Dept. Forensic Med. & Toxico.,
Cairo University

THE PROTECTIVE EFFECT OF SELENIUM AND ZINC AGAINST CADMIUM NEPHROTOXICITY IN ALBINO RAT

(With 4 Tables and 8 Figures)

By

A.A. KJALF, Kh. A.H. ABDO,*
*A.G. MOHAMED** and MANAL, Sh..HUSSEIN.***

* Dept. Of Forensic Med. & Toxico. , Cairo University (Beni Suef).
**Dept. of Theriogenology, Cairo University (Beni Suef).

(Received at 28/4/2001)

التأثير الوقائي للسلينيوم والزنك ضد التسمم الكلوي المسبب بواسطة الكادميوم في الفئران

عبد العظيم على أحمد خلف ، خالد عباس حلمى عبده ، أحمد جمعه محمد ، منال شعراوي حسين

تم تصميم هذه الدراسة لتقييم بعض الآثار السامة الناجمة عن التعرض لمعدن الكادميوم الذي كثر استخدامه في الأونة الأخيرة في الصناعات المختلفة .تم دراسة تأثير التعـــرض تحـــت المزمن للكادميوم على واحد من الأعضاء الهامة في الجسم وهى الكلى وذلك بتحديد تـــأثير الكادميوم على بعض مكونات الدم وهى (الكرياتينين، اليوريا ، حمض البوليك ، الكالسيوم ، الفوسفور ، الصوديوم ، البوتاسيوم) كذلك تأثير الكادميوم على وزن الحيوان بالإضافة إلـــى دراسة الأثر الوقائى للسيلينيوم والزنك في حالة التسمم تحت المزمن على وظائف الكلى. في هذه التجربة تم استخدام ١٨٠ فأر أبيض ، وقد قسمت هذه الفئران إلى ٦ مجموعات متساوية كل مجموعة تحوى ٣٠ فأر ، تم تعريض هذه المجموعات لكلوريد الكـــادميوم بتركـــيز ١٠ ملجم/لتر، كلوريد الكادميوم + السلينيوم كسيلينيات الصوديوم بجرعـــة ١٠ملجـــم/لـــتر ، ١ ملجم/لتر وكلوريد الكادميوم + الزنك كسلفات الزنك بتركيز ١٠ ملجم/لتر لكـــلا المـــادتين، السلينيوم كسيلنيات السلينيوم بتركيز ١ ملجم:لتر، الزنـــك كســـلفات الزنـــك بتركـــيز ١٠ ملجم/لتر على الترتيب ، بينما أستخدمت المجموعه السادسة كضابط للتجربه ، وقد استمرت التجربه لمدة ثلاثة أشهر . تم ذبح خمس فئران من كل مجموعه من المجاميع كـــل خمســـة عشر يوما وذلك بعد وزنها ثم تجميع الدم لفصل المصل منه وتحليل بعض مكونـــات هـــذا

المصل بيوكيميائيا ، كما تم استخراج الكلى لهذه الحيوانات وحفظها في محلـــول فورمـــالين متعادل ١٠% للفحص الهستوباثولوجي . أظهرت النتائج أن لعنصر الكادميوم تأثيرات سـلبية على الكلى ظهرت لوجود اضطرابات معنويه بالقياسات البيوكيماويه و هذا ما أكده الفحـــص الهستوباثولوجى للكلى. و أيضا أن لعنصرى الزنك والسلينيوم دور فعال للوقايه مـــن آثـــار الكادميوم السلبية.

SUMMARY

The wide spread applications of cadmium in industry make it one of the most important heavy metal of greatest toxicological concern. The present work was designed to evaluate the toxic effect of cadmium on kidney of albino rats with trials to minimize the toxicity of cadmium through usage of selenium and zinc. One hundred and eighty albino rat were classified into six equal groups. The first five groups were exposed respectively to 10 mg $CdCl_2$/l drinking water, the second, 10 mg $CdCl_2$ and 1 mg sodium selenite / L, the third, 10 mg $CdCl_2$ and 10 mg zinc sulfate/ L, the fourth, 1 mg sodium selenite /L, the fifth, 10 mg zinc sulfate/L, while the sixth remained as control. The experiment lasted for successive 3 month. Body weight gain, serum samples for determination of serum creatinine, urea, uric acid, calcium, phosphorus, as well as sodium and potassium concentration and kidney samples for histopathological examination were taken every 15 days.The obtained results revealed significant decrease in the body weight of cadmium treated rats, improvement in body weight was recorded in rats received cadmium and selenium and that treated with cadmium and zinc. While selenium or zinc alone has no remarkable effect in comparison with control group. Cadmium induced nephrotoxic effect in rats reflected by the significant disturbance in the biochemical parameters measured and confirmed with the macroscopic and microscopic changes in the kidney tissues. Also administration of zinc or selenium with cadmium plays an important role, as protective agents against cadmium while selenium or zinc alone have no remarkable histopathological lesions in comparison with other experimental or control group.

