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The Antidotal Effect Of Ascorbic Acid And Zinc Sulfate On Cadmium Toxicity In Male Albino Rats

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ABSTRACT

Fourteen male albino rats weighing 100-150 gm were divided into eight equal groups. The first group was kept as negative control. Group II was supplied with ascorbic acid at a concentration of 1 gm/l in drinking water. Group III was supplied with zinc sulfate at a concentration of 50 mg/l drinking water. Group IV was supplied with both ascorbic acid and zinc sulfate in drinking water as groups II & III. Group V received a single s/c injection of cadmium chloride at a dose of 20 mg/kg.B.W. Group VI received cadmium chloride injection as group V plus ascorbic acid as group II. Group VII take cadmium chloride plus zinc sulfate (the same doses as groups V& III). Group VIII was treated as group IV & V (cdcl2, ascorbic acid & zinc sulfate). The samples were taken after one week. The results revealed that cadmium elevated the serum levels of ALT (two folds increase), AST (4 folds increases), creatinine and urea and lowered calcium concentration in bones. The use of ascorbic acid and zinc sulfate lowered the serum levels of creatinine and urea and elevated calcium concentration in bones, while AST was still elevated without being affected by the use of ascorbic acid and zinc sulfate compared to positive control groups

INTRODUCTION

Cadmium is a wide-spread environmental pollutant. It is present in many types of food coming from soil contaminated with sewage sludge, super phosphate fertilizers. It is used in different industries as in plating material in food processing plants, battery electrodes, semiconductors, copper alloys, stabilizers in rubber and plastics. Cadmium used in industry finds its way in many water supplies.

When cadmium is taken up, it finds its way from the plasma to the red blood cells *(1)*. It is initially taken up by the liver where it binds with glutathione and can be excreted into the bile *(2)*. Some cadmium bound to metallothionein (MT), leaks into the plasma and is taken up by the kidney and is considered a potent nephrotoxicant *(3)*. Cadmium metallothionin is slowly released in the plasma, filtered through glomeruli and reabsorped in the proximal tubules *(4)*.

The severity of cadmium exposure is that it can produce pathological effects from a single dose and it perferentially binds to metallothionien in tissues 24 hours after cadmium chloride injection *(5)*. A decline in glomerular filtration rate in rats treated parentrally with cadmium chloride was reported *(6)* and on continued exposure, a progressive glomerular sclerosis was found.

Cadmium may also affect bone structure and blood calcium *(5)*. Cadmium in drinking water in rats fed normal diet resulted in decalcification and cortical atrophy in the skeleton. Moreover, cadmium may cause bone demineralization and decrease bone density and increase the risk of fracture *(6)*.

Several studies indicated that cadmium induced lethality can be prevented by pretreatment with antioxidant L-ascorbic acid without modifying the toxikinetics of

cadmium or markedly stimulating MT synthesis *(8)*.

Because cadmium has a similarity to zinc and its toxic effect in the body can be controlled by zinc *(9)*, zinc has been proposed to compete with cadmium for the uptake. Pretreatment of animals with zinc may protect against cadmium – induced lethality and hepatotoxicity *(10)* by induction of metallothionein. Metallothionin mediated hepatoprotection is due to the high affinity sequestration of cadmium by MT in the cytosol, thus reducing the amount of cadmium available to injure other critical organelles *(2)*.

The aim of the present study was to ameliorate the toxic affect of cadmium on the different tissues as well as liver and kidney enzymes in the serum by the use of an antioxidant, L-ascorbic acid and zinc sulfate as a cadmium competitive element.

MATERIAL AND METHODS

(1) **Animals:** Eight groups of male albino rats each of 5 rats weighing 100 – 150 grams were purchased form laboratory animal unit in the faculty of veterinary medicine Zagazig University. They were housed in stainless steel cages and maintained in natural daily light (12 h light /12 h dark) and temperature (22°c ± 2°c) during April and May 2007. Food and water were available *ad libitum*. The animals were randomly assigned into eight groups (Table 1):

1- **Group I:** control group supplied with normal drinking tap water and normal diet.

