EFFECT OF VITAMIN B6 TO RELIF COPPER SULFATE TOXICITY ON SOME PHYISOLOGICAL AND HISTOLOGICAL PARAMETERS OF FEMALE RABBITS

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Abstract

The present study was undertaken to investigate the role of vitamin B_6 in the improvement of the copper sulfate toxicity on some hematological parameters, biochemical parameters and histopathological changes in some internal organs of female rabbits. Eighteen female rabbits were divided to three groups(6 rabbit for each group) .The first group treated with normal saline orally daily for10 days (control),the second group treated with 6mg/kg live weight copper sulfate orally, and third group treated with 6mg/kg live weight copper sulfate orally and7mg/kg live weight vitamin B_6 intramuscular injection daily for10 days .The results showed positive effect of vitamin B_6 to improvement the different changes which resulted from the exposure to copper sulfate .There was significant increased PCV, Hb concentration and RBC count in third group when compared with second group .Total WBC count percentage improvement and decrease significantly total cholesterol ,ALT and AST in third group when compared with second group .In addition to that the histopathological changes in liver and kidney were return to the normal value with a little alteration as compared with control group.

تأ ثير ب $_6$ في ازالة التأثيرات السمية لكبريتات النحاس على بعض المعايير الفسلجية والنسيجية في أناث الارانب

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الخلاصة

اجريت الدراسة الحالية لمعرفة تأثير فيتامين ب$_6$ في تحسين التاثيرات السمية لكبريتات النحاس لبعض الاختبارات الدموية والكيماوية والتغيرات النسيجية في بعض الاعضاءالداخلية لأناث الارانب. استخدم (18) أنثى أرنب وبشكل متساوي الى ثلاثة مجاميع(6 حيوانات في كل مجموعة) المجموعة الاولى اعطيت المحلول الفسلجي عن طريق الفم (سيطرة) المجموعة الثانية اعطيت 6 ملغم/ كغم وزن حي من كبريتات النحاس عن طريق الفم ولمدة 10 ايام المجموعة الثالثة اعطيت كبريتات النحاس بالأضافة 7ملغم /كغم وزن حي من فيتامين **ب** $_6$حقن في العضلة يوميا لمدة 10 أيام.واشارت النتائج الى تحسن ملحوظ في التغيرات المختلفة نتيجة التعرض لكبريتات النحاس حيث وجدت زيادة معنوية

في كل من تركيز خضاب الدم وحجم خلايا الدم المضغوط وعدد كريات الدم الحمر مع وجود انخفاض معنوي في كل من عدد كريات الدم البيض الكلي وتركيز الكوليسترول الكلي وانزيمي الكبد AST وALT مقارنة مع المجموعة الثانية المعاملة بكبريتات النحاس. وحدوث تحسن ملحوظ في التغيرات النسيجية لكل من الكبد والكلية مع وجود تغيرات طفيفة عند مقارنتها مع مجموعة السيطرة.

Introduction

Vitamin B_6 is involved in many of metabolic reactions, such as metabolism of amino acid and proteins, lipids, carbohydrate, nucleotide, protein synthesis and cellular proliferation(1). Pyridoxal 5-phosphate (PLP) the active form of Vitamin B_6 is essential as cofactor metabolism of homocystein to the amino acid ,cystein (2) addition, the antoxidation properties of Vitamin B_6 have recently been discovered (3,4).

Copper is classified as a noble metal and occurs naturally in element form and as a component of many minerals (5).Copper is essential as a trace element for metabolic process. In spite of being an important biological trace element necessary for different physiological system copper has a toxic effect (6). 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 .Acute copper poisoning can occur at copper intake of 20-100mg/kg live weight in sheep and young calves 200-800mg/kg in adult cattle. The second form is chronic copper toxicity occur when high levels of copper are ingested over period of time (7).Copper sulfate of the most available salt of copper is a blue and odorless salt that is employed in various product such as fungicides, heisrbicides and insecticides(5,8).The copper sulfate ($cuso_4$) is strong irritant the usual routes by which human receive toxic exposure to copper in the form of copper sulfate are through skin or eye contact as well as by inhalation of powder and dusts(9).Copper sulfate has adverse range of usage as fungicides, algaecide, herbicide as well as molluscide. There are some case reports of death due to copper sulfate containing emetics, from china (8).Therefore, now days it use as an emetic is contraindicated (9).The purpose of this study was to investigate the effect of vitamin B_6 to remove copper sulfate toxicity on some hematological parameters, biochemical parameters and histopathological changing in liver and kidney.

