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Effects of experimental lead exposure and the therapeutic effect of defatted *Moringa oleifera* seed meal on serum electrolytes levels of Wistar rats

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Abstract

The study was conducted to evaluate changes in serum electrolytes concentrations following experimental lead acetate (Pb) exposure and the effects of treatment with defatted *Moringa oleifera* seed meal (DMOSM) in wistar rats. Eighty adult wistar rats divided into 5 groups of 16 per group were used for this study. Rats in group I received only distilled water, group II were administered Pb solution orally at 480 mg/kg body weight, group III were co-administered Pb and DMOSM orally at 480 mg/kg respectively, group IV were pre-administered Pb orally at 480 mg/kg for the first two weeks and then administered DMOSM orally at 480 mg/kg for the subsequent two weeks, group V received DMOSM orally at 480 mg/kg. Four rats were sacrificed weekly from each group and blood samples for serum electrolyte analysis were obtained. Phytochemical screening of DMOSM revealed the presence of alkaloids, reducing sugars, cardiac glycosides and saponins. A significant decrease ($P < 0.05$) in the mean serum Ca^{2+} concentration in the rats in group III (1.52±0.17) was observed at week 3 compared to group IV (2.24±0.16). Mean serum concentration of PO_4^+ at week 1 in rats in group III (3.62±0.15) was significantly higher ($P < 0.05$) compared to group IV (2.89±0.18). Similarly, serum PO_4^+ level significantly rose at week 3 in group II (4.40±0.36) compared to rats in groups IV (2.61±0.11) and V (2.99±0.07) respectively. By week 4, the serum PO_4^+ concentration rose significantly in the rats in group III (5.47±0.11) compared to those in group I (3.27±0.24). Mean serum concentrations of sodium (Na^+), chloride (Cl^-), potassium (K^+) and bicarbonate (HCO_3^-) were not significantly altered in all the test groups throughout the duration of the study. The result showed inability of DMOSM to mitigate Pb-induced alteration in serum Ca^{2+} and PO_4^+ levels in groups II and III.

Keywords: Lead, *Moringa oleifera*, Phytochemical screening, Serum electrolytes, Wistar rats

Introduction

Lead (Pb) is one of the most pervasive heavy metal contaminants in the environment (Krishna & Ramachandran, 2009). It is introduced into the environment and, consequently, to living organisms through air, water, food or soil (Ayodele & Abubakar, 2001; Ibeto & Okoye, 2010).

The induction of oxidative stress is a central sequelae in the mechanism of Pb toxicity (Olaleye *et al*., 2007; Ambali *et al*., 2011) via over production of reactive oxygen species (ROS) (Ibrahim *et al*., 2012) thereby enhancing lipid peroxidation, decreasing saturated fatty acids and increasing the unsaturated fatty acid contents of membranes (Malecka *et al*., 2001). The Ionic mechanism of action for Pb mainly arises due to its ability to substitute other bivalent cations such as Ca^{2+}, Mg^{2+}, Fe^{2+} and monovalent cations like Na^{+}, thus, affecting various fundamental biological processes of the body (Lidsky & Schneider, 2003) by damaging cellular components (Foyer *et al*., 2002). Lead can replace calcium, thereby affecting key neurotransmitters like protein kinase C, which regulates long term neural excitation and memory storage. It also affects the sodium ion concentration, which is responsible for numerous vital biological activities like generation of action potentials in the excitatory tissues for the purpose of cell to cell communication, uptake of neurotransmitters (choline, dopamine and GABA) and regulation of uptake and retention of calcium by synaptosomes (Bressler *et al.,* 1999; Gwaltney-Brant, 2002; Needleman, 2004; Garza *et al.,* 2006; Hontela & Lacroix, 2006).