2- **Group II:** supplied with 1gm ascorbic acid (chemical industries development Giza) /l of drinking water *(8)*.

3- **Group III:** supplied with zinc sulfate (Elgomhuria com) in the drinking water at a concentration of 50 mg/l.

4- **Group IV:** supplied with 1gm ascorbic acid /l of drinking water and 50 mg zinc sulfate /l of drinking water.

5- **Group V:** each animal received a single s/c injection of $CdcL_2$ (Prolabo-Rhone Poulenc) at a dose of 20mg/kg body weight *(4)*.

6- **Group VI:** the animal received single injections of $CdcL_2$ at a dose of 20mg/kg body weight 1gm and ascorbic acid/L of the drinking water.

7- **Group VII:** the animal received single s/c injections of $CdcL_2$ at a dose of 20mg/kg body weight and zinc sulfate in drinking water, (50mg/l).

8- **Group VIII**: each animal received single s/c injection of $CdcL_2$ at a dose of 20mg/kg body weight and supplied with ascorbic acid and zinc sulfate in the drinking water at a dose of 1gm/l.and 50 mg/l respectively.

(2) **Samples** from blood and bone of animals from the different group were taken after one week from the beginning of the experiment. Blood samples were collected from slaughtered animals without anticoagulant and the sera were separated and stored at -20°c for analysis. Bone samples from shaft of long bones were collected from each group cleaned wrapped in aluminum foil and stored until analysis was carried out. Cadmium was extracted from bone *(11)* and was determined in the serum and calcium in bone by atomic absorption

(3) **Biochemical analysis**: biochemical tests for serum creatinine were performed *(12)*, blood urea nitrogen *(13)*, total protein *(14)*, albumin *(15)*, serum transaminases, AST-ALT *(16)*.

(4) Histological examination. Tissues of liver and kidney were fixed in 10% neutral formalin and stained by H&E *(17)*.

(5) Statistical Analysis: MSTAT-C Computer program and f-test were used to compare the different groups.

Table 1. Experimental design of the animals subjected to cadmium chloride toxicity and treated with ascorbic acid and zinc sulfate

Group	CdcL2 20mg/kg B.W	Ascorbic acid lg/l water	Zinc sulfate 50mg/l water
I (control)	-	-	-
II	-	+	-
III	-	-	+
IV	-	+	+
V	+	-	-
VI	+	+	-
VII	+	-	+
VIII	+	+	+

RESULTS AND DISCUSSION

Table 2. The effect of intoxication of male rats with cadmium chloride and the treatment with ascorbic acid and zinc sulfate on serum parameters and calcium in bone

Parameter / Group	In serum							Calcium in mmol./gm dry bone
	Total protein gm/dl	Albumin gm/dl	sALT u/l	sAST u/l	Creatinine gm/dl	Urea gm/dl	Cd ppm	
I. Control	ab 7.6 ± 0.2	3.7 ± 0.11	D 37 ±2.8	B 41.33 ±4.3	C 1.03 ±0.079	C 42.3 ±3.8	b 0.06 ±0.028	b 8.430 ±0.230
II Ascorbic	b 7.403 ±0.2	3.52 ±0.15	C 42.66 ±4.33	b 45 ±8.5	C 0.95 ±0.035	bc 47 ±1.73	b 0.055 ±0.021	b 8.601 ±0.235
III Zinc	ab 7.68 ±0.37	3.43 ±0.2	C 41.66 ±6.69	b 47 ±3.05	C 0.906 ±0.064	C 41.3 ±3.28	b 0.057 ±0.005	b 8.412 ±0.253
IV Zinc+ Ascorbic acid	ab 7.593 ±0.19	3.35 ±0.2	C 42.66 ±3.64	b 46 ±4.72	C 0.94 ±0.13	C 39.3 ±3.52	b 0.0735 ±0.01	b 8.405 ±0.020
V Cadmium	ab 7.97 ±0.4	n.s. 3.8 ±0.18	a 83.33 ±6	a 260 ±28.21	a 1.77 ±0.107	a 80.66 ±7.8	a 0.476 ±0.28	a 6.310 ±0.250
VI Cadmium+ Ascorbic acid	ab 8.15 ±0.19	3.68 ±0.33	ab 68.6 ±3.4	a 252.6 ±48.7	b 1.38 ±0.08	b 60.6 ±5.69	b 0.065 ±0.0038	b 8.451 ±0.351
VII Cadmium + zinc	a 8.31 ±0.16	3.69 ±0.21	ab 72.33 ±5.36	a 283.3 ±26.4	C 0.946 ±0.101	C 38.3 ±1.66	b 0.069 ±0.0026	b 8.415 ±0.310
VIII Cadmium+ Ascorbic acid + Zinc	a 8.38 ±0.22	3.69 ±0.37	b 63.33 ±6	a 270.66 ±63.7	bc 1.14 ±0.18	bc 49.6 ±4.37	b 0.056 ±0.004	b 8.410 ±0.255