Material and methods

Eighteen healthy female domestic rabbits (*LEPUS CUNICULUS*) were brought from the local markets/Basra weighting (1300-1800) grams and age between (8-12) months .The rabbits were kept under observation for a week in an animal house of the college of Veterinary Medicine /University of Basra for one week. The animals were offered a balanced rabbits diet that consists of green leaves, fodder and water *ad libitum.*

The animals were divided into: three groups (6 rabbit for each group).Group 1: normal rabbits were served as control which was treated with 2ml of normal saline orally daily. Group 2: the animals were treated with 6mg/kg body weight copper sulfate (BDH chemical

Co. British, England) orally daily for 10 days and group 3: the animals were treated with 6mg/kg body weight copper sulfate orally and vitamin B_6 (china, MEHECO Corporation, Beijing) 7mg/kg body weight i.m injection daily for 10 days. All the rabbits were killed on the eleven day.

Blood Collection

At end of experiment the blood sample were collected from heart by using syringe (2ml) and then the blood sample was centrifuged to isolate blood serum to estimate the biochemical measurement .Other(1ml) blood samples were collected into tubes with anticoagulant(Ethylene Diamine Tetra Acetic acid) which were used for hematological study.

Hematology analyses

Packed cell volume (PCV) was determined by spinning about 75µl of each blood sample in heparinised capillary tube in hematocrit centrifuge for 5 minutes and read on hematocrit reader as described by (10). The hemoglobin (Hb) concentration was determined by using Sahli method (11).While red blood cells (RCB) and white blood cells (WBC) counts were determined by using hemocytometer method as described by (12).

Biochemical measurement

The total cholesterol was determined by using commercial kits(Spinreact/CHOD-POD, Spain).While the serum enzymes Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST)were obtained using kit(Biomagherb/GOD-PAP, Tunsia).

Histological techniques

After the blood collection, the organ samples removed and fixed in 10% buffered formalin, dehydrated progressively in increased ethanol concentrations, treated with xylene and embedded in paraffin. Five micron thickness sections cutting routinely prepared using a microtome, and then tissue were mounted on glass slides and stained with Heamatoxylin and Eosin stain (13).

Statistical analysis

The results were analyzed by using two –way covariance (ANOVA) test. The data were expressed as mean ± stander deviation. Lest significant different test (LSD) was calculated to test difference between means (groups) for (ANOVA) SPSS 1998.

Results and discussion

The results showed that treated group with copper sulfate at dose (6mg/kg) daily for 10 days caused a significant decrease($P \leq 0.05$) in red blood cells count, packed cell volume and hemoglobin concentration. While white blood cells count increase significantly ($P \leq 0.05$) compared with control group (table 1.) .The results are in agreement with (7), who found that

copper sulfate causes oxidative injury to hemoglobin, inducing Hinze –body formation and converting it to methemoglobin ,which cannot bind O_2 or CO_2 .The sulfadryl groups of RBC membrane also undergo oxidative change ,resulting in significant hemolysis. (14) showed that copper inhibits the function of glucose -6 phosphat dehydrogenease in the RBC. This enzyme is necessary for protection of RBCs against the hemolytic effects of oxidizing. On the other hand, (15) attributed the increment in WBC due to the inflammatory processes in tissue of somebody organs. The female rabbits treated with copper sulfate and vitamin B_6 showed significant improving ($P \leq 0.05$) in RBC, PCV, and Hb compared with animals treated with copper sulfate only while WBC decrease significantly ($P \leq 0.05$) compared with animals treated with copper sulfate only, these results consistent with (16, 17).

Vitamin B_6 is an antioxidant potential is currently associated with an inhibitory effect on lipid peroxidation caused by free radicals derived principally from oxygen. When these radical reacted with biological molecules, caused biochemical damage at cellular level (18, 19).

Table (1): Effect of vitamin B_6 to relief copper sulfate toxicity in some hematological parameter of rabbits

PARAMETERS/GROUPS	RBC count 10^6 cell/ml	PCV %	Hb gm/ml	WBC count 10^3cell/ml
CONTROL	5.04 ±0.22 a	37.83 ±0.752 a	11.33 ±1.032 a	4.21 ±0.521 b
TREATED CUSO4	3.73 ±0.571 b	28.83 ±3.311 c	7.85 ±1.020 b	6.35 ±0.446 a
TREATED Vit.B6	4.26 ±0.452 b	33.5 ±1.378 b	10.92 ± 0.801 a	5.06 ±0.744 b
LSD	0.541	2.604	1.178	0.716

Values are expressed as mean ± stander deviation. Small letters denote differences between groups, ($P \leq 0.05$) vs. control.