Key words: *Selenium, Zinc, Cadmium Nephrotoxicity, Rat.*

INTRODUCTION

Cadmium is a toxic transition metal of continuing occupational and environmental concern. As highly cumulative toxic agent, cadmium is estimated to have a biologic half-life in humans of approximately 20-30 years. Cadmium causes different kinds of toxicity among human and animal includes acute, subacute and chronic toxicity, nephrotoxicity, genotoxicity and affect male fertility. (IRAC, 1976; Friberg et al., 1986; Goyer, 1986; Kazantzis 1987; Waalkes and Oberdorster, 1990 and Waalkes et al., 1992). Significant decrease in body gain and, growth retardation, slight anemia, and decrease organs weight were recorded in all Cd treated animals Groten et al. (1991); Latino et al. (1997); Liao et al. (1997), and Pawaya et al. (1998). Several investigations were carried out on the nephrotoxicity of cadmium in rats and mice (Tandon, et al., 1992, Jun-Ichi Sudo et al., 1996; Nagwa El-Mossalamy et al., 1996; Wafaa El-Kholy, 1996; and Liu et al. (1998). They revealed that cadmium induce renal damage and dysfunction manifested by elevation in blood urea nitrogen, serum creatinine and decrease in serum total protein.

The present study aims to elaborate the protective effects of zinc and selenium supplementation against long term administration of cadmium (3 months), and the positive effect of zinc and selenium in improvement the kidney functions in case of cadmium nephrotoxicity.

MATERIALS and METHODS

180 apparently healthy adult albino wister rats weighing 120 g, supplied by Breeding Unit of the Egyptian Organization for the Biological and Vaccine production were fed on a commercially prepared diet and had free access to tap water continuously available throughout the study. Animals were classified into six equal groups (30 in each), rats were exposed to 10 mg cadmium chloride / L in drinking water, (group I) after Sayai et al. (1991), 10 mg cadmium chloride + 1 mg sodium selenite/L (group II) after Sugawara et al. (1989), 10 mg cadmium chloride + 10 mg zinc sulfate/L (group III) after Kudo et al. (1986), 1 mg sodium selenite/ L (group IV), 10 mg zinc sulfate / L (group V), while rats of group VI were given only tap water and considered as control group. The experiment lasted for three months,

animals were weighed every two weeks. Five animals from each group were sacrificed, to take serum and kidney samples every two weeks. Serum samples were taken for estimation of serum creatinine, urea, uric acid, calcium, phosphorus, sodium and potassium levels according to (Houto 1985; Patton & Grouch, 1977; Artiss & Entwistle 1981; Gindler, 1972; Goldenberg, 1966; and Bauer et al., 1974) respectively. Kidney tissues were taken for histopathological examination according to Carleton et al. (1967). The given data was analysed according to Snedecor (1961).

RESULTS

Effect on the body weight:

The obtained results revealed that body weight was significantly decreased in the cadmium exposed group from the second period and lasted to the end of the experiment. Regarding to groups that exposed to cadmium and selenium and that exposed to cadmium and zinc, the obtained results revealed improvement in body weight value. Selenium or zinc alone had no remarkable toxic effect in comparison with control group, Table (1).

Biochemical results:

In cadmium treated rats the effect was represented by significant and persistent elevation of serum creatinine, urea nitrogen, uric acid, calcium and phosphorus concentration with decrement in serum sodium and potassium concentration. Selenium or zinc in association with cadmium posses a protective effects against the nephrotoxicity. This was obvious by significant decrease in the previous elevated parameters and increase in concentration of sodium and potassium. Selenium or zinc alone has no remarkable toxic effect in comparison with control group, Tables, (2,3, & 4).

Macro and microscopical findings:

Macroscopic examination revealed swelling and congestion of kidney while Microscopic examination of kidney in cadmium treated group revealed hyperemia in glomerular tuft with vacuolation of the endothelial cell while epithelial cell lining the renal tubules showing granular degeneration Fig. (1). Few mononuclear leucocytic inflammatory cell infiltrations were observed in focal manner between the tubule Fig. (2 & 3). Histopathological examination of selenium in association with cadmium group shown histopathological lesion but less

than that in the cadmium treated group. This lesion were in form of aggregation of mononuclear leucocytic inflammatory cells in focal manner adjacent to the cortical blood vessels, edema was noticed surrounding the dilated blood vessels Fig. (4, 5 & 6). Zinc in association with cadmium has no marked .histopathological lesions except at the last month, a mononuclear leucocytic inflammatory cell infiltration were noticed in the periglomerular area with granular degenerative change in the cytoplasm of the epithelial cells lining the renal tubules Fig. (7&8). Selenium or zinc alone has no remarkable histopathological lesions in comparison with other experimental or control group.