n.s. = non significant
Means followed by different letters were significantly different at $p < 0.05$

The obtained results in Table (2) revealed that cadmium administration at a dose of 20mg/kg B.W led to a significant increase in cadmium level in blood compared to negative control group. The use of ascorbic acid and zinc sulfate resulted in lowering the cadmium level in blood to nearly the control levels.

The results also showed significant increase in ALT, AST, creatinine and urea levels without affecting the total protein or albumin as compared to negative control group.

In the present experiment cadmium produced alteration in serum enzymes while the use of ascorbic acid and zinc may protect the animals from Cd-induced toxicity. Ascorbic acid has been used as antioxidant and in the present experiment it returned the serum levels of AST, ALT, creatinine and urea to the normal levels as controls. Similarly, it has been reported that the use of ascorbic acid prevent cadmium induced lethality and hepatocellular necrosis induced by cadmium in male fisher rats *(8)*. This may be explained by the fact that cadmium causes depletion of glutathione and protein-bound sulfhydryle groups resulting in production of reactive oxygen species as peroxide ion, hydrogen peroxide and hydroxyle radical *(18)* and ascorbic acid participates in the detoxication of these compounds and in the maintenance of cytochrome P450 content in the liver *(19)*

As cadmium and zinc are metabolically competitive, cadmium may replace zinc in a number of metalloenzymes, proteins and ion channels *(9)*. The protective effect of zinc against cd^{+2} toxicity may occur by induction of metallothionein and the hepatoprotection of metallothionein is due to high sequestration of cadmium by MT in the cytosol, thus reducing the amount of cadmium which can injure the cell organelles *(20)*.

A proposed mechanism for the decreased calcium absorption and negative calcium balance seen in rats exposed to cadmium is that this metal inhibits activation of vitamin D in the renal cortex *(21)*. Cadmium inhibits vitamin D- stimulated intestinal calcium transport in rats *(22)*.

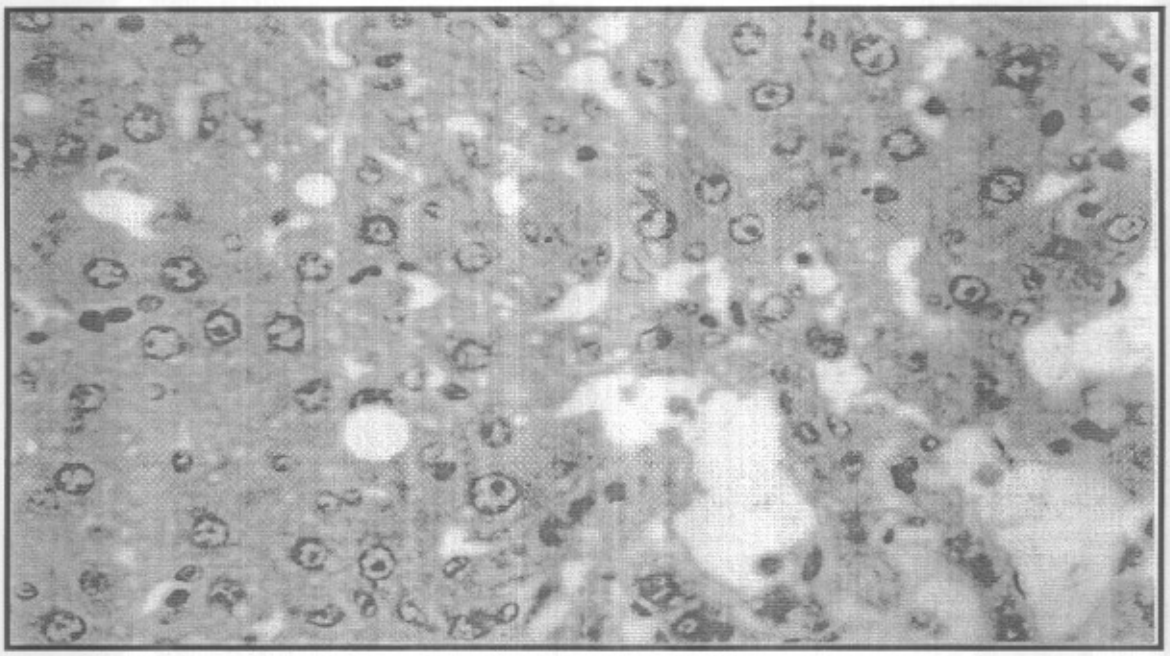
Cadmium was reported to inhibit the renal conversion of 25 – hydroxy calciferol to 1, 25-dihydroxy calciferol in rats when dietary calcium concentration is low *(23)*.

