International Journal of Biochemistry Research & Review
7(1): 36-44, 2015, Article no.IJBcRR.2015.055
ISSN: 2231-086X

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The Nephroprotective Effects of Graded Concentrations of Calcium and Magnesium on Nephrotoxicities Induced by a Constant Toxic Concentration of Cadmium and Lead in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author GAU designed the study, wrote the protocol and supervised the work. Authors JDD and PAO carried out all laboratories work and performed the statistical analysis. Author SYG managed the analyses of the study. Author JDD wrote the first draft of the manuscript. Authors SYG and PAO managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBcRR/2015/17119
Editor(s):
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Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1036&id=3&aid=8777

Original Research Article

Received 26th February 2015
Accepted 27th March 2015
Published 13th April 2015

ABSTRACT

Aims: To concurrently administer constant toxic concentrations of Cd and Pb with graded concentrations of Ca and Mg using a rat model to determine their nephroprotective effects against Cd and Pb nephrotixicities.
Study Design: Wistar rats were divided into five groups of four rats per group in metabolic cages. Group one was placed on tap water only, while group two to five were placed on a constant

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concentration of 0.327 mg/L lead and 0.079 mg/L cadmium concurrently with graded magnesium and calcium.
Place and Duration of Study: The animal House of Pharmacology Department, Anatomy and Biochemistry laboratories, University of Jos, Nigeria, were used for treatments, histochemical and biochemical analyses respectively, between December 2013 and April 2014.
Methodology: Their feed was mashed with the same water meant for each group. All the groups fed and freely drank from the water for a period of fourteen (14) days. Twenty-four hour (24h) urine samples were collected from the rats at their respective groups in the urine collector of the metabolic cages for fourteen days. The urine samples were kept frozen until needed for clinical analysis. At the termination of the experiments, the rats were humanely sacrificed, the kidneys identified and fixed in 10% formal saline for histopathological studies.
Results: Kidney biomarkers in urine decreased, while urinary excretion of urea and creatinine increased as the concentrations of calcium and magnesium were elevated. The histopathological analyses show that there was no significant difference ($P<0.05$) between control and groups 4 and 5, but there was significant difference ($P>0.05$) between control and groups 2 and 3.
Conclusion: Results suggest that calcium and magnesium could mitigate the nephrotoxicities induced by cadmium and lead. Therefore, good proportion of calcium and magnesium in the diet and water would enhance good health especially for those living in environments contaminated with heavy metals.

Keywords: Nephrotoxicity; nephroprotective; heavy metals; graded concentrations; environmental pollution; mutual exclusivity.

1. INTRODUCTION

Mining and smelting operations are important causes of heavy metal contamination in the environment due to activities such as mineral excavation, ore transportation, smelting and refining, and disposal of the tailings and waste waters around mines [1-3]. Literatures abound on the adverse environmental impact of excessive heavy metals dispersed from mine and smelter sites contamination of water and soil, phytotoxicity, soil erosion, and potential risks to human health [4-7]. Studies on the mining sites of Plateau State, Nigeria, show that in the recent past decades, the natural environmental concentrations of several chemical elements (toxic and essential) have largely increased on the Jos Plateau, mostly as a result of anthropogenic activities.

Metals and metalloids have been reported to occur in the mining pond waters of Plateau State at levels above World Health Organisation tolerable limits for drinking water [8-12]. In solution, these elements may exist either as free ions or as various complexes associated with organic or inorganic ligands or as suspended colloidal particles. In the solid phase, they may be adsorbed (or absorbed) on organic and inorganic soil components, exist as minerals ions, or co-precipitated with other minerals. Generally, ions in solution are more available for plant and animal uptake, and immediately entering the food chain [13-16].

In our previous work, varying concentrations of Ca and Mg were found to have nephroprotective potential against varying concentrations of Cd and Pb induced nephrotoxicity as determined by the urinary excretion of cadmium and lead [17]. The mining pond waters of Plateau state contain Cd and Pb in concentrations above WHO permissible limits, and also contain Ca and Mg in high concentrations. The local inhabitants of these areas use the pond waters for their domestic use. What could be the effect of the concurrent occurrence of these four metals from using this pond water on the inhabitants? This present work seeked to determine the nephroprotective effect of graded concentrations of the combination of Ca and Mg against the nephrotoxicity of a constant toxic concentration of the combination of Cd and Pb in rats, in order to have an idea of the possible effect of using the pond waters on the inhabitants of the area.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Twenty (20) adult male Wistar strain rats weighing 178g on the average were obtained from the University of Jos Animal House. Commercial feed produced by Grand Cereal and Oil Mill Limited, Jos, Nigeria, was used to feed the animals.

