The Hepatoprotective Effects of Concomitant Administration of Calcium and Magnesium on Cadmium and Lead Co-intoxicated Rats

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ABSTRACT

Aims: To determine the hepatoprotective effects of concomitant administration of calcium (Ca) and magnesium (Mg) on cadmium (Cd) and lead (Pb) co-intoxicated rats.

Study Design: Wistar rats were divided into five groups of four rats per group in metabolic cages. Group one was fed with tap water only, while group two to five were fed with the combination of 0.327 mg/L Pb and 0.079 mg/L Cd concurrently with graded Mg and Ca.

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 food 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. At the termination of the experiments, the rats were humanely sacrificed under anaesthesia, sample of blood was obtained from each rat by decapitation. Serum was obtained from clotted blood by centrifugation and kept
frozen until required for the measurement of liver biomarkers, while the liver was identified and fixed in 10% formal saline for histopathological studies.

**Results:** Liver biomarkers in serum decreased as the concentrations of Ca and Mg were elevated. There was no significant difference (p>0.05) in the liver biomarkers concentrations of all the groups as compared with control. The histochemistry show that there was mild damage to the liver integrity at the lower concentrations of Ca and Mg but as their concentrations were elevated, there was no significant difference between the liver integrity of control and the test groups.

**Conclusion:** Results suggest that Ca and Mg could mitigate the hepatotoxicities induced by Cd and Pb in the rats.

**Keywords:** Hepatotoxicity; hepatoprotective; 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 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 determine the hepatoprotective effect of graded concentrations of the combination of Ca and Mg against the hepatotoxicity 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

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 178 g 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 Pb and 0.079 mg/L Cd; while Mg and Ca were varied thus: 0.165, 0.193, 0.221, and 0.248 mg/L respectively as shown in Table 1 below. The choice of Cd and Pb concentrations of (0.327 mg/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 test graded concentrations of Ca and Mg 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-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.

2.2.1 Blood collection

The rats were humanely sacrificed, and five to ten milliliters 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 [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 520 nm. 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 510 nm. 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 [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 [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.

| Metals | Group 1 (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
at x 400 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 and ALT, total proteins and albumin concentrations, and results expressed as mean ± S.D. The INSTAT3 statistical software was used.

3. RESULTS AND DISCUSSION

The results are presented in Table 2 and Plates 1-5. When the highest concentrations of Cd and Pb in the first phase were kept constant while Ca and Mg where concurrently varied, results of the liver biomarkers and histochemistry showed that the protection to the liver integrity increased as the concentrations of Ca and Mg were elevated. There was no significant difference (p>0.05) in the liver biomarkers concentrations of all the groups as compared with control. The histochemistry show that there was mild damage to the liver integrity at the lower concentrations of Ca and Mg (groups 2 and 3), but as their concentrations were elevated, there was no significant difference between the liver integrity of control and groups 4 and 5.

Table 2. Effect of concurrent administration of a constant concentrations of Cd and Pb with varying doses of Ca and Mg on liver as determined by liver biomarkers

| Groups | Treatments (ppm) | AST activity (I.U/L) | ALT activity (I.U/L) | Total protein (g/100 ml) | Albumin (g/100 ml) | Globulins (g/100 ml) |
|--------|------------------|---------------------|---------------------|------------------------|------------------|--------------------|
| 1.     | Control          | 8.10±0.11           | 14.32±0.01          | 16.60±0.40             | 7.70±0.18        | 8.90               |
| 2.     | Pb+Mg+Cd+Ca      | 9.80±0.06*          | 16.27±0.05*         | 18.81±0.04*            | 8.88±0.38*       | 9.93*              |
| 3.     | Pb+Mg+Cd+Ca      | 9.73±0.16*          | 15.97±0.18*         | 17.62±0.03*            | 8.75±0.18*       | 9.12*              |
| 4.     | Pb+Mg+Cd+Ca      | 7.88±0.12           | 14.17±0.21          | 16.48±0.14             | 7.60±0.44        | 8.88               |
| 5.     | Pb+Mg+Cd+Ca      | 7.98±0.30           | 14.20±0.31          | 16.98±0.09             | 7.88±0.36        | 9.10               |

Concentrations of Cd (0.079) and Pb (0.327) were constant for groups 2, 3, 4 and 5, while Ca and Mg had equally varied concentrations of 0.165, 0.193, 0.221 and 0.348 mg/L added to groups 2, 3, 4, and 5 respectively.