The same condition of calcium deficiency was produced by oral administration of cadmium *(24)* in which decalcification and cortical atrophy in the skeleton was recorded .This decalcification may be due to reduced absorption of calcium and phosphorus from the intestine *(25)*. Moreover, administration of cadmium to beagle dogs was proved to reduce the bone turn over rate, consistent with calcium deficiency and osteomalacia *(26)*. All the previous studies *(21-26)* explained the proposed mechanism for decreasing calcium concentration in bone of group V and supported by its increased level in groups VI, VII and VIII due to the effect of zinc sulfate and ascorbic acid.

The histopathological findings in the present experiment confirm the previous findings *(27)* who reported cloudy swelling of renal tubular epithelium. Further more a decline in the glomerular filtration rate in rats treated parentrally with cadmium chloride was reported and on continued exposure a progressive glomerular sclerosis and impairment of glomerular filtration were produced *(6)*.

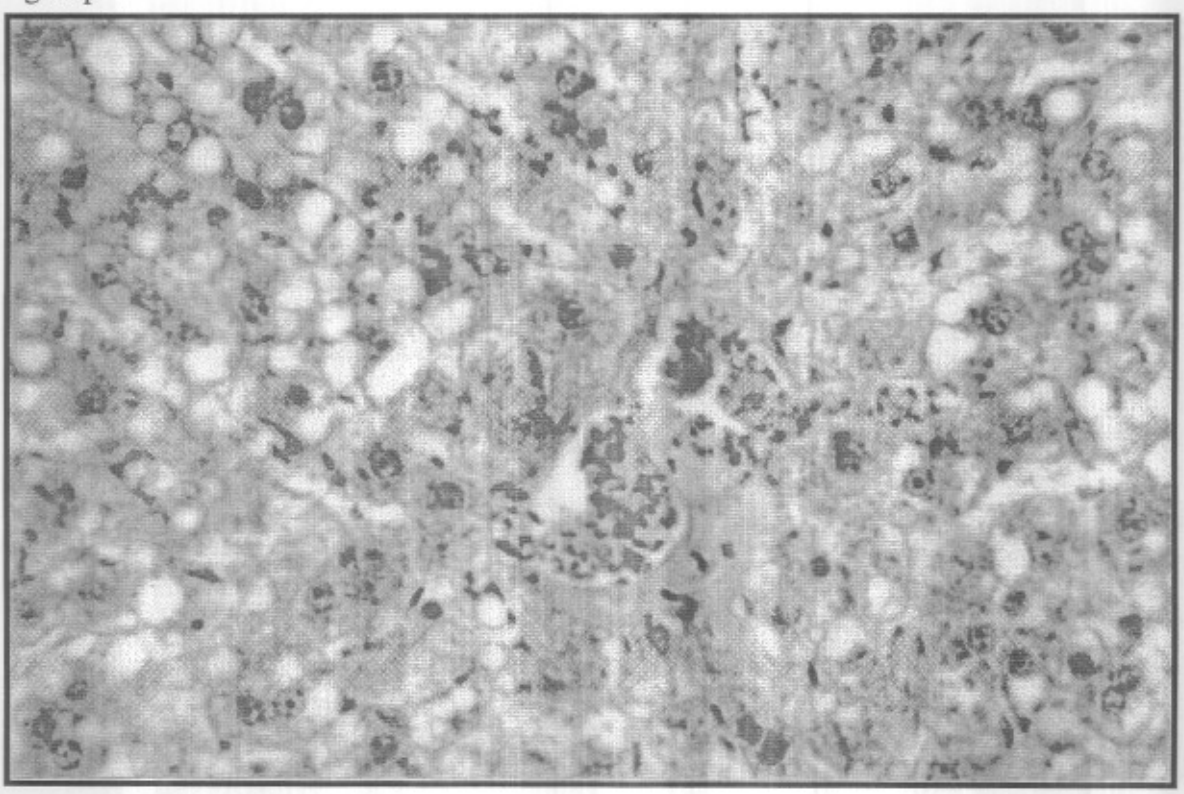
The cytotoxicity of cadmium to proximal tubule cells was recently explained *(3, 28)*. Once cadmium metallothionein has been endocytosed, it is transported to the lysosomes where metallothionein-1 moity is degraded by acid protease and the release of free cytosolic Cd^{+2} leads to mitochondrial damage and cell death.

