Comparative hepatoprotective effects of Concomitant Administration of Ca, Mg and the Combination of Ca and mg Against Cd and Pb co-Intoxicated Rats.

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***Abstract:** This study was designed to determine the comparative hepatoprotective effects of concomitant administration of calcium (Ca) alone, magnesium (Mg) alone and the combination of Ca and Mg against cadmium (Cd) and lead (Pb) co-intoxicated rats. Wistar rats were divided into five groups of four rats per group in metabolic cages. Group one was fed with tap water only; group two with the co-administration of cd and Pb; group three with co-administration of Cd and Pb and concomitant addition of mg; group four with co-administration of Cd and Pb and concomitant addition of Ca; while group five with co-administration of Cd and Pb and concomitant addition of Ca and Mg. All the groups fed and freely drank from the water meant for each group for a period of fourteen (14) days. The rats were humanely sacrificed under anaesthesia, sample of blood was obtained from each rat by decapitation. Serum was obtained by centrifugation of clotted blood for liver biomarkers determination, while the liver was identified and fixed in 10% formal saline for histopathological studies. Results show that liver biomarkers were significantly different (p<0.05) between control and the groups treated with the combination of Cd+Pb and Cd+Pb+Mg, while there was no significant difference ((p>0.05) between control and the groups treated with Cd+Pb+Ca and Cd+Pb+Ca+Mg. The histochemistry also show that there was mild damage to the liver integrity of the group treated with Cd+Pb+Mg and a marked damage to the group treated with Cd+Pb. Results suggest that ca has a better hepatoprotective property than mg and the two metals have synergistic effect in mitigating the hepatotoxicities induced by co-administration Cd and Pb in rats.*
***Keywords:** Comparative, Hepatoprotective, concomitant, Calcium, Magnesium, Cadmium, Lead, Co-intoxicated.*

I. 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, 2, 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, 5, 6, 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 chief amongst them is mining. 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, 9, 10, 11, 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, 14, 15, 16].

In our previous work, varying concentrations of Ca and Mg were found to have hepatoprotective potential against varying concentrations of Cd and Pb induced hepatotoxicity as determined by the urinary excretion of cadmium and lead [17], and graded concentrations of Ca and Mg had nephroprotective effect on the nephrotoxicity induced by a constant toxic concentrations of Cd and Pb [18]. 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 (drinking, cooking and washing). 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 compare the hepatoprotective effects of calcium alone, magnesium alone and the combination of calcium and magnesium against the hepatotoxicity of a constant toxic concentration of the combination of Cd and Pb in rats. This is to determine whether calcium alone, magnesium alone or the combination of calcium and magnesium have more efficient hepatoprotective effect against the hepatotoxicity induced by co-administration of cadmium and lead in rats.

II. Material And Methods

2.1 Experimental Animals

Ethical Clearance was obtained from The University of Jos Committee on Care and Use of Laboratory Animals before the commencement of this work. 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.

2.1.2 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.

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 was placed on 0.327mg/L Pb and 0.079 mg/L Cd only; group three was placed on 0.327mg/L Pb and 0.079 mg/L Cd with the addition of 0.221mg/L of Mg; group four was placed on 0.327mg/L Pb and 0.079 mg/L Cd with the addition of 0.221mg/L of Ca; while group five was placed on 0.327mg/L Pb and 0.079 mg/L Cd with the addition of equal concentrations 0.221mg/L of Ca and Mg respectively as shown in table 1 below. The choice of Cd and Pb concentrations of (0.327mg/L Pb and 0.079 mg/L Cd) 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 comparatively test the protective effects of Mg alone, Ca alone and the combination of Ca and Mg. 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-four (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* [19]. Their feed was mashed with the same water meant for each group. All the groups fed on the mashed vital growers' food, and freely drank from the water for a period of fourteen (14) days.

Table 1: Experimental design

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

Concentrations in mg/*L*

2.2.1 Blood Collection

The rats were humanely sacrificed, and five to ten milliliter sample of blood was obtained from each rat. To prevent mechanical lyses, the blood was allowed to flow along the wall of the tubes, which was brought close, to the bottom. The blood was allowed to clot at room temperature after which a gentle ringing was carried out to dislodge the clot from the walls of the tubes. The serum was then separated from the clot by centrifugation, using MSE Mistral 2L Centrifuge, and kept frozen until required for the measurement of the following biochemical parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total proteins, and albumin. The liver was excised and fixed in 10% formal saline for histopathological studies.