Twenty (20) rats were treated for fourteen (14) days. *Significant difference (p<0.05) between control and treatments 2 and 3 for all the parameters determined.

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)
Plate 2. Liver section of the rats treated with the addition of 0.327 mg/L, 0.079 mg/L, 0.165 mg/L and 0.165 mg/L of Pb, Cd, Mg and Ca respectively, showing round nodules of regenerating hepatocytes, signifying damage caused by the treatment (arrows)

Plate 3. Liver section of the rats treated with the addition of 0.327 mg/L, 0.079 mg/L, 0.193 mg/L and 0.193 mg/L of Pb, Cd, Mg and Ca respectively, showing mild hepatic damage as seen from the regenerating hepatocytes (arrows)

4. DISCUSSION

The results show that when 0.327 mg/L and 0.079 mg/L of Pb and Cd concentrations respectively were kept constant while Ca and Mg where concurrently varied, the liver biomarkers indicate that the protection to the liver integrity increased as the concentrations of Ca and Mg
were elevated. This is in agreement with our previous work [17] where kidney protection increased with increase in the concentrations of Ca and Mg. There was significant difference (p<0.05) in the serum AST, ALT, Albumin and total proteins concentration at the lower concentrations of Ca and Mg but there was no significant difference (P>0.05) at the higher concentrations of Ca and Mg as compared with control (Table 2). This suggests that the liver integrity was compromised. This is in agreement with the fact that animals fed with Cd in foods and water had compromised liver and other organ functions in animals [23-25]. Cd is first transported to the liver through the blood, where it binds with proteins to form complexes that are transported to the kidneys. This makes the liver the first target of attack, hence the injury to the liver as measured by the concentration of AST, ALT, total proteins and albumin in the serum.

Plate 4. Liver section of the rats treated with the addition of 0.327 mg/L, 0.079 mg/L, 0.221 mg/L and 0.221 mg/L of Pb, Cd, Mg and Ca respectively, showing a relatively normal hepatocytes radiating from central veins

Plate 5. Representative hepatic section of the rats treated with the addition of 0.327 mg/L, 0.079 mg/L, 0.348 mg/L and 0.348 mg/L of Pb, Cd, Mg and Ca respectively, showing normal hepatocytes (arrow)
The histochemistry of the liver showed that there was damage to the liver integrity at the lower concentrations of the combination of Ca and Mg, but as their concentrations were elevated, there was no significant difference between the liver 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 [26,27].

Pb 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 Pb is widely acknowledged. The greatest risk for harm, even with only minute or short term exposure, is to infants, young children, and pregnant women [28,29]. Toxic effects of Pb are typically broken down into two (2) categories: Acute (short term) and chronic (long term) effects [30].

In acute toxicity, effects show up relatively soon after exposure occurs. However, following ingestion of large amount of Pb, 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 [31].

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 [32]. For Pb, 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 liver cirrhosis, impaired Vitamin D balance and red blood cell problems [33].

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 liver disease.

Reproductively, effects of Pb 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 [34,35].

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 [36]. 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 [37,38].

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 [39,40]. 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 Ca and Mg treatment on the hepatotoxicities induced by a constant toxic concentrations of Cd and Pb.
showed that as the combination of Ca and Mg were increased, the toxic effects of Cd and Pb were obliterated just as was observed with the kidney in our recent work [18]. This raises some salient questions because it is a known fact that whenever Cd and/or Pb is/are present in drinking water above WHO's admissible limits of 0.001 mg/L and 0.05 mg/L respectively, liver diseases occur. 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 [18,41,42].

5. CONCLUSION

Our results provide evidence that Ca and Mg have hepatoprotective effects when concomitantly administered to Cd and Pb co-intoxicated rats. Therefore, people living in polluted areas could be advised to take foods rich in Ca and Mg so as to mitigate the hepatotoxicity that could occur as a result of ingesting foods or water (or both) containing concentrations of Cd and Pb above World Health Organization 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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