Other pathological changes in the kidney also reported increased number of lysozyme and swelling of mitochondria in addition to pathological changes in mesangial cells and increased thickening of the glomerular basement membrane *(5)*.

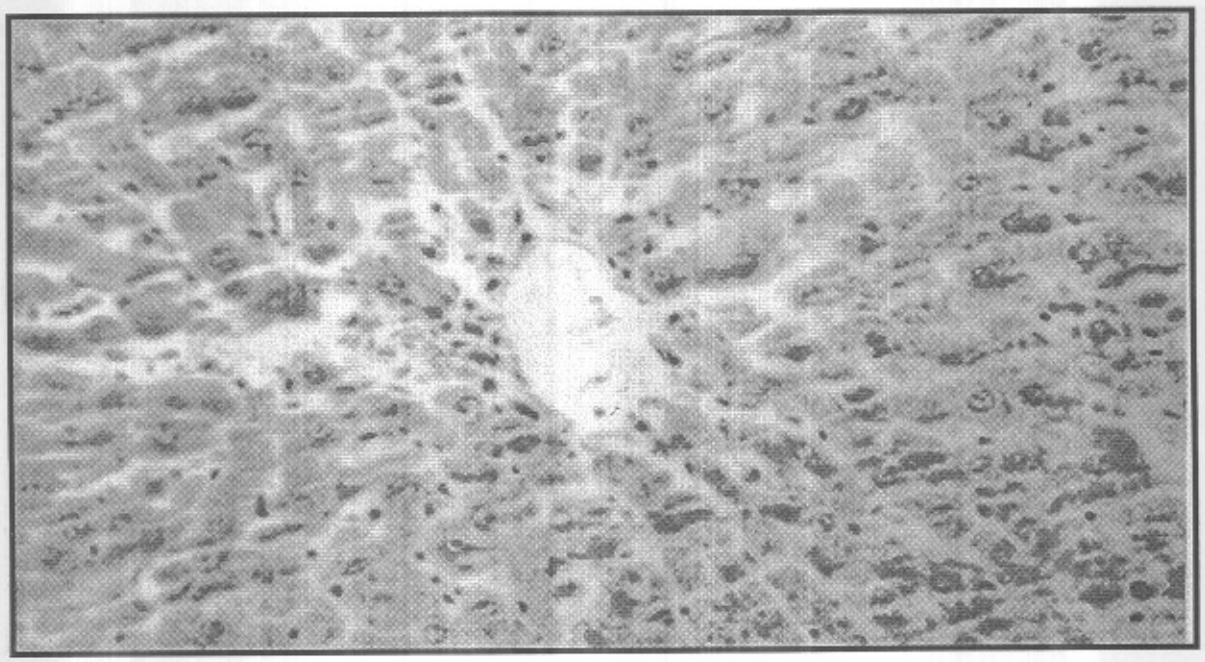