DISCUSSION

In the present study, our results revealed that exposure to cadmium in concentration of 10-mg/L drinking water for successive three months to albino rats induced marked nephrotoxicity. Our results are consistent with the previous studies indicating that cadmium is a well-known nephrotoxic agent (Chapatwala et al., 1982; Sato and Nagai, 1989; Tandon et al., 1992, Tewari et al., 1991; Shiraishi et al., 1993 and Nagwa El-Mossalamy et al., 1996). The significant reduction in body weight of cadmium treated animals may be attributed to the severe alterations induced in different tissues due to cadmium toxicity especially in the liver and kidneys. Our results were in agreement with the data obtained by Latino et al. (1997) and Liao et al. (1997).

Chronic exposure to cadmium causes renal tubular cell injury and dysfunction that may progress to a chronic interstitial nephropathy. These changes characterized by significant elevation of serum creatinine, urea nitrogen, uric acid , and calcium and phosphorus concentration.

Concerning the significant elevation in serum creatinine concentration in cadmium intoxicated rats, it was shown that creatinine metabolism was thought to reflect the amount of glomerular filtration, the changes in its concentration in the serum was followed. Previously, creatinine excretion was found to be decreased with Cd injection (Kudo et al., 1986) leading to decreased creatinine clearance (Hopf et al., 1990 and Zhao et al., 1990). These observations are consistent with the present data which showed high levels of serum creatinine two weeks following Cd-intoxication, reflecting depressing glomerular filtration rate and hence, glomerular dysfunction. The present results are also in

accordance with the previous studies of Zhao et al. (1990); Nagyova et al. (1994); Nagwa El-Mossalamy et al. (1996) and Wafaa El-Kholy (1996) who reported that elevated serum creatinine levels in guinea pigs and rats intoxicated with cadmium. The significant elevation in creatinine level may be recorded in acute or chronic renal insufficiency, urinary tract obstruction, and impairment of renal function induced by some drugs (Murray et al., 1988). The persistent increase in serum creatinine level in Cd intoxication may be closely resemble those observed by Lin et al. (1992); Dorian et al. (1995) and Nagwa El-Mossalamy et al. (1996).

The present results illustrated that serum urea levels in rats treated with Cd were much higher than that of control. Similarly, elevated levels of urea nitrogen were recorded by Nagyova et al. (1994) in sera of guinea pigs intoxicated with Cd. indicating impaired kidney function. Also Wafaa El-Kholy (1996) and Nagwa El-Mossallamy et al. (1996) and Jun-ichi Sudo et al. (1996) recorded similar observation in rats treated with cadmium. Elevated level of urea in blood may be noted in renal insufficiency acute and chronic nephritis-acute renal failure (tubular necrosis) and urinary tract obstruction. Also it was recorded in cases of increased nitrogen metabolism associated with diminished renal blood flow or impaired renal function (Sonnenwirth and Jarett, 1980). The significant elevation in serum urea level in the intoxicated rats may be attributed to the toxic effect of cadmium on the liver and kidneys where the histopathological examination of the kidneys revealed granular degeneration in the epithelial cell lining the renal tubules indicating kidney dysfunction.

In cadmium treated rats, serum uric acid showed marked elevation. The kidney excretes uric acid, an end product of nucleoprotein metabolism. Gout, a genetically transmitted metabolic error, is characterized by increased plasma or serum uric acid concentration, an increase in total body uric acid and deposition of uric acid in tissues. An increase in uric acid concentration in plasma and serum may accompany increased nucleoprotein catabolism. The elevated serum uric acid level may be noted in cases of gout, polycythemia, therapy with anti leukemic drugs and a variety of other agents and renal insufficiency (Murray et al., 1988).

The present study revealed that cadmium toxicity in rats induces significant increase in serum calcium and phosphorus concentration,

while serum sodium and potassium concentration showed significant decrease. Goyer (1986) reported that cadmium intoxication have dramatic effects on calcium excretion. Osteomalacia and osteoporosis accompanied by bone pain are part of the syndrome, termed, Itai-Itai. Also Tsuchiya (1969) recorded that cadmium is reported to cause osteomalacia, perhaps by interfering with calcium and phosphorus balance in the kidney. Kido et al. (1988) stated that cadmium toxicity affects calcium metabolism and individuals with severe cadmium nephropathy may have renal calculi and excess excretion of calcium. Also cadmium can affect calcium, phosphorus and bone metabolism in both industrial workers and in people exposed in the general environment (Nogawa et al., 1989). Similar elevation in serum phosphorus level of the intoxicated rats was also recorded by Cousins et al. (1973) in pigs fed on a basal diet containing 50, 150, 450, 1350 ppm Cd for 6 weeks. The significant decrease in serum sodium and potassium concentration was parallel to the result of Kim et al. (1988) who found that S/C injection of $CdCl_2$ (2 mg Cd/kg/day) for rats for 16 days induce increasing in sodium and potassium excretion.