2.1.1 Chemicals

Lead acetate and magnesium sulphate, both analar, were products of British Drug House (BDH), Poole, England. Bovine Serum Albumin (BSA) was a product of Sigma Chemicals. Cadmium chloride and calcium sulphate were products of May and Baker (M & B) Limited, Dagenham, England. All other chemicals used were of analytical grade purchased by the Department of Biochemistry, University of Jos, from reputable chemical companies in Jos, Plateau State, Nigeria. All chemicals were of analytical grade.

2.2 Experimental Design

The rats were randomly divided by body weight equally into five groups of four per group in metabolic cages. Group one (control) was placed on tap water only, while group two to five were placed on a constant concentration of 0.327 mg/L lead and 0.079 mg/L cadmium; while magnesium and calcium were varied thus: 0.165, 0.193, 0.221, and0.248 mg/L respectively as shown in Table 1 below. The choice of cadmium and lead concentrations of (0.327 mg/L lead and 0.079 mg/L cadmium) is based on the fact that the combination of the two concentrations caused the most damage to the kidney in our previous work, hence the need to test graded concentrations of calcium and magnesium on the toxic effect of the combined concentrations of these toxic metals. The mining pond waters of Plateau state contain Cd and Pb in concentrations above WHO permissible limits, and also contain Ca and Mg in high concentrations, which the inhabitants of the areas use for their domestic purposes.

Twenty fours (24) hours prior to the commencement of the experiment the rats were fasted to clear the gastrointestinal tract of any other food eaten before, according to Rodriguez-de Fonseca et al. [18]. Their feed was mashed with the same water meant for each group. All the groups fed on the mashed vital growers feed, and freely drank from the water for a period of fourteen (14) days.

2.2.1 Urine collection and preparation

Clean, dry, leak proof sterile containers were used in collecting a twenty-four hour (24h) urine sample from the rats at their respective groups in the urine collector of the metabolic cages for fourteen days. The urine samples were kept frozen until needed for clinical analysis [19]. At the termination of the experiments, the rats were humanely sacrificed, the kidneys identified and fixed in 10% formal saline for histopathological studies.

2.2.2 Methods used in the determination of biochemical parameters

Urine total proteins concentration was determined by Biuret method [20], while alkaline phosphatase was determined by King and Armstrong method [21]. Urea estimation in urine was done by diacetylmonoxime method [22]; estimation of creatinine in urine was done by Jaffe's method [23].

2.2.3 Histopathological studies

The kidney was fixed in 10% neutral formalin solution. After a week of fixing, the kidney tissues were dehydrated with a sequence of ethanol solutions, embedded in paraffin, cut into 5 µm section, stained with haematoxylin eosin dye (H & E stain) and observed under a microscope at x400 magnification. Morphological changes were observed including cell gross necrosis, sinusoidal congestion, fatty changes, ballooning degeneration, infiltration of lymphocytes and kuffer cells.

2.3 Statistical Analysis

The analysis of variance (ANOVA) at 95% level of confidence was used to test for the significant differences in the activities of urinary alkaline phosphatase, concentrations of urinary protein, urea and creatinine, and results expressed as mean±S.D. The INSTAT3 statistical software was used.

3. RESULTS

The results are presented in Table 1 and Plates 1-5. As the concentrations of Ca and Mg were elevated while that of Cd and Pb kept constant, results of the kidney biomarkers and histochemistry showed that the protection to the kidney integrity increased as the concentrations of Ca and Mg were elevated. There was significant difference ($p<0.05$) in the urinary alkaline phosphatase activity and total proteins concentration at the lower concentrations of calcium and magnesium but there was no significant difference ($P>0.05$) at the higher concentrations of calcium and magnesium as compared with control. At the higher concentrations of calcium and magnesium, there was increase in the urinary excretion of urea and

creatinine, which was insignificantly different from control (Table 2).

The histochemistry show that there was mild damage to the kidney integrity at the lower concentrations of Ca and Mg but as their concentrations were elevated, there was no significant difference between the kidney integrity of control and groups 4 and 5 (Plates 1-5).

