increase in AST activity as suggested by [23], and hepatic damage in rats increased serum (AST) at 7.9 mg/kg/day and necrosis at ≥ 40 mg/kg/day, and in pigs increased (AST) level at 36 mg/kg/day [4]. Prolong administration of 4.6 mg/kg/ for 8 weeks copper nicotinate significantly ($P < 0.05$) increased AST, ALT, Urea, Creatinine, and Cholesterol in serum of rats [24].

AST, ALT activies were not or less effected significantly in (Cu+Vit group) as compared with control due to Vit C which were prevented liver damage.

In the present study the copper toxicity caused histopathological change in liver and kidney tissues. The main change found in the liver are shown in the fig (1).

The histopathological damages seen in liver with the (Cu) treated group dose (4 mg/ kg/day), include severe mononuclear infiltration in parenchyma tissue, focal necrotic area and inflammatory cell infiltration, vein congestion, hepatocytes with irregular shaped nucleus, nuclear degeneration, sinusoidal dilation, and abnormal organization in hepatocytes and polymorphism, these symptoms in Cu group more than in CU + Vit group.

These finding are in agreement with those described by [25, 26] in rainbow-rout fish(*Ocorhynchus mykiss*) after exposed to sub lethal dose of copper sulfate ($CuSO_4$) and Neotropical fish species (*Prochilodus lineatus*) respectively. Copper is one of the essential metals that is transported by metallothionein to the blood circulation after absorption from intestine, skin, inhalation, and some of it accumulate in the different internal organs especially in the liver and kidney, these organs were most associated with detoxification and biotransformation process and due to its function, position and blood supply [27].

It has been known that copper storage begin in the centrilobular hepatocytes, where most of copper is sequestered in hepatic lysosomes [28,29] lysosomal membranes lose integrity as copper accumulates and copper lysosomal hydrolyses are released, irreversibly injuring the cell [28], hepatocellular necrosis and apoptosis occur. The accelerated loss of hepatocytes leads to acute massive copper release causing hemolytic and accumulation of hemoglobin cast in the renal tubules [28, 29].

The main change found in the kidney are shown in the (fig. 2). The kidney of Cu group–intoxicated mice show sever acute damage to glomerulus and proximal tubules. The glomeruli are swollen and dwindle in the lumen of Bowman's corpuscle being signs of glomerulonephritis .The epithelial cells of renal proximal convoluted tubule degeneration and necrobiotic loss of cell – cell contacts were seen in epithelial of proximal convoluted tubule, the cellular glomeruli swelling, and congestion of blood vessels , monocellular infiltration and cloudy swelling degeneration, hyaline droplets degeneration, protein liquid filled in renal tubules. However the (Cu + Vit group) were showed mailed affect or showed similar to those of the control group. Similar findings were also reported by [26, 30,31] on the rainbow – trout fish (*Ouconynchus mykiss*) and on the teleostean kidney respectively.

Moreover the microscopic finding in the kidney of industrial chronic copper poisoning in Iranian fat – Tailed Sheep showed the corpuscle thickening, tubular dilation, regenerated tubules with flattened epithelium glomerulonephritis, brown pigment of lipofuscin in tubular epithelia due to hemolytic epithelium [32] in the same contest damages of renal proximal tubular cells are clearly observed in mice exposed to nano – copper particles [33].

The preventive effect of Vit C on renal damage induced by Arsenic may be associated with its antioxidantion capacity [34] Vit C is the most important hydrophilic Free radical scavenger in extracellular fluids, trapping radicals in the aqueous phase and protecting biomembranes from peroxidative damages [35], in addition to its antioxidant effects, Vit C is involved in regeneration of tocopherol from tocopheroxyl radicals in the membrane. Other finding were also reported by [36] that antioxidants nutrients, as ascorbic acid, tocopherol, B- carotene, etc, are considered to give protection against damage induced by different toxicants and reduce the activity of free radical – induced reaction

In Conclusions, the administration of Vit C in copper intoxicated mice caused a pronounced protective effect to liver and kidney damage. Further study may be needed to achieve its optimal effect by increasing its dose.