Liver

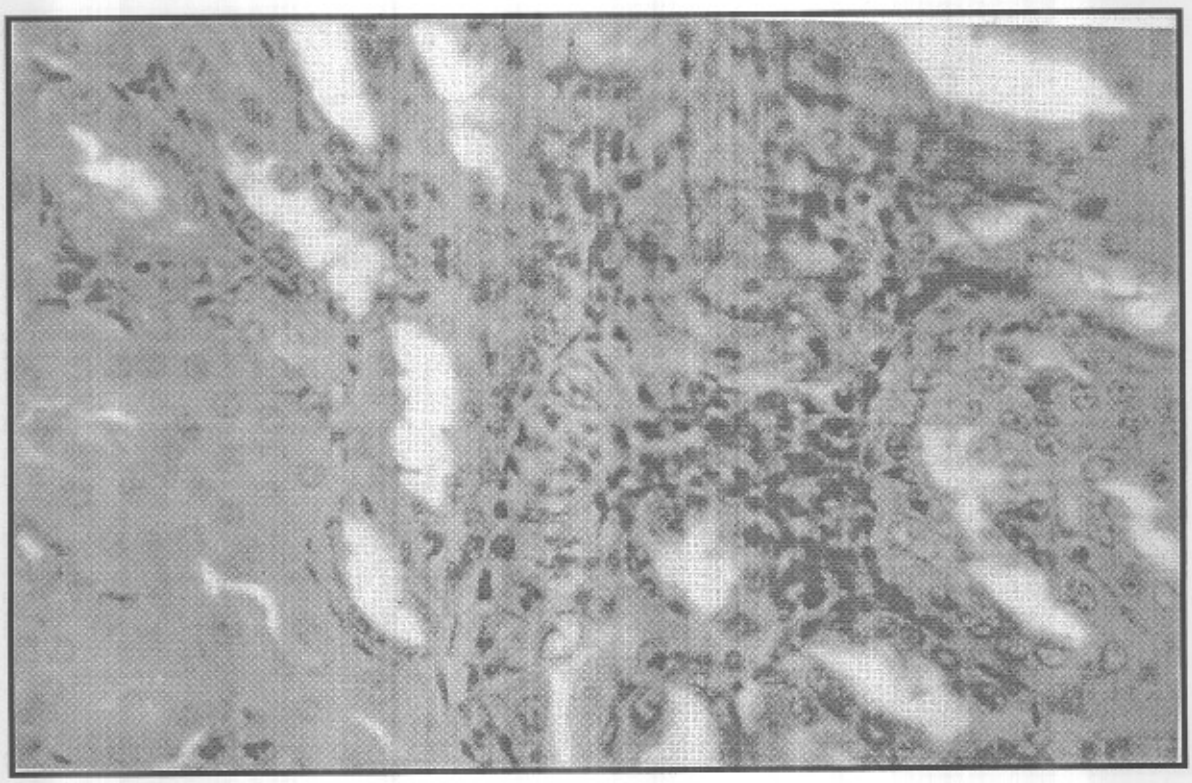
1- Coagulative necrosis of some hepatocytes represented by karyolysis, H&E, X 1200. Seen in group V



2- Congested blood vessels and hepatic sinusoides besides vaculations of hepatocytes, H&E, X 1200. Seen in groups VI, VII, and VIII.