Our data revealed that cadmium causes marked and severe histopathological alterations in kidneys of the intoxicated rats in the form of degeneration in the epithelial cell lining the renal tubules, vacuolation of the endothelium in association with periglomerular mononuclear leucocytic inflammatory cell infiltration. Several investigators recorded similar histopathological lesions in kidneys of intoxicated animals (Nagwa El-Mossalamy et al., 1996; Nagwa El-Mossalamy and Amna Khamis, 1996 and Jun-Ichi Sudo et al. (1996).

The severe nephrotoxicity induced by cadmium toxicity may be attributed to various hypotheses have been proposed to explain the pathogenesis of Cd nephrotoxicity (Dudley et al., 1985; Hussain et al., 1987 and Robinson et al., 1993). The hypothesis most commonly accepted is that: (1) Cd is taken up by the liver (2) Cd bound to metallothioneins (MTs-Cd) synthesized by the liver (3) the MTs-Cd is released from the liver into the plasma (4) the MTs-Cd in the plasma is filtered through the glomeruli and taken up by the proximal tubules of kidneys; and (5) Cd taken up by the proximal tubular cells damages the cells. Also, Cd other than MTs-Cd in the proximal tubular cells is suggested to play a critical role in producing this injury (Nomiyama & Nomiyama, 1986 and Goyer et al., 1989).

On the other hand, it is clear, from the obtained data in the present study, that the selenium and zinc supplementation provided a marked protective effect against the cadmium nephrotoxicity. Recent studies has been found that Cd cause oxidative damage in different tissues by increasing lipid peroxidation and by inhibiting certain enzyme responsible for deactivation of oxygen species (Shukla and Singhal, 1984 ; Hussain et al., 1987 and Sole et al., 1990).

Considerable attention has been paid towards the development of safe and effective chelation and protection therapy in management of cadmium poisoning. Optimal intake of different nutrients like minerals (Groten et al., 1991) and vitamins in the diet can favorably affect the Cd-toxicity (Nagyova et al., 1994). However, the free radical scavengers (antioxidant) such ascorbic acid, tocopherol and selenium present in the tissue are known to protect against oxidative damage and signs of Cd-toxicity (Shukla et al., 1988; Fariss, 1991 and Hudecova and Ginter, 1992). On the other hand, among the protective agents used zinc has been shown to modulate both the toxicity (Jacobs et al., 1983; Herkovits and Perez-Coll, 1989 and Coogan et al., 1992) and carcinogenicity (Waalkes et al., 1989) associated with cadmium exposure.

Regarding the protective role of both selenium and zinc against the nephrotoxicity of cadmium. The obtained results denote that both elements provided either partial or complete protection against the induced renal damage. Significant decrease in serum concentration of creatinine, urea, uric acid, calcium and phosphorus in the treated rats represented this improvement. Also significant increase in serum concentration of sodium and potassium in the treated animals manifested the protective action . The histopathological examination was nearly correlated with the biochemical analysis, and selenium and zinc supplementation was at least partially ameliorated to large extent the renal damage induced by cadmium toxicity. Although the exact mechanism of zinc protection is unknown. It is reasonable to assume that zinc stimulating MT plays a role in this protection, at least at the level of sequestered cadmium away from nucleic materials (Yoshijawa and Ohra, 1982). However, it has been suggested that the thiolate clusters in MT are particularly efficient at scavenging hydroxyl free radicals (Thormally and Vasak, 1985 and Coppen et al., 1985). MT has been localized within the cell nuclues, in addition to being found in cytosol (Banerjee et al., 1982). Nuclear MT may stimulate both to generate radicals due to Cd-

binding, as well as Scavenge those radicals formed (Coogan et al., 1992). Further, the reduction of the toxic effects of Cd by zinc may be attributed to altered subcellular distribution of Cd (Goering and Klaassen, 1984). Lastly, Cd replaces Zn in Zn-thionein synthesized after Zn pretreatment (Tanaka et al., 1977).

Our results are inagreement with the previous findings indicating that zinc has been shown to reverse Cd-induced tissue damage (Cheng, 1988) and diminish some toxic effects of cadmium such as hepatotoxicity and renal toxicity (Sato and Nagai, 1989, Wafaa El-Kholy, 1996 and Nagwa El-Mossalamy et al., 1996). Regarding the role-played by the antioxidant in protection against toxicity of cadmium, Manca et al. (1991) suggested that lipid peroxidation (LPO) is an early and sensitive reaction to Cd-exposure. It is known that ascorbic acid (vit C), tocophorol (vit E) and selenium plays some role in the antioxidant mechanism against LPO (Hudecova and Ginter, 1992 and Shiraishi et al., 1993).

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Table (1): Body weight values (gm) in treated and control rat groups.