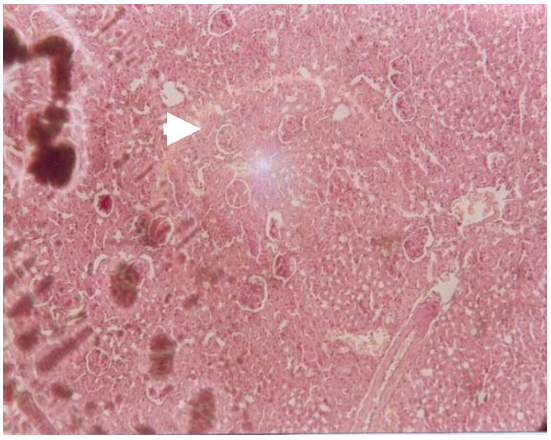

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Plate 1. Kidney section of the rats fed without the addition of cadmium, lead, magnesium and calcium (control), showing normal cells of the glomerulus and convoluted tubules

4. DISCUSSION

The results show that when the highest concentrations of Cd and Pb in our previous work [17] were kept constant while calcium and magnesium where concurrently varied, the kidney biomarkers indicate that the protection to the kidney integrity increased as the concentrations of Ca and Mg were elevated. There was significant difference ($p<0.05$) in the urinary alkaline phosphatase activity and total proteins concentration at the lower concentrations of calcium and magnesium but there was no significant difference ($P>0.05$) at the higher concentrations of calcium and magnesium as compared with control (Table 2). This suggests that the kidney integrity was compromised. This is in agreement with the fact that animals fed with cadmium in foods and water had high blood pressure, kidney damage, Iron–poor blood, liver diseases and nerve or brain damage [24-26]. Cadmium is first transported to the liver through the blood, where it binds with proteins to form complexes that are transported to the kidneys. It accumulates in the kidney, thus damaging the filtering mechanism and capacity of the kidney; causing excretion of essential protein and sugars from the body [27-29].

At the lower concentrations of calcium and magnesium, there was decrease in the urinary excretion of urea and creatinine, which was significantly different from control (Table 2). This suggests that urea and creatinine clearance capacity of the kidneys were less efficient as compared to control. This observed result was supported by the histochemistry of the kidney which show that there was damage to the kidney integrity at the lower concentrations of the

Table 1. Experimental design

Metals	Group1 (control)	Group 2	Group 3	Group 4	Group 5
Pb	-	0.327	0.327	0.327	0.327
Cd	-	0.079	0.079	0.079	0.079
Mg	-	0.165	0.193	0.221	0.248
Ca	-	0.165	0.193	0.221	0.248

Concentrations in mg/L

Table 2. Effect of concurrent administration of a constant toxic concentrations of Cd and Pb with graded doses of Ca and Mg on kidney integrity as determined by kidney biomarkers

Groups	Treatments (mg/L)	Alkaline phosphatase (I.U/L)	Total protein (mg/L)	Urea (mmol/L)	Creatinine (umol/L)
1.	Tap water only	467.00±70	289.29±126	160.36±25	5058.79±847
2.	Pb+Mg+Cd+Ca	593.33±142*	538.20±354*	138.33±38*	4353.80±505*
3.	Cd+Mg+Pb+Ca	597.00±97*	386.20±135*	130.33±39*	4446.00±435*
4.	Cd+Mg+Pb+Ca	481.67±101	304.00±123	175.20±24	5193.13±702
5.	Pb+Mg+Cd+Ca	470.27±106	292.07±139	163.933±30	4948.33±771

*Concentrations of Cd (0.079) and Pb (0.327) while Ca and Mg had equal concentrations of 0.165, 0.193, 0.221 and 0.348 mg/L respectively; *significant difference ($P>0.05$) between control and the treatments*

combination of Ca and Mg concentrations, but as their concentrations were elevated, there was no significant difference between the kidney integrity of control and groups 4 and 5 (Plates 1-5). This observation is consistent with the fact that when the availability of essential micronutrients is increased, the toxicity of toxic heavy metals is decreased [30,31]. Increase synthesis of metallothionein (MT), a thiol-rich protein that sequesters Cd and prevent acute nephrotoxicity, will lead to chronic toxicity as cadmium-MT is excreted from the liver and absorbed by the kidney. Although MT offers mostly protective effects against Cd toxicity, it has been shown that it is indirectly involved in contributing to Cd's main toxic effect: renal failure [31].

