Biochemical Evaluation of The Effects of Vitamin C in Rats Exposed to Sub-Chronic Low Doses of Cadmium

A Omonkhua, F Obi

Citation

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Abstract

In this study, the effects of vitamin C in cadmium-induced toxicity were investigated. Wister rats in different groups were exposed to cadmium (as CdSO4.8H2O), by sub-cutaneous injection, at doses of 1.0, 2.0 and 3.0 g/kg body weight, with or without vitamin C supplementation, for four weeks. The serum alkaline phosphatase, of group III of the vitamin C untreated rats significantly ($p<0.05$) increased, all the groups of the vitamin C treated rats also had significantly ($p<0.05$) increased serum alkaline phosphatase. The bone protein level and serum calcium of the vitamin C untreated group of rats, significantly ($p<0.05$) decreased relative to its control. The bone calcium of the vitamin C treated rats significantly ($p<0.05$) decreased (group IIIc from 2896.30±344.64 mg Ca/dl to 1049±101.43 mg Ca/dl) while the bone phosphate of this same group of rats, significantly ($p<0.05$) increased. For some parameters evaluated, the effects of cadmium on the vitamin C treated rats were less pronounced, indicating that vitamin C may be protective against cadmium-induced toxicity.

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INTRODUCTION

Cadmium is a natural element in the earth's crust. It is usually found as a mineral combined with other elements such as oxygen (cadmium oxide), chlorine (cadmium chloride), or sulfur (cadmium sulfate, cadmium sulfide)([1]). The major intentional uses of cadmium are Nickel-Cd batteries, cadmium pigments, cadmium stabilisers, cadmium coatings, cadmium alloys and cadmium electronic compounds such as cadmium telluride (CdTe). The major classes of products where cadmium is present as an impurity are non-ferrous metals (zinc, lead and copper), iron and steel, fossil fuels (coal, oil, gas, peat and wood), cement, and phosphate fertilisers ([2]).

For non-smoking, non-occupationally exposed population, food, especially of vegetable origin, is the main source of cadmium exposure ([3]). Much of the cadmium which enters the body by ingestion comes from terrestrial foods i.e. from plants grown in soil or meat from animals which have ingested plants grown in soil. It is estimated that 98% of the ingested cadmium comes from terrestrial foods, while only 1% comes from aquatic foods such as fish and shellfish, and 1% arises from cadmium in drinking water ([4]).

An acute intake of cadmium causes testicular damage. Within a few hours of exposure, there is necrosis and degeneration of the testes with complete loss of spermatozoa. This is thought to be due to an effect on the blood supply to these organs, reducing the blood flow ([5]). If the cadmium is inhaled, then severe lung irritation and damage (often called ‘fume fever') occur. Pleuritic chest pain, dyspnoea, cyanosis, fever and tachycardia, and the pulmonary oedema, are some of the symptoms which occurs that may be life-threatening ([6]). Acute ingestion of cadmium produces severe gastrointestinal irritation, which is manifested as severe nausea and vomiting, abdominal cramps and diarrhoea. A lethal dose of cadmium for ingestion is estimated to be between 350 and 8900 milligrams ([6]). The chronic effects of cadmium are dose-dependent and also depend on the route by which the metal enters the body. Chronic inhalation causes emphysema and obstructive airways disease, and these occur before kidney damage is seen ([7,6,5]). Long term ingestion causes kidney damage, which is first seen as proteinuria and $ß_2$microglobulinuria ([6,8]). In prolonged cadmium exposure, disorders of calcium metabolism occur, causing osteomalacia ([6,8]). This leads to painful fractures, hence the name given to the chronic exposure disease in Japan: Itai-itai disease (literally “ouch!-ouch!” disease) ([6,8]). Cadmium is

also known to be carcinogenic, and in studies has been linked with cancers in the lungs and prostate ([6,5,8]).