Groups / Time	Group (I)	Group (II)	Group (III)	Group (IV)	Group (V)	Group (VI)
2 weeks	128.3 ± 1.60	135.3 ± 3.33	137 ± 3.17	139 ± 3.40	140 ± 5.17	143.3 ± 3.33
4 weeks	138.3 ± 9.80	155 ± 4.99	150.6 ± 3.05	156 ± 3.45	163.3 ± 3.03	161.6 ± 4.40
6 weeks	146.6 ± 8.81	175 ± 5.01	180.5 ± 4.40	178.6 ± 4.39	180.6 ± 2.96	190.3 ± 3.33
8 weeks	157.6 ± 3.33	193.3 ± 3.55	205 ± 7.93	225 ± 7.53	210 ± 2.88	223.6 ± 4.10
10 weeks	165 ± 2.90	215 ± 2.88	220 ± 2.08	230 ± 5.71	226 ± 4.4	236.9 ± 2.80
12 weeks	160 ± 2.88	226.6 ± 4.40	225 ± 3.01	245 ± 2.83	240 ± 2.8	250 ± 5.70

The LSD at 5% is 42.901

The LSD at 1% is 57.776

Table (2): Serum creatinine, urea and uric acid concentration mg% in treated and control rat groups.

Time	Parameter	Group (I)	Group (II)	Group (III)	Group (IV)	Group (V)	Group (VI)
2 weeks	Creatinine	5.66 ± 0.161	5.83 ± 0.16	6.00 ± 0.28	5.23 ± 0.31	5.67 ± 0.161	5.9 ± 0.288
	Urea	50.3 ± 1.9	51.6 ± 0.4	51.5 ± 2.0	48.0 ± 1.8	48.9 ± 2.0	50.5 ± 5.4
	Uric ascid	12.26 ± 0.51	10.73 ± 0.31	11.44 ± 0.62	12.38 ± 0.81	10.61 ± 0.20	12.15 ± 0.59
4 weeks	Creatinine	6.94 ± 0.288	6.33 ± 0.43	6.61 ± 0.19	6.66 ± 0.16	6.63 ± 0.16	6.1 ± 0.29
	Urea	59.8 ± 0.2	52.7 ± 3.0	52.9 ± 1.2	50.7 ± 0.2	49.3 ± 9.0	51.5 +4.7
	Uric acid	19.24 ± 0.20	13.83 ± 0.30	12.50 ± 0.42	12.74 ± 0.46	12.36 ± 0.42	12.14 ± 0.13
6 weeks	Creat.	7.19 ± 0.29	6.53 ± 0.14	6.83 ± 0.16	6.33 ± 0.151	6.16 ± 0.188	6.16 ± 0.161
	Urea	63.7 ± 1.3	53.5 ± 2.2	52.9 ± 4.0	53.0 ± 5.0	51.0 ± 4.2	53.3 ± 4.0
	Uric acid	21.32 ± 1.04	15.47 ± 0.85	15.45 ± 0.75	16.98 ± 1.33	17.77 ± 1.29	12.03 ± 0.40
8 weeks	Creatinine	7.5 ± 0.32	7.23 ± 0.32	6.66 ± 0.32	6.13 ± 0.151	6.33 ± 0.17	6.65 ± 0.02
	Urea	74.2 ± 3.0	55.5 ± 1.5	57.5 ± 2.0	55.9 ± 3.1	54.3 ± 6.1	58.7 ± 4.3
	Uric acid	24.26 ± 1.58	14.47 ± 0.52	15.49 ± 2.42	17.22 ± 0.26	17.93 ± 0.283	13.56 ± 0.31
10 weeks	Creatinine	8.16 ± 0.36	7.04 ± 0.16	6.87 ± 0.16	6.5 ± 0.288	6.66 ± 0.23	6.3 ± 0.31
	Urea	88.9 ± 0.9	61.9 ± 7.0	60.2 ± 1.1	58.0 ± 1.3	57.9 ± 6.2	54.7 ± 1.7
	Uric acid	28.91 ± 0.31	20.8 ± 0.54	15.75 ± 1.97	13.8 ± 0.20	14.27 ± 0.67	13.85 ± 0.32
12 weeks	Creatinine	8.43 ± 0.18	7.06 ± 0.18	6.5 ± 0.288	5.83 ± 0.438	6.66 ± 0.44	6.36 ± 0.161
	Urea	98.7 ± 0.7	68.8 ± 4.9	58.9 ± 3.0	59.4 ± 5.0	56.0 ± 2.9	59.0 ± 1.3
	Uric acid	32.41 ± 0.82	20.23 ± 0.31	17.44 ± 0.85	15.33 ± 0.62	14.98 ± 0.54	13.91 ± 0.831

For creatinine the LSD at 5% is 0.835 & at 1% is 1.126 F values is 6.742 The prop > F is < 0.0003
For Urea the LSD at 5% is 9.00 & at 1% is 12.80 F values is 10.309 with 5/30 degree of freedom The prop > F is < 0.0001
For uric acid the LSD at 5% is 7.072 and at 1% is 9.524 F values is 6.055 The prop > F is < 0.000

Table (3) : Mean values of serum calcium & phosphorus levels mg% in treated and control rat groups.