The results of total cholesterol concentration, alanin transferase(ALT) and asperatet transferase(AST) in group of animals treated with copper sulfate increased significantly ($P \leq 0.05$) in comparison with control group (table-2). These findings are similar to that of (17, 20). Copper sulfate cause extensive tissue and hepatocyte destruction thereby liberating these enzymes into the serum due to increase the activity of free radicals (21, 22).Whereas the effect of vitamin B_6 able to decrease significantly ($P \leq 0.05$) cholesterol concentration, ALT and AST compared with treated group with copper sulfate. This finding consist with (23) who demonstrated that vitamin B_6 compound can prevent the oxygen radical generation on lipid peroxidation caused by hydrogen peroxide and that some of the protective effect of vitamin B_6 may occur via modification of mitochondria function .

Other studies showed that vitamin B_6 is closely related with the permeability of the cellular membranes and the secondary interaction takes place between the intracellular Krebs cycle and cholesterol levels. In other words, the vitamin B_6 may lead to increased Krebs cycle activity by inhibiting cholesterol synthesis (24, 25, and 26). Moreover, (27) proposed that PLP active form of vitamin B_6 is essential as cofactor of transaminase enzymes in liver.

Table (2): Effect of vitamin B_6 to relief copper sulfate toxicity in some biochemical parameter of female rabbits.

PARAMETERS/GROUPS	CHOLESTEROL concentration mg/dl	ALT concentration µl	AST concentration µl
CONTROL	109.2 ± 40965 b	50.5 ± 18.185 b	32.2 ± 6.112 b
TREATED CUSO4	176.3 ±40.123 a	75.7 ±14.207 a	50.3 ±11.656 a
TREATED VitB6	132.2 ±20.133 b	51.2 ±12.188 b	45.2 ±9.453 b
LSD	32.9	18.54	11.51

Values are expressed as mean ± stander deviation. Small letters denote differences between groups, ($P \leq 0.05$) vs. control.

The results of histopathological examination of liver and kidney tissue of animals treated with copper sulfate showed that the hepatocytes are swelling and vacuolated, the central vein dilution with aggregation of inflammatory cells pre-central vein (figure-2) compared with control group (figure-1). The kidney showed hemorrhage area with degeneration of renal tubules cells (figure-5) as compared with control animals (figure-4). These results agreement with (26, 27) who noted that copper is strong oxidizing agent. It binds to proteins in the liver and kidney cells and is stored in lysosomes with in hepatocyte and renal tubules cells ,and then cause degenerative changes in tissue cells .While the liver of animals treated with copper sulfate and vitamin B_6 appear more or less as normal with small area of swelling hepatocytes (figure-3),the kidney showed decrease in hemorrhage area, nucleus of cells lining are dances some of them are normal (figure-6) as camper with copper sulfate treated animals. Because vitamin B_6 is antioxidant and have an inhibitory effect on lipid peroxidation which prevent oxidative change in kupffer cells and renal tubules cells (28).

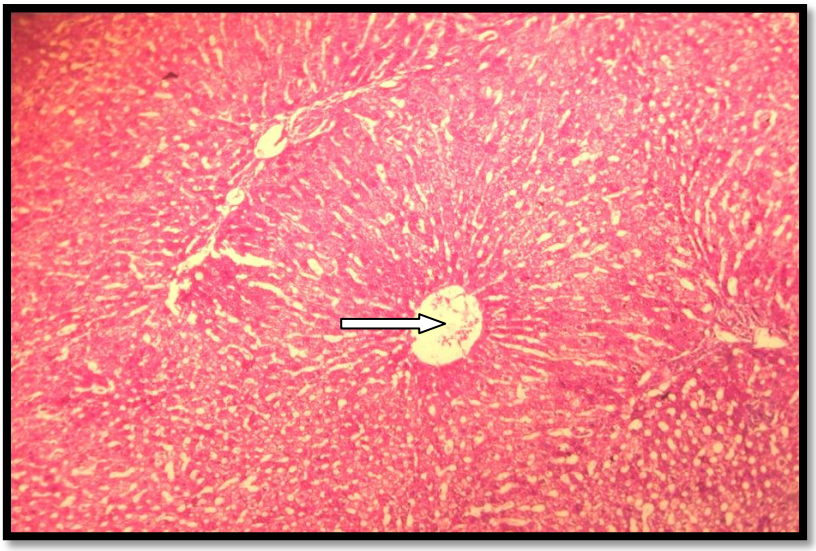

Figure (1) Normal liver of rabbit stained with H&E X10. The pointer indicates normal central vein.