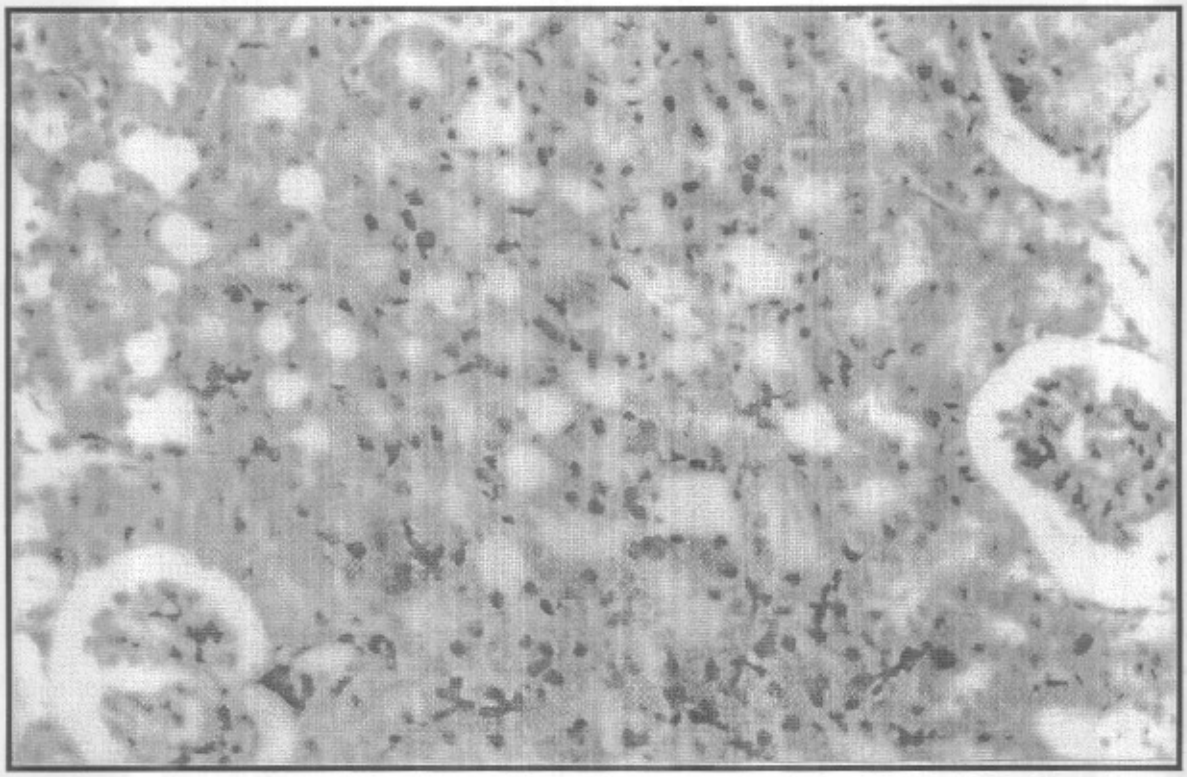


3- Few lymphocytic aggregation and slight congestion of hepatic sinusoids, H&E, X 300. Seen in safety groups II, III, and IV.