Time	Parameter	Group (I)	Group (II)	Group (V)	Group (V)	Group (V)	Group (V)
2 weeks	Ca	11.18 ± 0.59	9.01 ± 0.12	9.71 ± 0.19	8.91 ± 0.124	8.74 ± 0.235	9.39 ± 0.084
	P	3.94 ± 0.54	3.89 ± 0.59	4.59 ± 0.02	3.98 ± 0.27	4.66 ± 0.42	4.33 ± 0.14
4 weeks	Ca	11.77 ± 0.144	9.84 ± 0.137	9.82 ± 0.147	9.66 ± 0.166	9.54 ± 0.156	9.61 ± 0.164
	P	4.47 ± 0.29	4.20 ± 0.34	4.09 ± 0.56	4.2 ± 0.178	4.28 ± 0.31	4.07 ± 0.18
6 weeks	Ca	12.17 ± 0.16	11.03 ± 0.216	9.75 ± 0.15	10.06 ± 0.32	9.92 ± 0.148	9.51 ± 0.06
	P	5.13 ± 0.30	4.51 ± 0.47	4.332 ± 0.281	4.3 ± 0.63	4.58 ± 0.28	4.41 ± 0.18
8 weeks	Ca	13.25 ± 0.147	11.23 ± 0.084	9.273 ± 0.354	9.85 ± 0.148	10.02 ± 0.599	9.87 ± 0.497
	P	5.94 ± 0.128	4.64 ± 0.17	4.51 ± 0.76	4.76 ± 0.20	4.51 ± 0.52	4.27 ± 0.21
10 weeks	Ca	15.03 ± 0.207	10.46 ± 0.231	9.32 ± 0.266	10.02 ± 0.104	10.38 ± 0.595	9.92 ± 0.083
	P	6.141 ± 0.42	4.95 ± 0.06	4.30 ± 0.37	4.69 ± 0.33	4.08 ± 0.16	4.40 ± 0.19
12 weeks	Ca	15.7 ± 0.29	11.22 ± 0.219	9.92 ± 0.464	10.15 ± 0.116	10.21 ± 0.291	10.02 ± 0.064
	P	6.62 ± 0.59	4.773 ± 0.63	4.93 ± 0.47	4.92 ± 0.51	4.75 ± 0.168	4.52 ± 0.03

For Calcium The LSD at 5% is 2.291 For P the LSD at 5% is 0.654
The LSD at 1% is 3.086 at 1% is 0.881

Table (4) : Serum potassium & sodium concentration mg% in treated and control rat groups.

Time	Parameter	Group (I)	Group (II)	Group (III)	Group (IV)	Group (V)	Group (VI)
2 weeks	K	8.83 ± 0.44	8.90 ± 0.95	8.81 ± 1.07	8.97 ± 0.52	9.563 ± 0.43	9.26 .26 ± 0.61
	Na	43.85 ± 1.91	42.9 ± 0.42	42.43 ± 0.53	42.67 ± 1.01	43.68 ± 0.52	43.106 3.106 ± 1.6
4 weeks	K	7.85 ± 0.48	8.61 ± 0.57	8.81 ± 0.27	9.21 ± 0.32	8.87 ± 0.6	8.86 ± 0.46
	Na	42.61 ± 3.2	43.531 ± 0.97	43.49 ± 2.3	42.87 ± 0.96	43.08 ± 1.03	43.71 3.71 ± 1.9
6 weeks	K	8.43 ± 1.16	8.501 ± 0.56	8.78 ± 1.24	9.41 ± 0.27	9.17 ± 0.6	9.21 ± 0.9
	Na	38.57 ± 2.3	43.89 ± 0.83	43.81 ± 2.1	43.69 ± 0.98	43.42 ± 1.3	44.32 4.32 ± 1.2
8 weeks	K	7.81 ± 1.07	8.83 ± 0.63	8.92 ± 0.71	10.09 ± 0.49	9.85 ± 0.06	9.55 .55 ± 0.83
	Na	37.85 ± 4.3	43.702 ± 3.2	43.2 ± 0.83	43.903 ± 0.93	44.32 ± 2.3	44.19 4.19 ± 2.13
10 weeks	K	7.17 ± 0.6	8.43 ± 1.2	9.23 ± 1.22	9.70 ± 1.27	9.76 ± 0.57	9.08 ± 0.66
	Na	37.39 ± 2.5	43.202 ± 1.4	43.714 ± 0.41	43.86 ± 1.11	43.36 ± 1.2	44.29 4.29 ± 2.1
12 weeks	K	6.40 ± 0.45	8.30 ± 0.83	8.51 ± 1.14	8.92 ± 0.9	8.34 ± 1.16	8.89 .89 ± 0.03
	Na	37.6 ± 2.13	43.5 ± 1.28	44.49 ± 0.44	44.85 ± 1.12	43.906 ± 1.6	44.1 ± 2.1