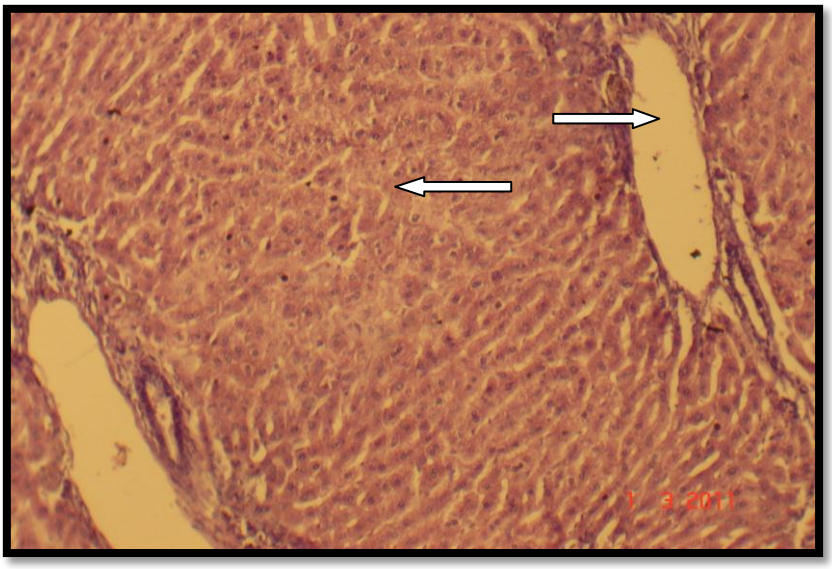

Figure (2) Effect of copper sulfate on liver stained with H&E X 10. The pointer indicates swelling and vacuolated of hepatocytes, dilatation of the central vein with aggregation of inflammatory cells pre-central vein.

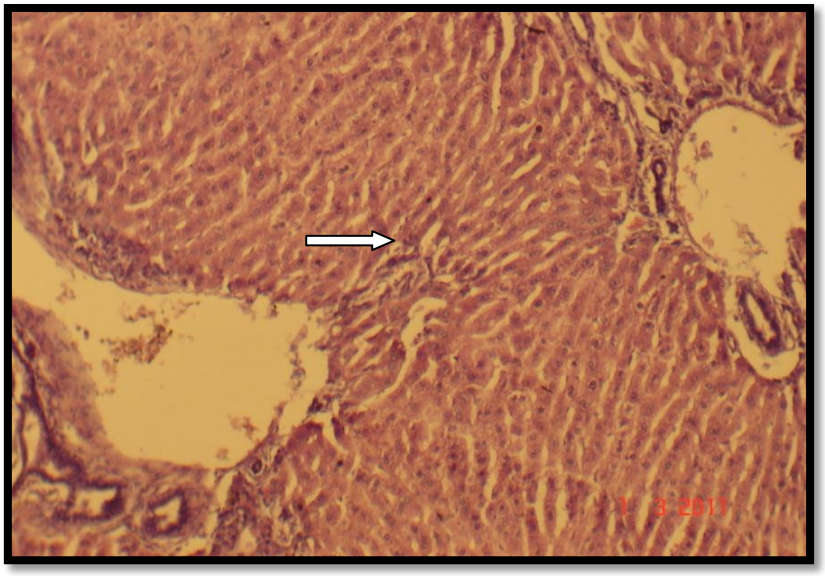

Figure (3) Effect of vitamin B_6 to relief copper sulfate toxicity in liver stained with H&E X10 The pointer indicates the liver more or less as normal with small area of swelling hepatocytes .