Electrolytes play significant role in several body processes, such as controlling fluid levels, acid-base balance (pH), nerve conduction, coagulation and muscle contraction (Hasona & Elasbali, 2016). Fluid and electrolyte homeostasis is usually maintained within narrow limits (Roberts, 2005), and therefore, it must be kept at a level that is suitable for normal biochemical and physiological functions (Kaneko *et al.,* 2008). Therefore, imbalance in serum electrolyte is an important indicator of pollution in identifying target organs and general health status of animals (Shaista *et al.,* 2010; Chezhian *et al*., 2011; Ergonul *et al*., 2012).

Interest in medicinal plants as a re-emerging health aid in the maintenance of personal health and well-being has been fuelled by rising costs of prescription drugs, and the bioprospecting of new plant-derived drugs (Sharma *et al*., 2010). This have sparked remarkable surge of interest in chemoprevention research which has led to the identification of many phytochemicals of dietary origin as effective and potential chemo-preventive agents (Bharali *et al*., 2003). Researchers have explored the possible use of medicinal plants with antioxidant activity in protection against heavy metal toxicity (El-Nekeety *et al*., 2009). *Moringa oleifera* was demonstrated not only to suppress formation but even have strong free radical scavenging effect (Sofidiya *et al.,* 2006; Pari *et al.,* 2007; Ogbunugafor *et al.,* 2011).

This study was designed to understand the pattern of serum electrolyte disorders in lead toxicity with a view to arriving at the right choice of fluid therapy to institute in order to restore normal tissue perfusion in lead exposed patients. The study also explored the potential of using defatted *Moringa oleifera* seed meal in an attempt to correct lead-induced electrolyte imbalance in Wistar rats.

Materials and Methods

Collection and identification of the plant material

Moringa oleifera is a plant commonly grown in most parts of Nigeria. The seeds of *Moringa oleifera* were collected from Ruma, Batsari Local Government Area of Katsina state, Nigeria and were authenticated by a taxonomist at the herbarium of Department of Biological Sciences, Ahmadu Bello University, Zaria, and given a voucher number, 571.

Extraction of Moringa oleifera

The fresh seeds were allowed to dry in a shed under room temperature for two weeks. The dried seeds of the plant were pulverized to powdered specimen using a mortar and pestle. Exactly 522.750 g of the powdered seeds was weighed out and utilized for non-solvent extraction.

Mechanical Cold Press Extraction

This was carried out according to the method described by Fils (2000) with little modification. Briefly, 522.750 g of pulverized powder of *M. oleifera* seeds was mixed with 500 ml of lukewarm deionized water and the mixture made into paste by stirring with glass rod. The paste material was then transferred into an oven and left for 5 minutes at 40°C to remove the water content, after which it was transferred into and tied in a clean cheese cloth. This was then pressed using the mechanical oil press. The oil was extracted and the defatted cake was left in the cheese cloth. The defatted cake was kept on a shelf for 3 days to dry at room temperature and then removed and stored in refrigerator at 4°C.

Identification of phytochemical groups in the defatted Moringa oleifera (MO) seed meal

The defatted *Moringa oleifera* seed meal (DMOSM) was tested for various classes of compounds using the methods described by Trease & Evans (1996). The compounds that were tested for included Alkaloids (Dragendoff's test), Steroids (Salkowski test), Tannins (Lead subacetate test), Anthraquinones (Bontrager test), Cardiac glycosides (Keller-Kiliani test), Flavonoids (Sodium hydroxide test) and Saponins (Frothing test), which were reported to have biological activities on animal tissues (Kapadia *et al.*, 1978; Okwu & Josiah, 2006; Calderón-Montaño *et al*., 2011).

Experimental site and animals

This study was carried out in the Experimental Animal room of the Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. A total of 80 apparently healthy 8 to 10-week old Wistar rats (*Rattus novergicus*) were obtained from the Laboratory Animal Unit of National Institute for Trypanosomosis Research, Kaduna, Kaduna State, Nigeria. They were kept in steel cages in the experimental animal room of the Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Kaduna State, at an average room temperature of around 27°C and under 12/12-hour light dark cycle. The rats were allowed to acclimatize for fourteen days in the experimental animal room before the commencement of the experiment. All animals were handled in accordance with the standard guide for the care and use of laboratory animals (NRC, 1996).

