*International Journal of Biochemistry Research & Review*
*4(3): 224-232, 2014*

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# Effects of Phosphide-powder Residue Contaminated Cowpea on Serum Magnesium and Trace Elements in Rats

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***Author's contribution***

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***Original Research Article***

***Received 25th January 2013***
***Accepted 21st April 2013***
***Published 30th January 2014***

## ABSTRACT

**Background:** Cowpea, a major source of vegetable protein for millions in Africa is mostly preserved using synthetic chemicals e.g. phosphide. Because a large number of people who carry out grain fumigation are largely untrained, sometimes contamination of fumigated grain with spent or unspent phosphide does occur.
**Objective:** The objective of this study is to determine if phosphide powder residue is capable of inducing alteration in serum concentrations of Mg, Zn, Cu, Se, Mn, Fe, Co, Mo, Cr in female rats.
**Methods:** Six rats were assigned to each of the three experimental groups. While the rats in the first group were fed untreated cowpea and served as control, the rats in the second and third groups were exposed to phosphide-powder residue contaminated and uncontaminated cowpea respectively.
**Results:** Estimation of elements in the serum of these rats revealed significant decreases ($p<0.05$) in the levels of Zn, Cu, Mn, Mg, Cr and Mo but non-significant ($p>0.05$) change in levels of Co, Se and Fe in the contaminated group. In the uncontaminated group, Mn and Mo were significantly decreased all other elements were not significantly changed.
**Conclusion:** The results of this study suggest that phosphide residue contamination of cowpea can induce serum Mg and trace element alteration in a mammalian species.

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*Keywords: Phosphide residue; cowpea; magnesium; trace elements.*

## 1. INTRODUCTION

Globally, growth in food production has been greater than population growth, with the amount of food per person increase occurring between the period of 1961 and 2005. But this pattern of growth is absent in Africa as well as some other parts of the developing world where food security is lacking. Food security is availability of food and one's access to it and as it has been well stated by World Resource Institute; it is a situation whereby the occupants of a household do not live either in hunger or fear of starvation. United Nations' Food and Agriculture Organization [1], though has defined food security as a situation in which all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life. While United States Department of Agriculture [2] revealed that food security exists for a household when access by all members at all times to enough food for an active, healthy life is guaranteed. According to this agency, this includes, that at a minimum availability of nutritionally adequate and safe foods, and ability to acquire acceptable foods in socially acceptable ways is ensured [2-4].

As well pointed out through an observation (World Resources Institute) that food security does not exist in many households in the developing world especially Africa, and buttressed by Ibeanu et al. [5], who have indicated that lack of food security may exist in Nigeria. The results of their study [5] carried out in two rural agricultural communities showed that more than a third of the households were not food secured and this was linked to spoilage and rodent/insect attack of crops as a result of inadequate preservation. Ideal food preservation has been described as the process of treating and handling food to stop or slow down spoilage, so as to prevent loss of quality, edibility or nutritional value and therefore allow for longer storage. To prevent such spoilage a number of preservative techniques have been put in place not only by the farmers but grain merchants in Nigeria. Although modified atmosphere has been suggested as a way of preserving grains [6,7], cowpea a cheap source of protein for many households in Nigeria is mostly chemically preserved using either acetallic acid or phosphide, although market survey proved that phosphide is the more commonly used of the two. A number of studies have been carried out to investigate the effect of phosphide contaminated feed on animals, but still there is dearth of data on the impact of phosphide on essential trace elements. This compound (phosphide) exists either as aluminum, zinc or calcium salt and one of the characteristics of trace elements is their ability to interact. According to Shenkin et al. [8] physiological interaction may occur between different trace elements and other essential major minerals. The aim of this study therefore, is to determine serum trace element concentrations in female rats fed phosphide powder-residue contaminated and uncontaminated cowpea.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Animals

The animal study was carried out in conformity with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research; as promulgated and adopted by United States Institutes of Health (1985). Female Wistar rats weighing between 240-270 g and supplied by the Animal House of the Department of Veterinary Physiology, University of Ibadan, Nigeria were used for the study. The animals were left to

acclimatize for one week prior to commencement of the experiment. All the animals were fed standard laboratory pellets and supplied water *ad libitum* during and prior to the commencement of the study. They were kept in cages at ambient temperature of 23±3ºC and a 12 h light, 12 h dark cycle. The eighteen rats used for the study were randomly divided into 3 groups of 6 rats each. While the rats in the 1st group were given Protex (manufactured by United Phosphorus Ltd, India) residue uncontaminated cowpea, those in the 2nd group were fed Protex residue contaminated cowpea. The 3rd group which served as the control was fed untreated cowpea.

## 2.2 Treatment of Cowpea

Protex **(**manufactured by United Phosphorus Ltd, India) which is made up of aluminum phosphide-57% and inert ingredients-43% was employed for the fumigation of cowpea. By using a ratio of 2 tablets of phosphide per $m^3$ of space, cowpea was fumigated at an average temperature of 29ºC for a period of 48 hours. Subsequent to the fumigation period, the wrapped phosphide powder residue was removed from the grains and the treated cowpea was divided into two. One part was deliberately contaminated with phosphide powder residue, i.e. residue of a quarter tablet of Protex was used to contaminate one kilogram of cowpea. Airing of the treated grains was carried out for 3 h before the commencement of the experiment at 32ºC. Specific cowpea type for each group was given to the rats over a period over 8 hours. The study lasted exactly 24 hours after which blood for trace elements analysis was obtained through retro-orbital bleeding.