2.2.2 Methods used in the determination of Biochemical parameters

The transaminases were determined according to Reitman and Frankel method of 1957 [20]. AST: The principle is based on the fact that the pyruvate produced by the transamination activities of AST reacts with 2, 4-dinitrophenylhydrazine to give a brown coloured hydrazine which is measured colourimetrically at 520nm. ALT: The principle is that the pyruvate produced by the transamination activities of ALT reacts with 2, 4-dinitrophenylhydrazine to give a brown coloured hydrazine which is measured colourimetrically at 510nm. The same method as for AST above was followed except the incubation of 40 minutes instead of 60 minutes and the substitution of AST substrate for ALT substrate. Serum protein concentration was determined by Biuret method of 1957 [21]. The principle behind this method is that protein forms complex with copper salts in alkaline solution, while the determination of serum albumin was done by the dye-binding method of Dumas et al,1971 [22]. This method is based on the fact that when a solution containing serum albumin is added to a buffered solution of bromocresol green (BCG) at pH 4.2, the BCG solution undergoes a change in colour as if there has been a shift in pH (especially to the alkaline side), when in fact there has been none. The colour produced is proportional to the albumin concentration.

2.2.3 Histopathological studies

The liver was fixed in 10% neutral formalin solution. After a week of fixing, the liver tissues were dehydrated with a sequence of ethanol solutions, embedded in paraffin, cut into 5µm 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 and infiltration of hepatocytes.

### 2.2.4 Statistical Analysis

Tukey-Kramer multiple comparisons test at 95% level of confidence was used to test for the significant differences in the activities of serum AST, ALT, total proteins, albumin and the globulins concentrations, and results expressed as mean ± S.D. The INSTAT3 statistical software was used.

III. Results

The results are presented in Table 2 and Plates 1-5. When the combination of Cd and Pb only were administered, the result of AST was significantly (P<0.05) different from control. But when Cd, Pb and Mg were administered concomitantly, the significant difference observed in the first instance was drastically reduced. There was no significant difference in the value of AST when Ca alone or the combination of Ca and Mg were concomitantly administered. The same trend was observed for the values of ALT, total proteins, albumin and the globulins (table 2). The histochemistry show that when Cd and Pb were administered without the addition of either Mg, Ca, or the combination of Ca and Mg, the liver histochemistry had enlarged nuclei with mass alteration of hepatocytes, diffused nuclei and wide sinusoids (Plate 2). There was enlarged nuclei with mild alteration of radial disbursement of hepertocytes when Mg alone was added to the combination of cadmium and lead (Plate 3). When Ca alone was added to the combination of Cd and Pb, the liver histochemistry showed normal hepertocytes and radial disbursement but with limited sinusoid (Plate 4). The addition of the Ca and Mg to the combination of Cd and Pb showed that the histochemistry of the liver had normal radial disbursement of hepertocytes.

Table2: The Effect of Concurrent Administering of Cadmium and Lead with the Addition of either Magnesium alone, Calcium alone or with the Combination of Calcium and Magnesium on Liver Enzymes, Total Proteins, Albumin and the Globulins in Rats.

| ALBUMIN (g/100ml)	GLOBULIN (g/100ml)	TOTAL PROTEIN (g/100ml)	ALT (IU/L)	AST (IU/L)	Treatments	Group
3.20±0.01	3.40	6.60±0.06	38.5±0.01	49.5 ±0.06	Control	1.
2.80±0.03	6.50	9.30±0.04	45.5±0.02	68.9±0.07	Cd+Pb	2.
3.01±0.05	4.39	7.40±0.01	40.0±0.03	59.1±0.04	Cd+Pb+Mg	3.
3.19±0.02	3.51	6.70±0.02	37.8±0.01	51.7±0.02	Cd+Pb+Ca	4.
2.29±0.03	3.40	6.69±0.06	39.0±0.01	50.1±0.08	Cd+Pb+Mg+Ca	5.
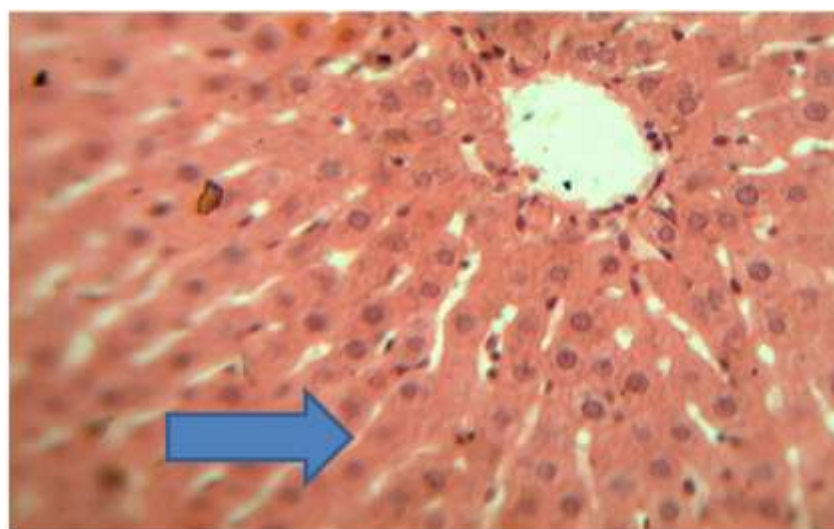
x 400