Rivers containing excess cadmium can contaminate surrounding land, either through irrigation for agricultural purposes, dumping of dredged sediments or flooding. It has also been demonstrated that rivers can transport cadmium for considerable distances, up to 50 km, from the source ([9]). Nonetheless, studies of cadmium contamination in major river systems over the past twenty to thirty years have conclusively demonstrated that cadmium levels in these rivers have decreased significantly since the 1960s and 1970s ([2,10,11,12]).

Studies have shown that vitamin C supplementation has varied effects on induced toxicity ([13]). Ascorbic acid has been found to interact with several elements in such a way as to render them less available for animals ([14]). Grosicki ([15]) reported a decrease in the carcass cadmium burden and the cadmium contents in the liver, kidney, testicles and muscles of cadmium exposed rats (1.0-1.2mgCd/kgb.w) given water supplemented vitamin C (1.5mg/L) for 28days.

Apart from accidental and occupational exposure to cadmium, the general population is facing an increasing risk of cadmium exposure, a comprehensive and continuous monitoring of Warri River (Nigeria) between 1986 and 1991, showed that the average level of cadmium was 0.3mg/litre ([16]); this far exceeds the minimum allowable level of cadmium in drinking water (0.005mg/litre) ([17]). This study was designed to evaluate the protective effects of vitamin C in rats exposed to low doses of Cd, i.e. doses that are frequently encountered in the natural environment, as a means of ameliorating its toxic effects.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Hydrated Cadmium Sulphate ($CdSO_4.8H_2O$) and other analytical grade chemicals used for this study were produced by E. Merch Darmstadt, Germany and BDH Chemical Limited, Poole, England. Pharmaceutical grade vitamin C tablets were obtained from Emzor Pharmaceuticals, Lagos, Nigeria. Alkaline Phosphatase and Calcium kits were products of Quimica Clinica Applicada SA (QCA), Spain, purchased from Equator Medics, Benin City, Nigeria.

EXPERIMENTAL ANIMALS AND MANAGEMENT

Forty-eight post weaning healthy albino rats (Rattus novergicus) of average weight 84.20g were obtained from the Animal Unit of Lagos University Teaching Hospital (LUTH), Nigeria, treatment of the animals was in accordance with the Principles of Laboratory Animal Care (NIH Publication 85-93, revised1985). They were divided into eight (8) groups of six (6) rats each. All the rats were maintained on commercial feeds, product of Bendel Feeds and Flour Mill, BFFM, Ewu, Nigeria. A set of four groups (I, II, III, and IV) corresponding to control, 1.0, 2.0 and 3.0µgCd/kg body weight were given water, while another set of four groups (Ic, IIc, IIIc and IVc) corresponding to control, 1.0, 2.0 and 3.0µgCd/kg body weight were given 5% vitamin C solution in place of water.

Once a day, for five days a week, the rats were given 0.25ml of the appropriate Cadmium solution or distilled water per 100g body weight by subcutaneous injection. Each rat was weighed weekly.

At the end of the fourth week, the rats were anaesthetized in a chloroform saturated chamber and while under the influence of the anaesthesia, the kidneys and femur bones were collected. The tissues were washed in ice cold physiological saline and then homogenized in ice cold physiological saline (1:4 wt/v of saline). The supernatant of the centrifuged homogenates were then separated, appropriately labeled and stored frozen until required. Blood was collected by heart puncture, allowed to clot on ice and then centrifuged at 5,000rpm for 5minutes, the sera was kept frozen until required.

One of the femur bones from each rat was ashed at 600 ^{o} C for 12hours in a Gallenkamp muffle furnace; model FR614, Gallenkamp &Co., England. The ashen bones (dissolved in 2M HCl) were used for the determination of bone calcium and inorganic phosphate.