Table(1) Body weight gain (gm) in all group (values are mean ± SD) of 7 mice in each group. The mean differences in significant at 0.05 level.

	Control group	(Cu) group	(Cu+Vit) group
Initial body weight (gm)	23.00±2.160	23.571±2.070	23.047±2.036
Final body weight (gm)	23.1429±1.573	$16.7143\pm1.9760^{A\,a}$	21.1429 ± 1.219^{A}

A $p<0.05$ (Cu), (Cu+VitC) groups compared with control

a $p<0.05$ (Cu) group compared with (Cu+VitC) group

Table(2) Hematological indices and activities of liver enzyme in all groups (Values are mean ±SD) of 7 mice in each group. The mean differences is significant at 0.05 level.

	Control group	(Cu) group	(Cu+Vit C) group
Hemoglobin (g/d L)	13.385±.425	$9.142\pm.808^{A\,a}$	$11.571\pm.834^{A}$
PCV (g/L)	40.157±1.97	$29.142\pm4.973^{A\,a}$	33.514 ± 2.083^{A}
RBC×10^6	8.928±1.205	$4.928\pm.672^{A\,a}$	$7.714\pm.909^{A}$
WBC×10^3	4.7±.637	$3.01\pm.348^{a}$	3.928±1.03
AST(U/L)	69.85±3.132	$98.428\pm8.618^{A\,a}$	73.14±5.490
ALT(U/L)	26.142±3.132	$47.571\pm4.577^{A\,a}$	33.85 ± 3.436^{A}

A $p< 0.05$ (cu) (cu+vit) group compared with control.

a $p< 0.05$ (cu) group compared with (cu+vit) group

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التأثير الوقائي لفيتامين (ج) على الفئران المختبرية عند تعرضها للتسمم بالنحاس

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تناولت هذه الدراسة التأثير الوقائي لفيتامين (ج) في التأثير السمي التحت مزمن للنحاس على وزن الجسم، معايير الدم، إنزيمات الكبد والتأثير المرضي النسيجي للكبد والكلية في الفئران المعملية. قسمت حيوانات التجربة إلى ،مجموعة السيطرة حقنت بالمحلول الفسلجي ، مجموعة النحاس حقنت ب4 ملغم/كغم من كبريتات النحاس المائية (CuSO4.H2O) ومجموعة النحاس + فيتامين (ج) حقنت ب[4 ملغم/كغم نحاس+60 ملغم /كغم فتامين (ج)] في منطقة الخلب (p.i) يوميا ولمدة شهر.

أظهرت النتائج وجود انخفاض معنوي في وزن الجسم، ومعايير الدم (الخضاب، كريات الدم الحمر. والبيض، والحجم المضغوط الكريات الدم) في مجموعة النحاس عند مقارنتها بمجموعة السيطرة ومجموعة النحاس + فيتامين (ج). وكان هناك تأثير معنوي اقل في مجموعة النحاس + الفيتامين عند مقارنتها بمجموعة السيطرة .

أشارت الدراسة أيضا بان هناك ارتفاع معنوي في إنزيمات الكبد(AST , ALT) لمصل الدم في مجموعة النحاس عند مقارنتها بمجموعة السيطرة ومجموعة النحاس + الفيتامين . وكان التأثير المعنوي في مجموعة النحاس + الفيتامين اقل عند مقارنتها بمجموعة السيطرة. وأظهرت النتائج تغيرات مرضية نسيجية في نسيج الكبد والكلية منها: ترشيح خلايا التهابية في نسيج الكبد، تنكس و تنخر الخلايا الكبدية . وظهر في الكلية تلف حاد في الكبيبة والأنابيب الجامعة القريبة في مجموعة النحاس عند مقارنتها بمجموعة السيطرة ومجموعة النحاس + الفيتامين . إما الأخيرة لم يظهر تلف واضح في نسج الكبد والكلية .