Plate 1: Liver section of the rats treated without the addition of Cd, Pb, Mg and Ca (control), showing normal hepatocytes radiating from central veins (arrow).
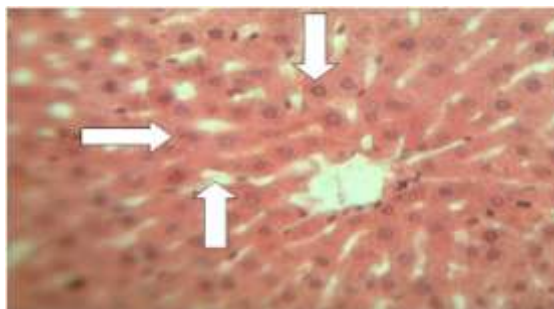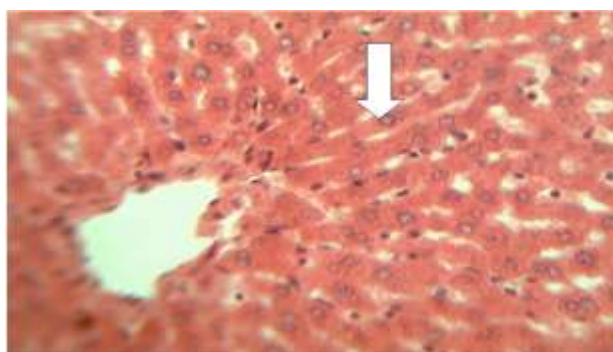
x 400

Plate 2: Liver section of the rats treated with the addition of 0.327mg/L and 0.079mg/L of Pb and Cd respectively, showing enlarged nuclei with mass alteration of hepertocytes (down-poiting arrow).The right-poing arrow shows diffused nuclei and the up-pointing arrow shows a wide sinusoid.
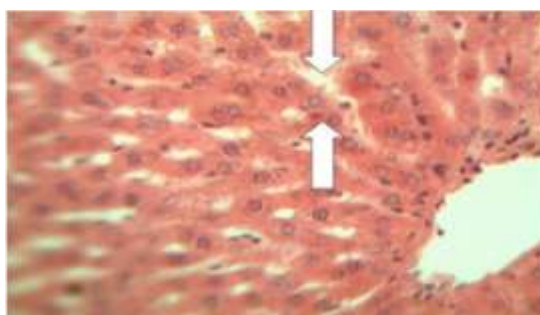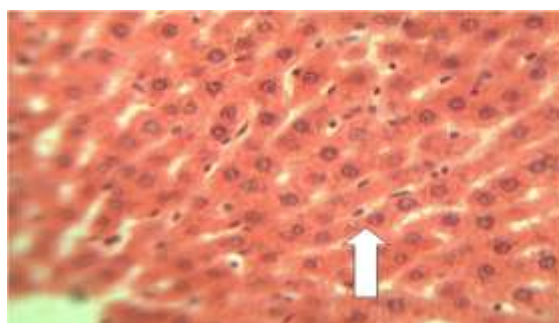

x400

Plate 3: Liver section of the rats treated with the addition of 0.327mg/L, 0.079mg/L and 0.248mg/L of Pb, Cd and Mg respectively, showing enlarged nuclei with mild alteration of radial disbursement of hepertocytes (up-pointing arrow). The down-pointing arrow shows wider sinusoids.

x400

Plate 4: Liver section of the rats treated with the addition of 0.327mg/L, 0.079mg/L0.248mg/L of Pb, Cd, and Ca respectively, showing normal and radial disbursement of hepertocyte with limited sinusoid.

x 400

Plate 5: Representative hepatic section of the rats treated with the addition of 0.327mg/L, 0.079mg/L, 0.248mg/L and 0.248mg/L of Pb, Cd, Mg and Ca respectively, showing normal radial disbursement of hepertocytes.