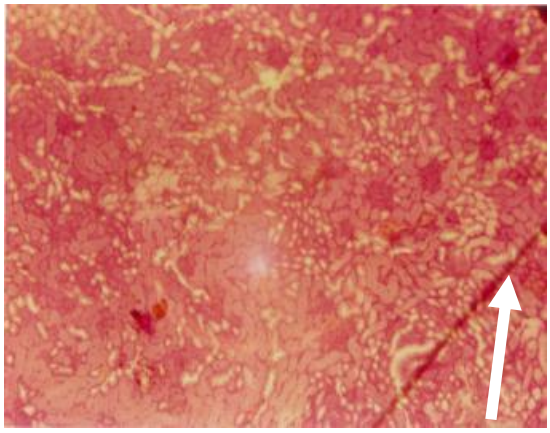

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Plate 2. Representative kidney section of the rats treated with the addition of 0.327 mg/L (Pb), 0.079 mg/L (Cd), 0.165 mg/L (Mg), and 0.165 mg/L(Ca), showing a rapidly progressive and severe glomerular damage

Lead has been shown to cause a wide variety of health effects. Many of the effects have been known since ancient times, although some of the more subtle effects have been discovered only recently. The toxicity of lead is widely acknowledged. The greatest risk for harm, even with only minute or short term exposure, is to infants, young children, and pregnant women [32,33]. Toxic effects of lead are typically broken down into two (2) categories: acute (short term) and chronic (long term) effects [34].

In acute toxicity, effects show up relatively soon after exposure occurs. However, following ingestion of large amount of lead, there will be a direct tissue interaction. This includes tissues desiccation, mucosal tissue damage in the gastro-intestinal tract (GIT) and convulsion possibly resulting in death. The most sensitive is the haematopoietic (blood forming) system, with hypochromic microcytic anaemia common. A variety of symptoms involved in acute toxicity include metallic taste in the mouth, stomach pain, vomiting, diarrhoea, black stools, constipation, drowsiness, fatigue and weakness [35].

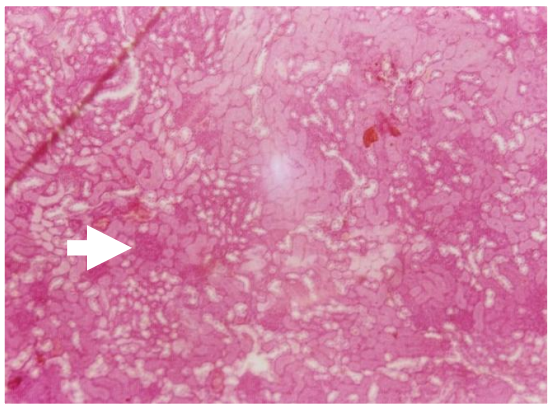

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Plate 3. Representative kidney section of the rat treated with the addition of 0.327 mg/L (Pb), 0.079 mg/L (Cd), 0.193 mg/L (Mg), and 0.193 mg/L (Ca), showing glomerular and renal tubular damage (arrow)

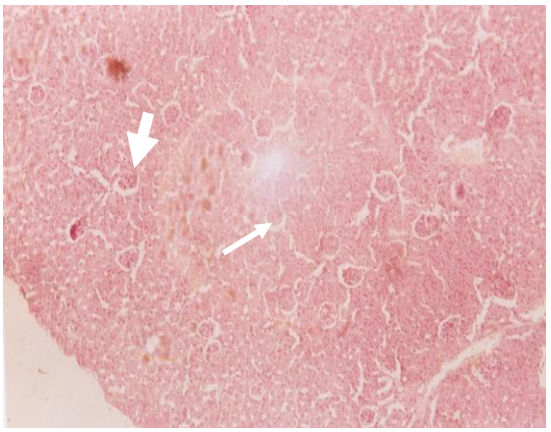

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Plate 4. Kidney section of the rats treated with the addition of 0.327 mg/L (Pb), 0.079 mg/L(Cd), 0.221 mg/L (Mg), and 0.221 mg/L(Ca), showing relatively normal glomerulus (arrows)

Furthermore, in chronic toxicity, there is no sudden onset of symptoms with a gradual build–up of a positive lead balance. As the lead level

rises, hyper–excitability is seen. Confusion, delirium and convulsions may occur in some cases, while in other cases, there is progressive lethargy leading to a comatose state. These types of effects take sometimes before they begin to develop [36]. For lead, a wide variety of chronic effects can be set in motion by continued exposure. However, the immediate symptoms often seen with significant long term exposure includes loss of appetite, nausea, lead colic (stomach pain), weight losses, insomnia, headache, nervous irritability, anxiety, weakness, hyper–activity, pallor (yellowing of the skin). Others are kidney failure, impaired Vitamin D balance and red blood cell problems [36].