Preparation of lead acetate solution and defatted Moringa oleifera seed meal

The lead acetate salt (Mayer and Baker®) solution was prepared by dissolving 8 g of lead acetate salt in 20 mL of deionized water to give 400 mg/mL concentration whereas defatted *Moringa oleifera* seed cake was ground to powder form using mortar and pestle, and then sieved. Exactly 5 g of the fine powder of the defatted *Moringa oleifera* seed cake was dissolved in 20 mL of distilled water to give the 250 mg/mL concentration of the seed meal used for this study. The respective concentrations were administered to the experimental rats at 480 mg/kg body weight. Unused solutions were stored in the refrigerator at 4^0C.

Determination of median lethal dose fifty (LD_{50})

The LD_{50} for lead acetate and DMOSM were carried out as described by Lorke (1983). A total of 12 rats for lead acetate and another 12 rats for DMOSM (test agents) were used for determination of the LD_{50}. The first phase involved nine rats divided into 3 groups with 3 rats in each group for each of the test agents. The respective test agents were administered at a dosage of 10 mg/kg, 100 mg/kg and 1000 mg/kg, once, orally, for the first phase and observed for 48-72 hours for neurological, behavioural changes and or, mortality. The second phase involved three rats which were divided into 3 groups with 1 rat in each group. The test agents were administered at a dosage of 1600 mg/kg, 3200 mg/kg and 4800 mg/kg respectively, once, orally. The rats were then observed for 48-72 hours for any sign of toxicity or mortality. Following the absence of obvious clinical signs and or mortality after the two phases of the LD_{50} determination by Lorke's method, one tenth ($1/10^{th}$) of the highest dosage (4800 mg/kg) was selected for the study *i.e.* 480 mg/kg.

Experimental Design

Eighty (80) wistar rats were randomly divided into 5 groups of 16. All the rats were fed daily with pelleted growers' marsh (Vital Feeds Ltd®., Jos) and water provided *ad libitum*. The grouping was as follows:

Group I: Rats served as negative control and received distilled water via drinkers daily for 28 days.

Group II: Rats received lead acetate solution (480mg/kg) by oral gavage daily for 28 days.

Group III: Rats received lead acetate solution and defatted *Moringa oleifera* seed meal at a dose of 480mg/kg, each, simultaneously by oral gavage daily for 28 days.

Group IV: Rats in this group received lead acetate solution at a dose of 480mg/kg by oral gavage daily for the first 14 days and, subsequently, defatted *Moringa oleifera* seed meal for the next 14 days through the same route and dose.

Group V: Rats in this group received defatted *Moringa oleifera* seed meal at a dose of 480mg/kg by oral gavage daily for 28 days.

Blood sampling for serum electrolytes determination

Blood samples were collected weekly from 4 rats per group during the 28-day experimental period. Five milliliter of blood was collected via jugular venesection from each of the four rats that were randomly selected from the respective experimental groups following light ketamine/xylazine (ketamine at a dosage of 22-50 mg/kg; xylazine at a dosage of 2.5- 10 mg/kg soaked in cotton wool) anaesthesia. The blood samples were dispensed into sterile sample bottles without anticoagulant and then

centrifuged at 3000 g for 15 minutes to obtain serum for electrolytes determination.

Serum electrolyte determination

The sera were carefully harvested into appropriately labeled plastic tubes and transported in ice packs to the laboratory for analysis. Serum samples were used for measuring the concentrations of sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), phosphate (PO_4), chloride (Cl^-) and bicarbonate (HCO_3^-) using the automated Audiocomb analyser (Bayer Express Plus, Bayer Germany, Serial Number 15950) in the Chemical Pathology Laboratory of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria.