IV. Discussion

From the results, when $0.327 mg/L$ of Pb and $0.079 mg/L$ of Cd concentrations were administered without the addition of either Mg, Ca or the combination of Ca and Mg, there was significant difference between the liver of control group with this group as indicated by liver biomarkers and the histochemistry.This indicate that there was marked compromise of the liver integrity of this group. This is in agreement with the fact that when Cd and Pb are ingested either in food, water, or breathe in the air beyond the WHO admissible limits liver toxicity occurs [12, 23, 24]. But when the Cd and Pb concentrations above were administered concurrently with $0.248 mg/L$ of Mg, the hepatocytes showed enlarged nuclei with mild alteration of radial disbursement and wider sinusoids than those of control. This shows that Mg ameliorated the damage to the liver as was observed in group two (2), but not obliterated. Increasing the availability of essential micronutrients had proved in various studies to decrease the toxicity of toxic heavy metals. Zinc can increase synthesis of Metallothionine (MT), a thiol-rich protein that sequester cadmium and prevent acute hepatotoxicity, leading to chronic kidney toxicity as Cadmium-MT is excreted from the liver and absorbed by the kidney. Gastrointestinal lead absorption and retention constitutes the major pathway of lead intake and depends on the micronutrients status of the individual. Adults are said to absorb approximately 10% of ingested lead and small children absorb approximately 50% of ingested lead. From this studies, magnesium decreased the susceptibility of cadmium and lead intoxication in rats. This could be as a result of decrease intestinal absorption of cadmium and lead as a result of competition for similar binding sites on intestinal proteins which are important in the absorptive process. These shared binding sites on absorptive proteins could explain why sufficient dietary magnesium could decrease lead and cadmium absorption [25, 26, 27].When Ca was concurrently administered with Cd and Pb concentrations above, the histochemistry showed normal and radial disbursement of hepatocyte with limited sinusoid. There was no significant difference between the liver biomarkers of this group and control. This means that calcium alone has more hepatoprotective effect on Cd and Pb co-intixicated rat liver than Mg alone. The interaction between calcium and cadmium was made clear in the case of the itai-itai disease in Japan where women developed bone deformities, osteomalacia and an increased in osteoporosis. It was discovered that the women normal diet consisted of mostly rice and other grains. This is in agreement with the work of Ahamed and Siddiqui [28], who reported that Cd transport occurred by temperature-insensitive processes, probably Ca channel, and carriers that involved interaction with sulfhydryl groups. If that is so, then Ca channel will preferentially transport Ca leaving the other toxic metals Cd and Pb, since Ca was present in high concentration, leading to the insignificant difference between control and this group in the parameters analysed [29, 30].

When Ca and Mg were concurrently administered with the Cd and Pb concentrations above, there was no significant difference between the liver biomarkers of control and this group. The histochemistry of this group showed normal radial disbursement of hepertocytes which was not significantly different from that of control. This means that the combined hepatoprotective effect of Ca and Mg against Cd and Pb co-intoxicated rats was higher than Ca or Mg alone. This could be as a result of the fact that Cd and Pb have no specific transport proteins, but rather rely on their similarities in chemical and physical properties to the essential metals Ca and Mg for their transport and uptake into the cells by a process referred to as "ionic and molecular mimicry" [31]. Some studies have demonstrated that Cd and Pb ions are taken up by the divalent metal transporter 1 (DMT1), and the metal transporter protein 1 (MTP1), which are located in the basolateral and the apical membranes of the enterocytes respectively [32, 33, 34]. This work therefore points to the fact that disruption of essential metals homeostasis can lead to liver diseases and adequate intake of essential metals (Ca and Mg) can mitigate the toxicities of non-essential metals (Cd and Pb).

V. Conclusion

This study shows that the synergistic action of Ca and Mg have the greatest hepatoprotective effect, followed by Ca alone and then Mg alone last, against Cd and Pb hepatoxicities in rats. Based on these results, we recommend that people around the world who are at risk of exposure to toxic metals Cd and Pb should ensure a sufficient intake of Ca and Mg through enhance consumption of vegetables, fruits and foods which are known to be high in Ca and Mg. These metals are important natural antagonists to Cd and Pb toxicities and should be consumed on a regular basis. Providing livestock and farmed fish with the above-mentioned food interventions may also be helpful to reduce Cd and Pb exposure in humans.

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"

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