BIOCHEMICAL ANALYSIS

Alkaline phosphatase (ALP) activity was measured spectrophotometrically at 550nm, in the serum, kidney and bones, using QCA alkaline phosphatase kits. The method measures spectrophotometrically, the intensity of the pink colour of phenolphthalein which is obtained by the hydrolysis of a colourless substrate phenolphthalein monophosphate by ALP ([18]).

Protein levels were measured spectrophotometrically at 540nm, in the serum, kidney and bone, using the Biuret method ([19]).

Serum and bone calcium were measured

spectrophotometrically at 565nm, using QCA calcium kits. At alkaline pH, calcium forms a coloured complex with O-cresolpthalein, 8-hydroxyquinolein is added to the reagent as a chelating agent of magnesium ions which can interfere with the reaction ([20]).

Serum and bone inorganic phosphate were measured spectrophotometrically at 625nm, according to the method described by Plummer ([21]). In this method, ammonium molybdate-H_2SO_4 reagent reacts with inorganic phosphate to form complexes which is reduced to give a blue colour that can be read spectrophotometrically by adding 0.2% ascorbic acid.

Statistics: Values are expressed as means of 5 or 6 determinations ± SEM. The results obtained for the vitamin C treated and untreated groups were analyzed separately (within the column) by the Independent Samples T-test, each group was compared to its respective control on SPSS 11.0, SPSS Inc., Chicago, Illinois, USA.. A $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

This study was designed to investigate the effects of vitamin C in rats exposed to sub-chronic (4 weeks) low doses of sub-cutaneously injected cadmium, with particular reference to kidney and bone toxicities. The overall toxic effects of continuous exposure to Cd were assessed by monitoring rat body weight gain and organ-body weight ratio.

Figure 1

Table 1: Body Weight of rats administered doses of cadmium

Group Number	Cd Dosage µgCd/kg Bd wt	Body Weight (g) Week 0 (Initial)	Week 1	Week 2	Week 3	Week 4 (Final)
I	0	78.60 ± 5.036	80.10 ± 5.675	90.70 ± 5.682	94.80 ± 5.472	108.10 ± 6.346
II	1.0	85.67 ± 5.137	81.50 ± 5.130	90.83 ± 5.320	101.67 ± 5.664	102.25 ± 5.470
III	2.0	69.42 ± 3.348	66.25 ± 3.007	82.50 ± 3.601	96.67 ± 3.712	103.67 ± 3.803
IV	3.0	75.00 ± 4.645	83.75 ± 4.727	87.25 ± 4.765	93.75 ± 5.028	102.17 ± 5.143
Ic	0	86.12 ± 6.360	94.7 ± 6.878	99.39 ± 7.328	110.00 ± 7.292	111.88 ± 7.920
IIc	1.0	81.21 ± 6.084	72.4 ± 5.597	85.30 ± 5.98	89.20 ± 5.245	88.10 ± 4.488
IIIc	2.0	104.50 ± 8.24	95.10 ± 8.141	102.60 ± 8.95	115.30 ± 10.14	124.80 ± 10.99
IVc	3.0	93.10 ± 5.382	96.10 ± 4.831	99.30 ± 5.408	102.80 ± 5.126	108.80 ± 4.596

Values are mean of five or six determinations ±standard error of the mean. Values carrying b and b* are significantly ($p<0.05$) different from control (a and a*).

Table 1 shows a general steady increase in body weight gain in the vitamin C untreated and vitamin C treated groups of rats, however, while the final body weight of all tests groups of the vitamin C untreated rats decreased compared to control, only Group IIc of the vitamin C treated groups showed a statistically significant ($p<0.05$) decrease in final body weight compared to control, group IVc actually recorded a significantly ($p<0.05$) higher body weight compared to control. Asagba et al, ([22]) reported that rats given 3 mg Cd/ kg body weight (as $CdCl_2$) for 4 weeks, had statistically ($p<0.05$) lower final body weight compared to control. Ribas et al, ([23]) also reported that the pups of female rats supplied with drinking water containing 300 mg/l of $CdCl_2$ during lactation, showed significantly lower ($p<0.01$) body weight than control pups. Grosicki and Kowalski ([24]), reported that rats exposed for 4 weeks to $CdCl_2$ (labeled with cadmium-109) at 10 mg/ kg diet, showed a steady increase in average body weight gain throughout the experiment, however the final body weight of the rats exposed to Cd was markedly lower in comparison to the control rats. This observation totally correlates with the results obtained for the vitamin C untreated rats in this study.