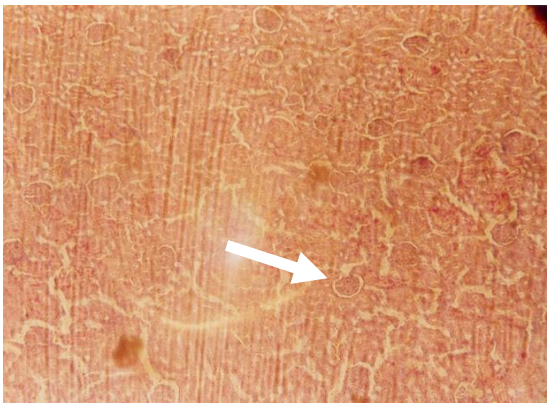

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Plate 5. Representative kidney section of the rats treated with the addition of 0.327 mg/L (Pb), 0.079 mg/L (Cd), 0.348 mg/L (Mg), and 0.348 mg/L (Ca), showing a relatively normal glomerulus (arrow)

Moreover, because of these symptoms, a variety of health problems are associated. These health problems are more noticeable and unusual, which is in most cases associated to classic lead poisoning. These include gum discolouration (a blue line on the gum), wrist drops, foot drops, severe stomach pain, tremor, and kidney failure. Reproductively, effects of lead toxicity include abnormal reproductive cycles, menstrual disorders, sterility, spontaneous miscarriages, still births and premature births in women; while in men it includes decreased sexual drive, impotence, and infertility [37,38].

All mining operations have a disruptive effect on the environment and subsequently adverse health effects on animals and man via the food chain because of the volume of materials involved that make the impact on health acute or chronic. For example, the high levels of environmental contamination which led to the poisoning of children < 5 years of age with elevated blood lead levels (97%, > 45 µg/dL), and incidence of convulsions among children before death (82%) in two villages in Zamfara state, Nigeria, suggest that most of the recent childhood deaths in the two surveyed villages were caused by acute lead poisoning from gold ore–processing activities [39]. The outbreak of itai-itai disease, which was the most severe stage of chronic Cd poisoning, occurred in the Cd-polluted Jinzu River basin in Toyama, Japan. In this area, the river was contaminated by slag from a mine upstream; as a consequence, the soil in rice paddies was polluted with heavy metals including Cd through irrigation water from around 1910 to the 1960s [40,41].

From the foregoing, it can be seen that the emphasis on heavy metals and their toxic effects, that may occur in portable water has almost obscured the fact that important beneficial constituents are commonly present [12]. The concentrations of essential macro and micro elements that occur in natural, portable waters vary greatly, depending on their sources and geographic considerations, which are very important in any study attempting to relate water quality to health [42,43]. The importance of many natural, portable waters in human nutrition has been largely ignored by the concern for health-threatening, toxic heavy metals that some waters contain. In the context of positive contributions to human health, the beneficial qualities of the drinking water should also be emphasized. This is more so as the result of the graded calcium and magnesium treatment on the nephrotoxicities induced by a constant toxic concentrations of cadmium and lead showed that in all the parameters assayed, as the combination of calcium and magnesium were increased, the toxic effects of cadmium and lead were obliterated. This raises some salient questions because it is a known fact that whenever cadmium and/or lead is/are present in drinking water above WHO's admissible limits of 0.001 mg/L and 0.05 mg/L respectively, nephrotoxicity occurs. Even the histopathological studies of the liver show that there is no observable difference between groups 4, 5, and the control, but there was significant difference between groups 2 and 3 with control. This work agrees with the fact that chemical substances in water that make positive contributions to human health act mainly in two ways: (a) nutritionally, by supplying essential macro and micro elements

that the diet (excluding water) may not have provided in adequate amounts (for example Ca, Mg, I and Zn); and (b) by providing macro and micro elements that inhibit the absorption and/or effects of toxic elements such as Hg, Pb and Cd [44,45].

5. CONCLUSION

Our results provide evidence that high calcium and magnesium intake could mitigate cadmium and lead induced nephrotoxicity in rats. This observation could be as a result of the mutual exclusivity that may exist between cadmium and lead on the one hand and calcium and magnesium on the other hand as a result of their similar chemistry. Therefore, people living in polluted areas could be advised to take foods rich in calcium and magnesium so as to mitigate the nephrotoxicity that may occur as a result of ingesting foods or water (or both) containing concentrations of cadmium and lead above World Health Organisation Permissible limits.

ETHICAL APPROVAL

All authors hereby declare that the principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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