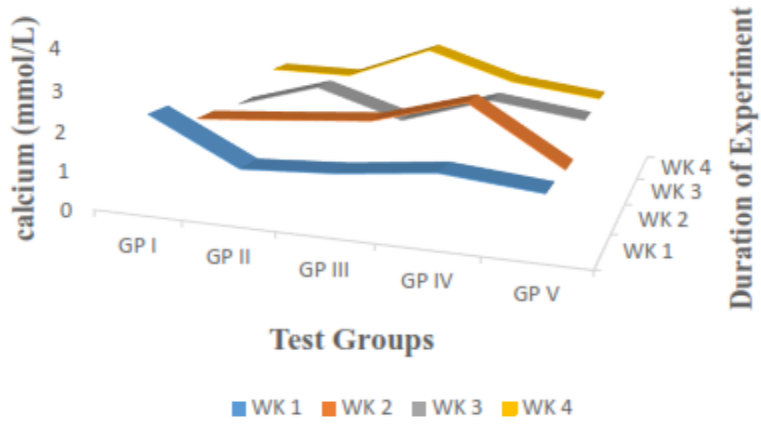

Figure 1: Mean ± SD of serum calcium (Ca) concentration from weeks 1-4. (P < 0.05)

Key: GP I (control group), GP II (administered Pb acetate), GP III (co-administered Pb acetate and DMOSM), GP IV (administered Pb acetate for 14 days and DMOSM for the subsequent 14 days), GP V (administered DMOSM) 480 mg/kg orally

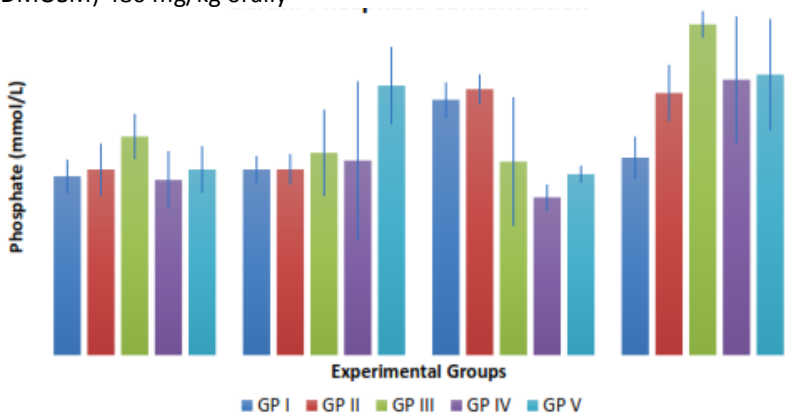

Figure 2: Mean ± SD of serum phosphate (PO4) concentration from weeks 1-4. (P < 0.05)

Key: GP I (control group), GP II (administered Pb acetate), GP III (co-administered Pb acetate and DMOSM), GP IV (administered Pb acetate for 14 days and DMOSM for the subsequent 14 days), GP V (administered DMOSM) 480 mg/kg orally

Data analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) using Graph Pad Prism version 5 and Tukey post hoc test. Values of P< 0.05 were considered to be significant. Data obtained were expressed as in charts.

Results

A significant (P < 0.05) decrease in the mean serum calcium (Ca^+) concentration was observed at day 21 (week 3) in the group co-administrated Pb acetate and DMOSM (1.52±0.17) when it was compared to the group that received only Pb acetate (2.25±0.17) as shown in figure 1 below. Significant (P < 0.05) increases in the mean serum phosphate (PO_4^+) concentration were observed in the rats' co-administered Pb acetate and DMOSM (3.62±0.15) at day 7 (week 1) of the study compared to those pre-administered Pb acetate and later treated with DMOSM (2.89±0.18) and also at day 28 (week 4) (5.47±0.11) when compared to the control group (3.27±0.24). At day 14 (week 2), a significant (P < 0.05) rise in the mean serum (PO_4^+) levels was also observed in the group administered DMOSM only (4.46±0.32) when compared to those in Group I (3.07±0.22), Group II (3.07±0.13), Group III (3.35±0.12) and Group IV (3.22±0.23) respectively. The mean serum phosphate (PO_4^+) levels rose significantly (P < 0.05) at day 21 (week 3) in the rats administered Pb acetate only (4.40±0.36) when it was compared to the group that was first pre-administered Pb acetate and subsequently treated with DMOSM (2.61±0.11), and the group that received DMOSM only (2.99±0.07) as shown in figure 2. The other electrolytes (Na^+, Cl^-, K^+, HCO_3^-) investigated were not significantly (P < 0.05) different when compared to the control group.