Figure 2

Table 2: Organ- Body Weight Ratio of rats administered doses of cadmium.

Group Number	Cd Dosage µgCd/kg bd wt	Organ-Body Weight Ratio ($X\ 10^{-3}$) Bone	Kidney
I.	0	7.5 ± 0.42	8.8 ± 0.28
II.	1.0	7.2 ± 0.23	8.1 ± 0.30
III.	2.0	6.6 ± 0.05	8.4 ± 0,27
IV.	3.0	7.9 ± 0.30	8.1 ± 0.25
Ic	0	8.8* ± 0.78	8.1* ± 0.62
IIc	1.0	8.7 a* ± 0.32	8.7* ± 0.10
IIIc	2.0	7.7* ± 0.30	7.3* ± 0.26
IVc	3.0	7.0* ± 0.14	7.3* ± 0.28

Values are mean of five or six determinations ±standard error of the mean. Values carrying b and b* are significantly ($p<0.05$) different from control (a and a*).

Table 2 shows the general decreases observed in the bone- and kidney-body weight ratios for both set of rats, however, group IIc bone-body weight ratio was statistically similar to control, the kidney-body weight ratio of this same group was significantly ($p<0.05$) higher than control. Since chemical toxicity can be assessed by changes in the body weight and organ-body weight ratio ([25]), the significant alterations of these parameters in the Cd-treated rats, especially the vitamin C untreated rats implies Cd toxicity. The fact that only group IIc of the vitamin C treated rats showed a

significant ($p<0.05$) decrease in body weight, while all the vitamin C untreated rats had significantly ($p<0.05$) decreased body weights, implies that vitamin C had a protective effect against the Cd-induced body weight reduction.

Since the kidney and bone are implicated in non-acute cadmium exposure, the serum, kidney and bone ALP were determined in this study.

Figure 3

Table 3: Serum, kidney and bone alkaline phosphatase of rats administered doses of cadmium.

Group Number	Cd Dosage µgCd/kg bd wt	Alkaline Phosphatase U/L		
		Bone	Kidney	Serum
I	0	$215.16^{a} \pm 2.56$	$466.32^{a} \pm 14.69$	$99..51^{a} \pm 2.22$
II	1.0	$193.78^{b} \pm 15.14$	$411.28^{b} \pm 12.26$	$94.62^{a} \pm 2.63$
III	2.0	$192.51^{b} \pm 13.48$	$467.19^{a} \pm 8.51$	$146.09^{b} \pm 6.11$
IV	3.0	$182.63^{b} \pm 12.77$	$435.01^{b} \pm 14.71$	$118.73^{a} \pm 2.40$
Ic	0	$286.60^{a*} \pm 15.83$	$494.15^{a*} \pm 15.30$	$74.26^{a*} \pm 10.15$
IIc	1.0	$195.81^{b*} \pm 10.21$	$436.84^{b*} \pm 16.49$	$123.22^{b*} \pm 3.10$
IIIc	2.0	$203.80^{b*} \pm 12.55$	$448.67^{b*} \pm 5.10$	$123.22^{b*} \pm 3.44$
IVc	3.0	$240.57^{b*} \pm 16.77$	$478.27^{b*} \pm 12.07$	$124.17^{b*} \pm 2.88$

Values are mean of five or six determinations ±standard error of the mean. Values carrying b and b* are significantly ($p<0.05$) different from control (a and a*).