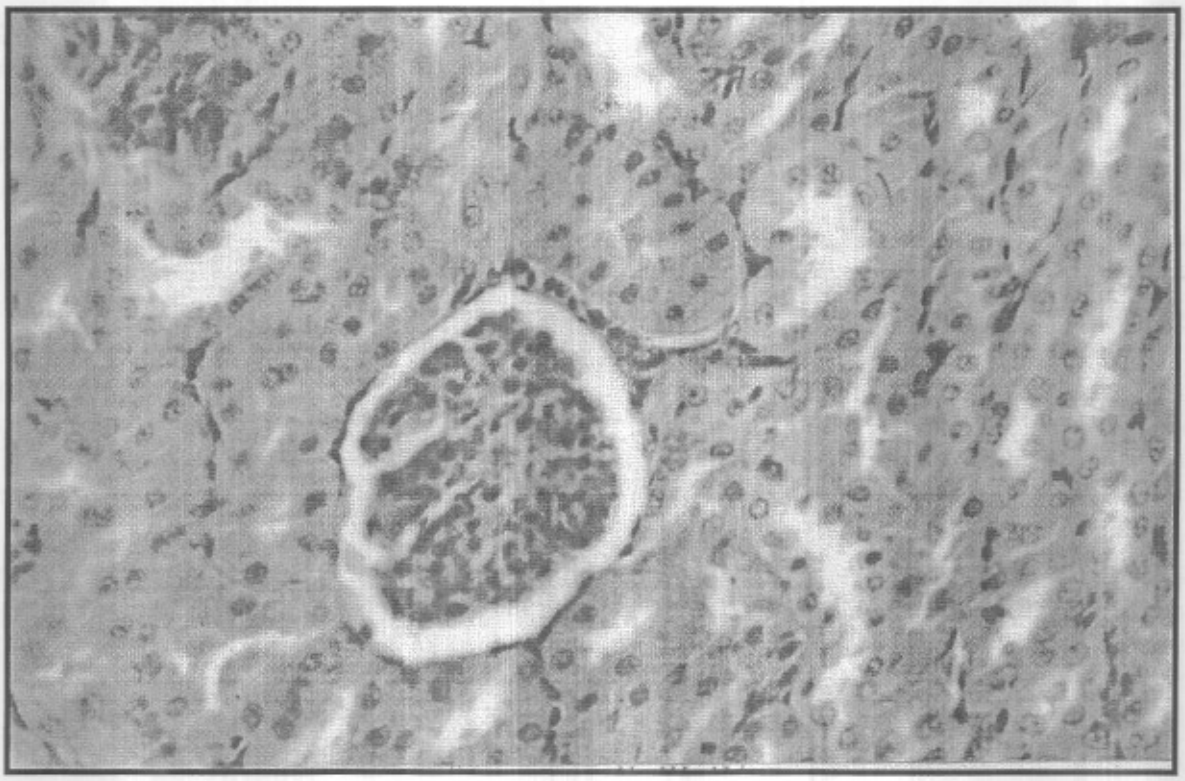


Kidney

1- Coagulative necrosis represented by pyknotic nuclei, H&E, X 1200. Seen in group V



2- Perivascular edema and cloudy swelling of some renal tubules, H&E, 1200. Seen in groups VI, VII, and VIII.



3- Hypercellularity of the glomerular tufts, H&E, X 1200. Seen in groups II, III, and IV.

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الملخص العربى

دراسة تأثير حمض الأسكوربيك وكبريتات الزنك كمضادات لسمية الكادميوم فى ذكور الفئران البيضاء

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أستخدم فى هذه الدراسه أربعون من ذكور الفئران التى تزن من ١٠٠-١٥٠جم والتى قسمت ألى ثمانى مجموعات متساويه. أستخدمت المجموعه الأولى فيها كمجموعه ضابطه، وتم أمداد المجموعه الثانيه بحمض الأسكوربيك فى ماء الشرب بمعدل ١جم/لتر،والمجموغه الثالثه بكبريتات الزنك بمعدل ٥٠مجم/لتر من ماء الشرب. وقد تم معالجة المجموعه الرابعه مثل المجموعتين الثانيه والثالثه مجتمعتين (حمض أسكوربيك وكبريتات الزنك). أما المجموعه الخامسه فقد تم حقنها بجرعه واحده من كلوريد الكادميوم تحت الجلد بمعدل ٢٠مجم/كجم من وزن الجسم. وفى المجموعه السادسه تم حقنها بكلوريد الكادميوم مثل المجموعه الخامسه بالأضافه ألى وضع حمض الأسكوربيك فى ماء الشرب (١جم/لتر). أما المجموعه السابعه فتم حقنها بكلوريد الكادميوم مثل المجموعه الخامسه بالأضافه ألى وضع كبريتات الزنك فى ماء الشرب. وقد عوملت المجموعه الثامنه مثل المجموعتين السادسه والسابعه مجتمعتين.

وقد أوضحت النتائج أن الكادميوم قد سبب أرتفاعا فى معدل أنزيمات الكبد فقد زاد (ALT) (حوالى ضعفين) و (AST) (حوالى أربعة أضعاف) وزاد معدل الكرياتينين والأمونيا ونقص مستوى الكالسيوم فى العظام

وعند أستخدام حمض الأسكوربيك وكبريتات الزنك أنخفض معدل الكرياتينين والأمونيا وزاد تركيز الكالسيوم فى العظام بينما أستمر معدل أنزيمات الكبد عاليا ولم يتأثر بأستخدام حمض الأسكوربيك أو كبريتات الزنك