Discussion

The biochemical basis of lead toxicity is fundamental to understanding serum electrolytes alterations in lead-exposed patients. This is important with a view to proposing alternatives to diagnosis, treatment and prevention of lead poisoning menace.

The result from this study showed significant reduction in the mean serum Ca level in the group of rats' co-administered Pb acetate and DMOSM at the 3rd week post administration. The hypocalcaemia that was evident showed that DMOSM could not counteract the antagonistic effect of Pb on Ca metabolism. One of the toxic mechanisms of Pb on the molecular machinery of living organisms is its covalent binding to the Ca carrier proteins albumin and calmodulin, oxidative damage to cell membranes (Gurer-Ohrnan *et al.*, 2004) and close interaction on stereospecific sites for cations such as Ca^{2+} in bones (Garza *et al.*, 2006). The causes of hypocalcaemia in lead-exposed patients are complex and multifaceted, some of which include; competitive binding to the Ca-receptor sites at the epithelial surfaces of small intestine, thus, reducing Ca absorption (Bodgen *et al.,* 1995), negative Ca balance arising from Pb inhibition of the activation of vitamin D in the renal cortex (Chichovska & Anguelov, 2006; Gavazzo *et al*., 2008; Herman *et al*., 2009), hypoalbuminaemia, renal impairment and depressive effect of Pb on parathyroid function (Kristal-Boneh *et al*., 1998; Pekcici *et al*., 2010; Dongre *et al*., 2013) and increased intracellular accumulation of Ca due to lead-induced oxidative stress (Oyama *et al.,* 1996; Rajanna *et al.,* 1996). Reduction in free Ca and increased intracellular accumulation may disrupt Ca related signaling mechanisms and protein kinase C in the hippocampus. This causes long-term memory and cognition impairment in affected subjects (**Vazquez & Ortiz, 2004)**.

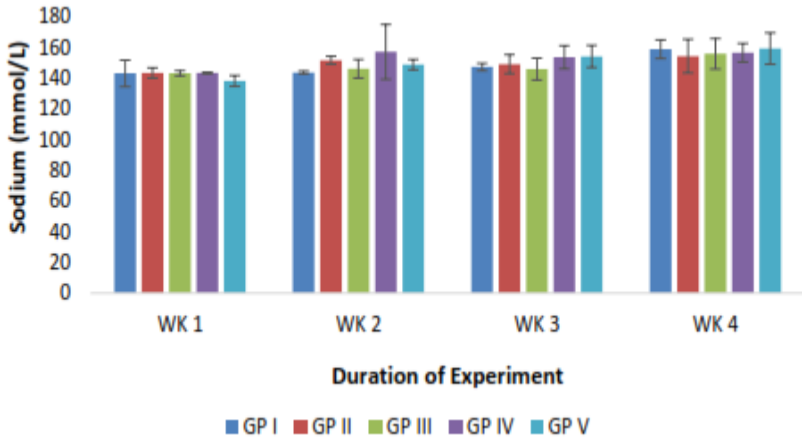

Figure 3: Mean of serum sodium (Na) from weeks 1-4. (P < 0.05)
Key: GP I (control group), GP II (administered Pb acetate), GP III (co-administered Pb acetate and DMOSM), GP IV (administered Pb acetate for 14 days and DMOSM for the subsequent 14 days), GP V (administered DMOSM) 480 mg/kg orally