The bile canaliculli of the liver, osteoblasts in the bone, proximal tubules in the kidney and mucosal cells of the small intestine, are rich sources of alkaline phosphatase ([26]). Damage to any of these organs or tissues would lead to elevated activity of its isoform of ALP in the serum ([27,28]). Table 3 shows significant ($p<0.05$) decreases observed in tissue ALP of almost all the groups of the vitamin C untreated and vitamin treated rats. Axelsson and Piscator ([29]), reported a reduction in the ALP activity of renal cortex in rabbits given 0.5mgCd/kg body weight. Saillenfait et al, ([30]) also reported that pups from pregnant rats intraperitoneally injected with $CdCl_2$ at 2.5 mg/kg body weight for up to 14 days of gestation, exhibited significant decreases in renal ALP activity.

The significantly high serum ALP (Table 3) observed in this study could be as a result of the leakage of this enzyme from damaged tissues, rich in ALP, into the blood stream, this is particularly evident in the vitamin C treated groups where both bone and kidney ALP were significantly ($p<0.05$) decreased while the serum enzyme significantly ($p<0.05$) increased. Itokawa et al ([31]) observed an increase in the activity of serum ALP coincident with changes in the skeleton after 120days of oral exposure to 50ppm cadmium. Akesson et al, ([32]), using a population based women's health survey in Southern Sweden to investigate the association between low-level cadmium exposure and osteoporosis, reported that urinary cadmium displayed a near-significant ($p<0.06$) association with serum bone-alkaline phosphatase (bALP). Since the kidney and bone, are established target organs of cadmium toxicity ([33,34]); in addition to the decreased tissue ALP observed in this study, it is plausible to conclude that the increase in serum ALP is a reflection of the decreased bone and kidney ALP.

Serum total protein level is a rough measure of protein status but reflects major functional changes in kidney and liver functions ([35]).

Figure 4

Table 4: Serum, kidney and bone protein levels of rats administered doses of cadmium.

Group Number	Cd Dosage µgCd/kg bd wt	Protein Levels mg/ml		
		Bone	Kidney	Serum
I	0	$5.38^{a} \pm 0.41$	$12.13^{a} \pm 0.74$	$47.80^{a} \pm 1.04$
II	1.0	$6.00^{b} \pm 0,33$	$14.50^{b} \pm 0.67$	$52.50^{b} \pm 0.48$
III	2.0	$6.00^{b} \pm 0.42$	$12.50^{a} \pm 0.44$	$48.90^{a} \pm 1.26$
IV	3.0	$2.70^{b} \pm 0.27$	$11.50^{b} \pm 0.54$	$47.75^{a} \pm 0.95$
Ic	0	$6.67^{a*} \pm 0.52$	$15.00^{a*} \pm 0.90$	$52.75^{a*} \pm 2.31$
IIc	1.0	$4.80^{b*} \pm 0.51$	$8.88^{b*} \pm 0.71$	$42.80^{b*} \pm 1.15$
IIIc	2.0	$5.63^{b*} \pm 0.28$	$9.10^{b*} \pm 0.49$	$49.80^{b*} \pm 0.55$
IVc	3.0	$6.88^{a*} \pm 0.43$	$11.25^{b*} \pm 1.32$	$46.75^{b*} \pm 1.48$

Values are mean of five or six determinations ± standard error of the mean. Values carrying b and b* are significantly ($p<0.05$) different from control (a and a*).

Table 4 shows the serum, kidney and bone protein levels. Low doses of cadmium administration for 4 weeks appears to have a general lowering effect on the bone, kidney and serum protein levels of the vitamin C treated groups. Also, the significant reduction in the bone protein level of group IV of the Vitamin C untreated rats, show that cadmium interferes with bone protein levels. The reductions observed in the bone, kidney, and serum protein levels of all test groups of the vitamin C treated rats are puzzling, however, since statistical analysis show that vitamin C alone

significantly ($p<0.05$) increased the bone, kidney and serum protein levels of group Ic (Table 7), the reductions observed can be attributed to cadmium-induced toxicity. Proteinuria due to kidney impairment in cadmium toxicity may be the cause of protein loss in the test groups since inhibitory role of cadmium in protein synthesis has not been established.