For K The LSD at 5% is 0.78
The LSD at 1% is 1.05

For Na the LSD at 5% is 1.10
The LSD at 1% is 1.48

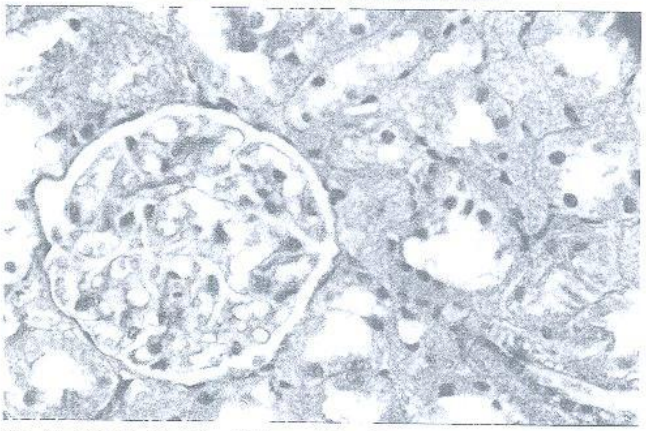

Fig. (1) :Kidney of rat treated by cadmium for one month showing vacuolar endothelium and hyperemia in the glomerular tuft with granular degeneration in the epithelial cells linning the renal tubules. H & E x 160

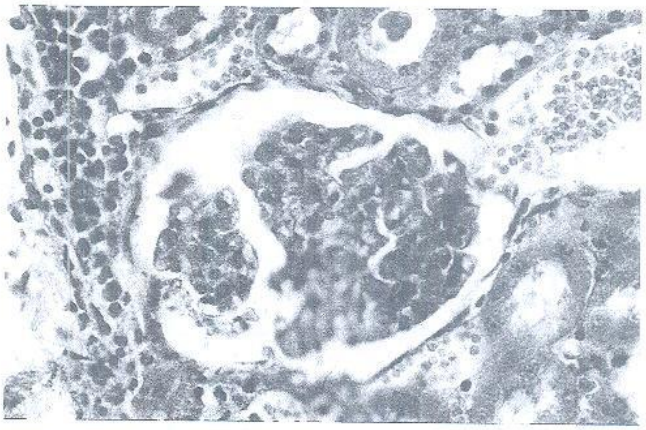

Fig.(2): Kidney of rat treated by cadmium for two months showing hyperemic glomerular tuft with periglomerular mononuclear leucocytic inflammatory cells infiltration as well as focal extravasation of red blood cells (haemorrhage) beside granular degeneration in the epithelial cells linning the renal tubules in the cortex.
H & E x 160

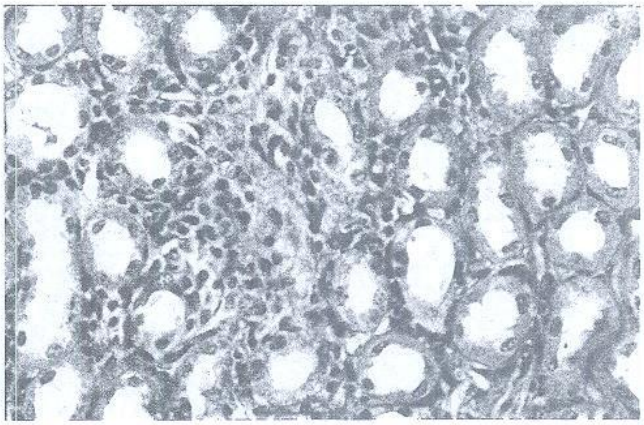

Fig.(3) :Kidney of rat treated by cadmium for two months showing focal mononuclear leucocytic inflammatory cells infiltration in between the renal tubules of the medullary portion. H & E x 160

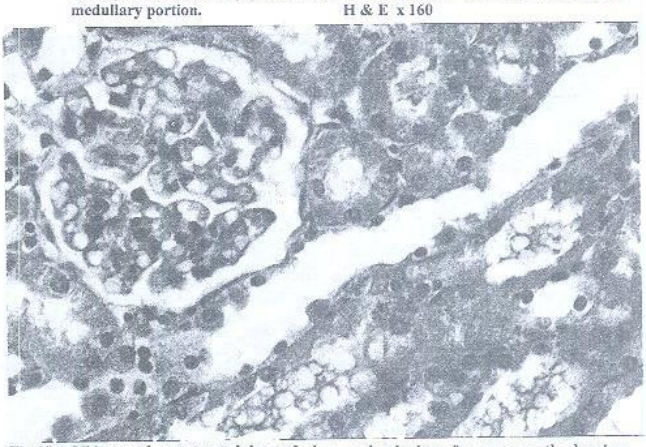

Fig.(4): Kidney of rat treated by cadmium and selenium for one month showing vaculation of the endothelial cells linning the hypermic tuft while the tubules showing granular degeneration . H & E x 160