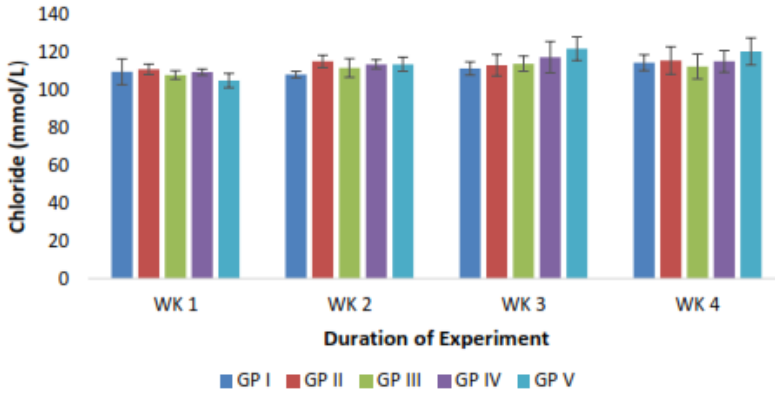

Figure 4: Mean of serum chloride (Cl^-) from weeks 1-4. (P < 0.05)
Key: GP I (control group), GP II (administered Pb acetate), GP III (co-administered Pb acetate and DMOSM), GP IV (administered Pb acetate for 14 days and DMOSM for the subsequent 14 days), GP V (administered DMOSM) 480 mg/kg orally

The hypocalcaemia observed in this study was similar to those by Hamadouche *et al.* (2009) who reported significant reduction in plasma Ca level following oral administration of Pb acetate to Wistar rats. It was also similar to those observed in Baladi goats following oral administration of Pb acetate (Abd El-Hameed *et al.,* 2008) as well as those of other authors in Pb associated toxicity (Srivastav *et al*., 2013; Kaya *et al*., 2015;). The steady rise in serum Ca level towards the end of the study may be due to the body's compensatory response to the shortfall in Ca via the secretion of parathyroid hormone (PTH) by the parathyroid glands. This in turn mobilized Ca from the hard tissues such as bones, increased intestinal absorption and stimulated the production of vitamin D by the kidneys (Esmaeeili *et al*., 2015; Cianciolo *et al*., 2016; Dhondup & Qian, 2017). Ultimately, serum Ca levels were restored in addition to maintaining normal phosphorus levels (Moe, 2008) in the absence of Pb exposure. The result also showed significant increases in the mean serum phosphate levels in the rats co-administered Pb acetate and DMOSM at weeks 1 and 4 of the study; rats administered only DMOSM at the 2nd week; and those that were

administered Pb acetate only at the 3rd week as shown in figure 2. Hyperphosphataemia upregulate phosphate-regulating hormone, which invariably suppresses renal 1-alpha hydroxylation of 25-hydroxyvitamin D3 (1, 25 $(OH)_2$ D_3) (Portale *et al.,* 1989; Bouillon, 2001; Holick, 2001; Gutierrez *et al.,* 2005. This may predispose to secondary hyperparathyroidism (Slatopolsky *et al*., 2001) and may hamper bone Ca release during resorption (Pastore *et al*., 2012). This in part, might be responsible for the hypocalcaemia observed in the Pb exposed group. The hyperphosphataemia in the DMOSM group might be due to excessive dietary levels that exceeded renal excretory ability and/or decreased urinary excretion (Weisinger & Bellorin, 1998). *Moringa oleifera* seeds have been reported to contain significant amount of phosphorus and proteins (**Compaoré *et al*., 2011**; Ogbe & Affiku, 2012). In contrast to our result, the serum level of phosphorus was unchanged in rabbits (Walid, 1997) and goats (Abd El-Hameed *et al.,* 2008) following oral administration of Pb acetate.