The reduction in serum calcium in the vitamin C untreated groups is a reflection of the effect of cadmium on calcium metabolism (Table5)

Figure 5

Table 5: Serum and bone calcium levels of rats administered doses of cadmium.

Group Number	Cd Dosage µgCd/kg bd wt	Calcium mg Ca/dl	
		Bone	Serum
I	0	1641.98^{a}±119.19	12.23^{a} ±0.54
II	1.0	1323.46^{b} ±94.25	11.16^{b} ±0.18
III	2.0	1033.33^{b} ±129.00	8.24^{b} ±0.49
IV	3.0	1739.46^{b} ±28.21	8.48^{b} ±0.10
Ic	0	2896.30^{a*}±344.64	10.10^{a*}±0.14
IIc	1.0	1017.28^{b*}±40.65	8.46^{b*}±0.20
IIIc	2.0	1049.99^{b*}±101.43	8.99^{b*}±0.38
IVc	3.0	1370.37^{b*}±108.56	12.2^{b*}0±0.20

Values are mean of five or six determinations ±standard error of the mean. Values carrying b and b* are significantly ($p<0.05$) different from control (a and a*).

The decreased Ca^{2+} absorption and negative Ca balance in cadmium exposed rats could result from the inhibitory effect of cadmium on the activation of vitamin D in renal cortical cells ([36]). Nogawa et al ([37]) reported that serum 1, 25-dihydroxylcholecalciferol levels were lower in Itai-Itai disease patients and cadmium exposed subjects with renal damage. A reduction in serum calcium level (hypocalcaemia) stimulates the release of calcium from bone, this correlates well with the reduced bone calcium observed in the test groups of the vitamin C untreated and vitamin C treated rats. Itokawa et al ([31]) reported that simultaneous administration of cadmium with a low-protein, low-calcium diet led to a decrease in calcium and zinc content of the bone.

Available data show that cadmium can affect calcium, phosphorus and bone metabolism ([11]). Reductions in serum 1, 25-dihydroxycholecalciferol in cadmium-exposed subjects, are closely related to serum concentrations of parathyroid hormone (PTH), β_2-microglobulin and the percentage tubular reabsorption of phosphate ([37]), suggesting that cadmium-induced bone effects may also be due to disturbances in vitamin D and PTH metabolism ([38]). Phosphorus is important for the modulation of calcium mobilization from the bone and the regulation of plasma calcium ([39]). Increase in serum calcium is associated with decrease in serum phosphorus and increased urinary phosphorus excretion and vice versa ([39]).

Figure 6

Table 6: Serum and bone phosphate levels of rats administered doses of cadmium.

Group Number	Cd Dosage µgCd/kg bd wt	Phosphate Levels µg/ml	
		Bone	Serum
I	0	1397.50^{a}±37.68	253.75^{a}±7.37
II	1.0	1310.00^{b} ±19.68	196.67^{b} ±0.96
III	2.0	1362.50^{a} ±33.38	188.75^{b} ±9.96
IV	3.0	1479.17^{b} ±20.98	240.00^{a} ±5.93
Ic	0	1200.00^{a*}±15.10	318.33^{a*}±26.94
IIc	1.0	1295.00^{b*}±30.57	70.00^{b*}±2.83
IIIc	2.0	1400.00^{b*}±30.77	176.25^{b*}±12.15
IVc	3.0	1347.50^{b*}±19.24	211.67^{b*}±14.84

Values are mean of five or six determinations ±standard error of the mean. Values carrying b and b* are significantly ($p<0.05$) different from control (a and a*).