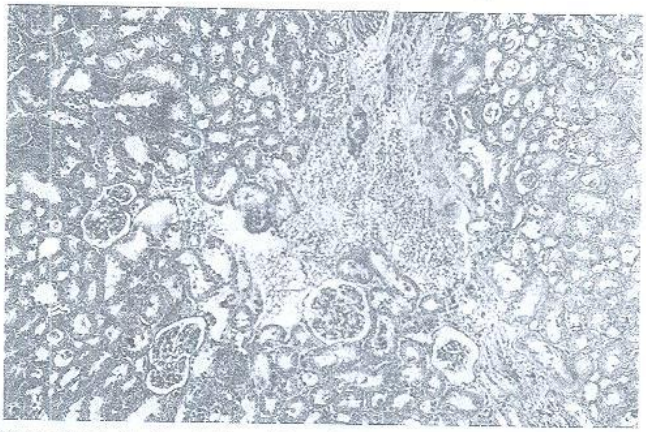

Fig.(5) :Kidney of rat treated by cadmium and selenium for one month showing focal extravasation of red blood cells (haemorrhage). H & E x 160

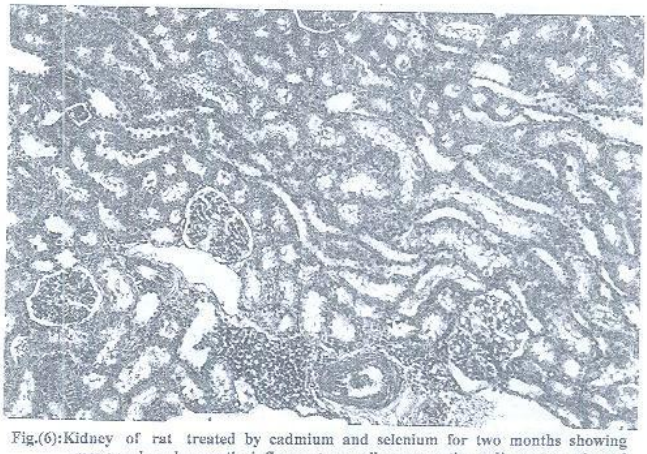

Fig.(6):Kidney of rat treated by cadmium and selenium for two months showing mononuclear leucocytic inflammatory cells aggregation adjacent to the red blood vessel in the cortical portion H & E x 160

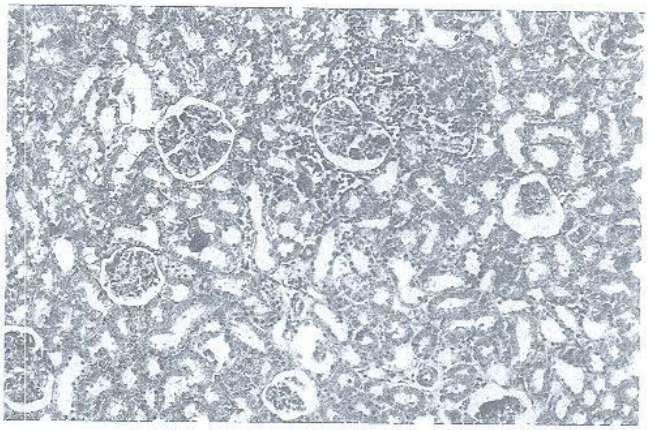

Fig.(7):Kidney of rat treated by cadmium and zinc for three months showing periglomerular leucocytic inflammatory cells infiltration. H & E x 160

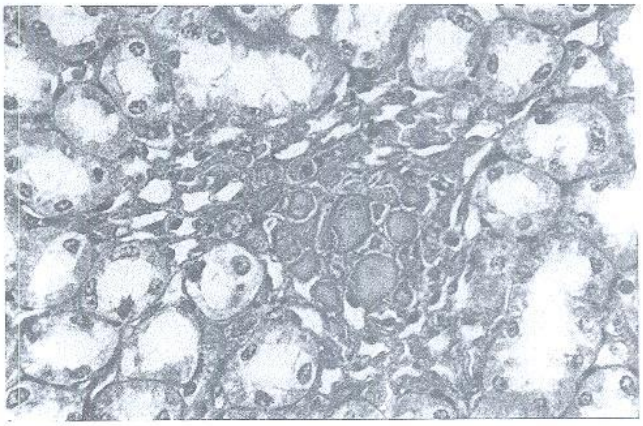

Fig.(8):Kidney of rat treated by cadmium and zinc for three months showing hemolysed blood in the lumen of the renal tubules. H & E x 160