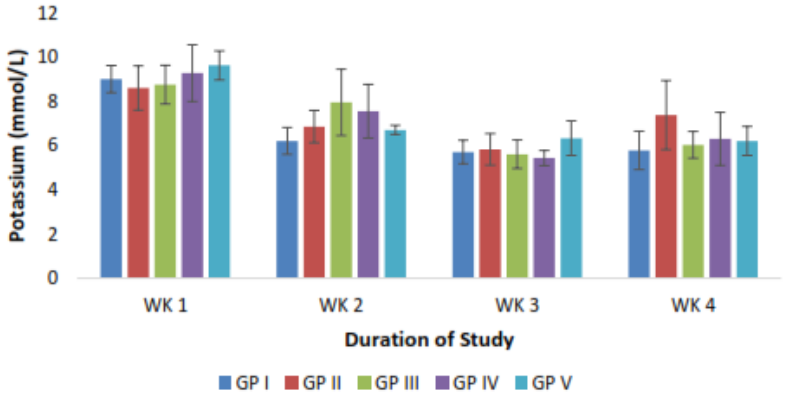

Figure 5: Mean of serum potassium (K) from weeks 1-4. ($P < 0.05$)
Key: GP I (control group), GP II (administered Pb acetate), GP III (co-administered Pb acetate and DMOSM), GP IV (administered Pb acetate for 14 days and DMOSM for the subsequent 14 days), GP V (administered DMOSM) 480 mg/kg orally

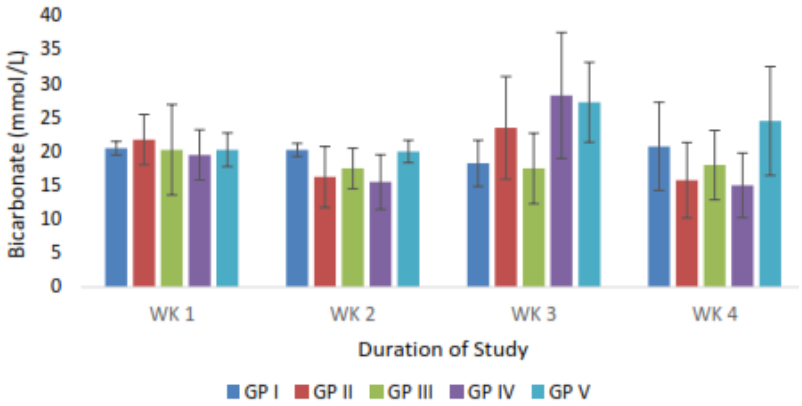

Figure 6: Mean of serum bicarbonate from weeks 1-4. ($P < 0.05$)
Key: GP I (control group), GP II (administered Pb acetate), GP III (co-administered Pb acetate and DMOSM), GP IV (administered Pb acetate for 14 days and DMOSM for the subsequent 14 days), GP V (administered DMOSM) 480 mg/kg orally

The homeostatic regulation of electrolytes maintains serum and intracellular levels, likewise, the optimal mineral contents in tissues. From our result, imbalances in Ca and PO_4 levels were most evidenced in all the electrolytes investigated. This imbalance might affect their homeostatic distribution and physiological roles, which is maintained through coordinated interaction of the intestine, the site of net absorption; the kidney, the site of net excretion; and the skeleton, the largest repository of these ions in the body (Favus *et al.,* 2006).

The findings from this study showed that Pb toxicity was the principal culprit in the observed hyperphosphataemia. The study also showed that defatted *Moringa oleifera* seed meal could not counteract the deleterious effects of Pb acetate in the exposed groups.

In conclusion, the results from this study showed significant decrease in serum levels of calcium in rats co-administered Pb acetate and defatted *Moringa oleifera* seed meal. The study also revealed significant increase in serum concentration of phosphate in rats administered Pb acetate only; those co-administered Pb acetate and defatted *Moringa oleifera* seed meal; and those that received defatted *Moringa oleifera* seed meal only. The study showed detrimental effect of Pb acetate on serum electrolytes characterized by hypocalcaemia and hyperphosphataemia. *Moringa oleifera* seed meal showed minimal effect in counteracting or reversing the detrimental effect of Pb acetate. It will be necessary to explore other extraction methods, modify the selected doses and to extend the duration of the experiment in future research.

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