The results obtained for serum calcium and phosphate in this study (Tables 5 and 6) does not sufficiently reflect the reciprocal relationship between calcium and phosphorus, however, group IVc, of the vitamin C treated rats, shows an increased calcium level while its phosphate level is decreased. The results for bone calcium and phosphate levels of the vitamin C treated rats, correlates better with the reciprocal relationship between calcium and phosphorus.

The statistical analysis of groups I and Ic, using Independent Samples T-test on SPSS 11.0, showed that vitamin C alone positively influenced most of the parameters evaluated (Table 7).

Figure 7

Table 7: Effect of Vitamin C on all Parameters Evaluated

Parameter	Group I	Group Ic
Final Body Weight (g)	108.10±6.346 [a]	111.88±7.920 [a]
Bone-Body Weight Ratio	7.5±0.42 [a]	8.8±0.78 [b]
Kidney-Body Weight Ratio	8.8 ±0.28 [a]	8.1±0.62 [b]
Bone ALP U/L	215.16 ±2.56 [a]	286.60±15.83 [b]
Kidney ALP U/L	466.32 ±14.69 [a]	494.15±15.30 [b]
Serum ALP U/L	99..51 ±2.22 [a]	74.26±10.15 [b]
Bone Protein Level mg/ml	5.38 ±0.41 [a]	6.67±0.52 [b]
Kidney Protein Level mg/ml	12.13 ±0.74 [a]	15.00±0.90 [b]
Serum Protein Level mg/ml	47.80 ±1.04 [a]	52.75±2.31 [b]
Bone Calcium Level mgCa/dl	1641.98±119.19 [a]	2896.30±344.64 [b]
Serum Calcium Level mgCa/dl	12.23 ±0.54 [a]	10.10±0.14 [a]
Bone Phosphate level µg/ml	1397.50±37.68 [a]	1200.00±15.10 [b]
Serum Phosphate level µg/ml	253.75±7.37 [a]	318.33±26.94 [b]

Values are mean of five or six determinations ±standard error of the mean. Values carrying different notations are significantly different at ($p<0.05$).

For some of the parameters evaluated, recognizable differences were observed between the vitamin C untreated and the vitamin C treated groups of rats, these include:

The final body weights of groups IIIc and IVc of the vitamin C treated rats did not decrease relative to control, while all the groups of the vitamin C untreated rats decrease compared to their control

The bone protein concentration of group IV of the vitamin C untreated groups of rats showed a statistically significant ($p<0.05$) decrease compared to its control, while there was no significant difference in group IVc of the vitamin C treated groups, compared to its control.

While a significant ($p<0.05$) decrease in serum calcium was observed in all the vitamin C untreated groups, group IVc of the vitamin C treated groups, showed a significant increase compared to its control

Cadmium may also exert its toxicity via oxidative damage. Valko et al ($_{40}$) reported that the primary route for cadmium toxicity is depletion of glutathione and binding to sulfhydryl groups of protein. It is therefore not surprising that vitamin C, an antioxidant, would play a protective role against cadmium toxicity.

CONCLUSION

The findings from this study have shown that subcutaneous administration of low doses of cadmium to rats either with or without vitamin C supplementation caused an increase in serum ALP and bone phosphate. It also caused a decrease in bone protein concentration, serum calcium and bone calcium, indicating that under the conditions of this study, the bone is the major target of cadmium toxicity. The result also shows that vitamin C may play a protective role against cadmium toxicity, implying that vitamin C can be used to ameliorate the toxic effect of low doses of cadmium.

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CORRESPONDENCE TO

Name: Omonkhua Akhere A. Address: Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. E-mail: aaomonkhua@yahoo.com

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Author Information

Akhere A. Omonkhua, M.Sc Biochemistry
Adekunle Ajasin University

Fredrick O. Obi, PhD Biochemistry
University of Benin