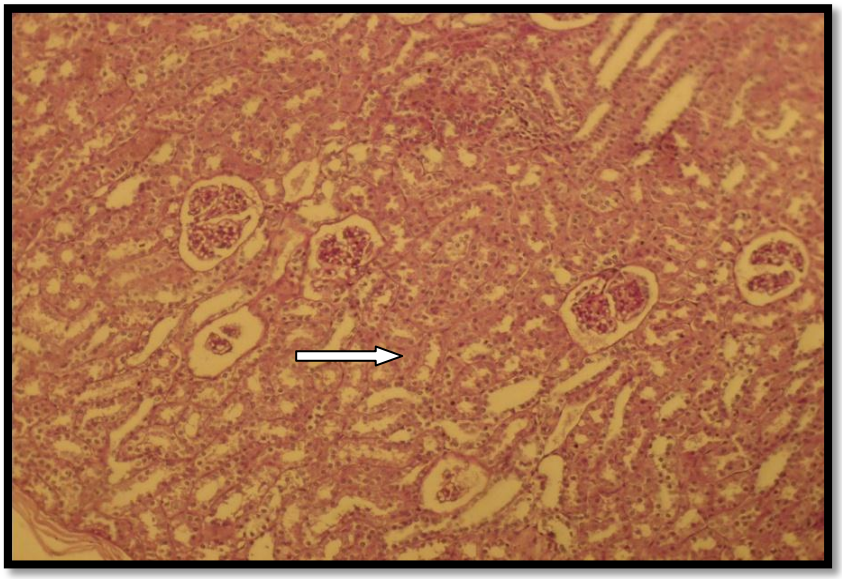

Figure (4) Normal kidney of rabbit stained with H&E X10.The pointer indicates renal tubules cells

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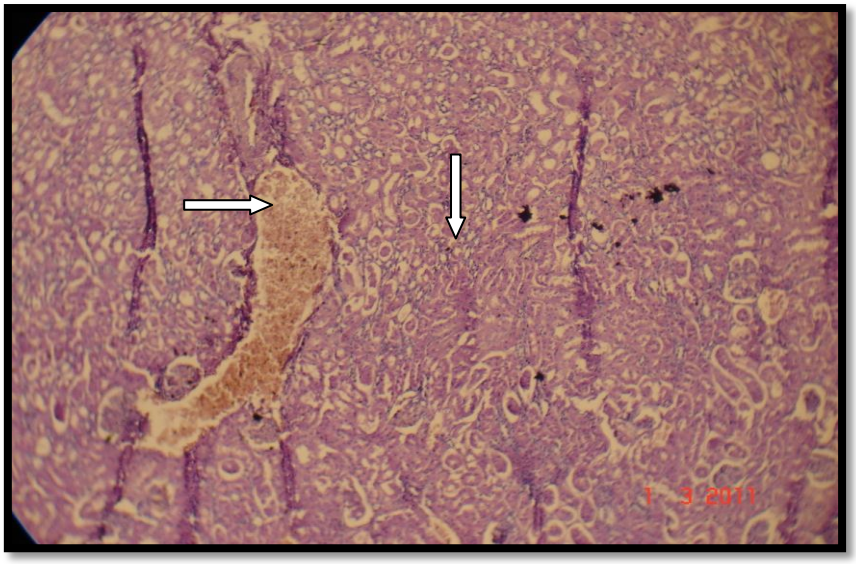

Figure (5) Effect of copper sulfate on kidney stained with H&E X 10. The pointer indicates hemorrhage area with degeneration of renal tubules cells

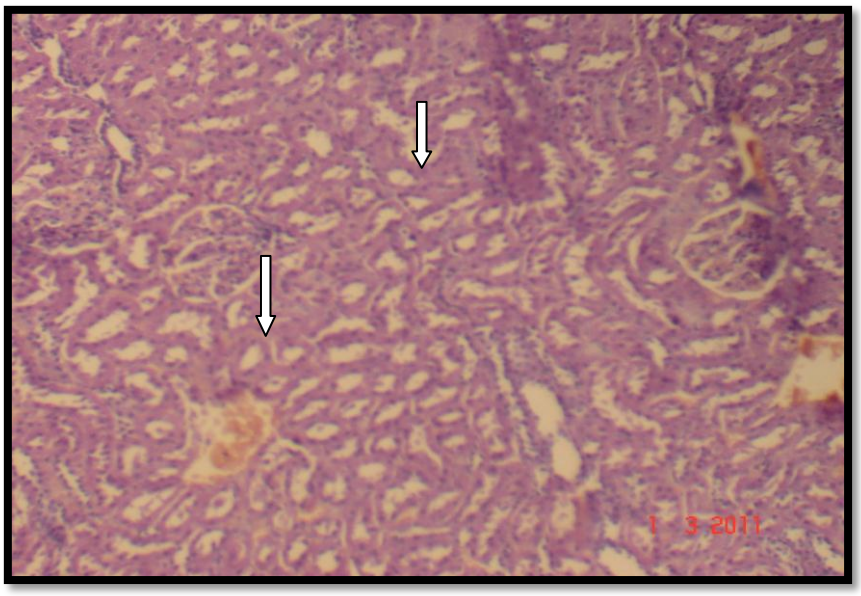

Figure (6) Effect of vitamin B_6 to relief copper sulfate toxicity in kidney stained with H&E X10. The pointer indicates decrease in hemorrhage area and nucleus of cells lining are dances some of them are normal

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