## 2.3 Serum Trace Element

Blood obtained from each rat was discharged into an anticoagulant free bottle and left to clot. After centrifugation at 3000 g for ten minutes serum was separated from cells and stored at -20ºC until required for analysis. The estimations of serum concentrations of trace elements (Zn, Cu, Se, Mn, Fe, Co, Mo, Cr) and Mg were carried out using the atomic absorption spectrometric method. Buck Scientific 205 Atomic Absorption (Buck Scientific, East Norwalk, Connecticut, USA) was used for this purpose. Some other materials used for these estimations included high-purity analytical grade reagents supplied by Merck, Darmstadt, BDH, Chemicals Ltd; deionized, doubly distilled, and re-deionized water with specific resistance of > 3 MΩ was used for the preparation of reagents and working standard (Millipore Co., Bedford, MA). Working standard on the other hand, was prepared from the Spectrosol stock standard 1g/L (Buck Scientific). Samples and standards were diluted with a 2mM/L of aqueous solution of Triton X-100 (BDH Chemicals, Ltd). Both Bovine Reference Material and human serum Standard Reference Material were used to ascertain accuracy while dilutions were carried out with the aid of automatic pipetting system (PD 100; Crony Instruments s. r. l., Rome, Italy). Prior to the commencement of this analytical procedure all disposable apparatus was vigorously washed before use by immersing in concentrated nitric acid and thoroughly rinsed with the same deionized, doubly distilled and redeionized water. The operating characteristics of atomic absorption spectrometry for the analyzed elements are shown below Table 1.

**Table 1. The operating characteristics of AAS for trace elements and magnesium**

| | |
|---|---|
| Slit width | 0.7nm(Cu & Zn); 0.2nm (Se), Fe (0.2), Mg (0.7), Cr (0.7), Mo (0.7), Co (0.2) |
| Wave lengths(nm) | Fe (248), Mg (285), Cr (357), Mo (313), Co (240) |
| Burner height | Low |
| Gas mixture | Air – acetylene |
| Acetylene | 12 psi |
| Air pressure | 50 psi |
| Temperature | 2300ºC |
| Analytical mode | Concentration |
| Measurement mode | Peak area |
| Linear range (mg/L) | 5.00 (Cu); 2.50 (Zn); 25.00 (Se), 5.00 (Fe), 1.50 (Mg), 5.00 (Cr), 20.00 (Mo), 5.00 (Co) |
| Sensitivity (mg/L) | 2 (Cu); 0.5 (Zn); 15 (Se), 2.5 (Fe), 0.015 (Mg), 2 (Cr), 0.15 (Mo), 3.5 (Co) |
| Detection limit (mg/L) | 0.005 (Cu); 0.005 (Zn); 0.5 (Se), 0.05 (Fe), 0.005 (Mg), 0.04 (Cr), 0.80 (Mo), 0.05 (Co) |
| Atomizing air flow | 83µl / second |
| Lamp current | 18mA |
| Scale expansion | 3 |
| Noise suppression | 2 |

## 2.4 Statistical Analysis

The results obtained were subjected to statistical analysis using SPSS version 15. Results were expressed as mean±SEM. While Student's t test was used to determine the level of significance between each group and control, analysis of variance (ANOVA) was used to test inter-group differences; value of $P \leq 0.05$ was considered as significant.

## 3. RESULTS

Using Student's t test, female Wistar rats fed cowpea contaminated with phosphide powder residue exhibited significant changes ($p<0.05$) in the levels of many of the elements. Zinc and Cu were significantly decreased, Mn significantly increased although Se was not significantly different ($p>0.05$) when compared with control (Table 2). In Table 2 as well, results showed that this same set of rats manifested significant decreases ($p<0.05$) in the serum levels of Mg, Mo and Cr but levels of both Fe and Co were not significantly different ($p>0.05$) when compared with control. Rats fed phosphide residue uncontaminated cowpea on the other hand did not show significant alterations ($p>0.05$) in the levels of Zn, Cu and Se but the level of Mn was significantly reduced (Table 2). Moreover, results in Table 2 showed that exposure to phosphide residue uncontaminated cowpea did not result in any significant change in the levels of serum Fe, Mg, Cr and Co but the level of Mo was significantly decreased. Statistical analysis using analysis of variance revealed significant differences for Mg, Fe, Cu, Zn, Cr, Mn and Mo; Se and Co though were not significantly different.

**Table 2. Serum levels of elements in rats fed with phosphide residue contaminated cowpea**

| | Zn (µg/dl) | Cu (µg/dl) | Mn (µg/dl) | Se (µg/dl) | Fe (µg/dl) | Mg (mg/dl) | Cr (µg/L) | Mo (µg/L) | Co (µg/L) |
|---|---|---|---|---|---|---|---|---|---|
| A | 104.1±5.7 | 142.2±6.7 | 11.4±1.0 | 97.1±7.00 | 106.1±6.6 | 1 .7±0.1 | 2.2±0.3 | 2.3±0.2 | 0.14±0.0 |
| B | 80.6±7.4 | 119.1±7.1* | 14.6±1.3 | 100.5±10.1 | 110.1±9.1 | 1.2±0.1 | 1.4±0.2 | 2.0±0.1 | 0.14±0.1 |
| p-value | 0.002* | 0.021* | 0.035* | 0.659 | 0.381 | 0.009* | 0.006* | 0.005* | 0.241 |
| C | 98.5±5.0 | 140.9±4.0 | 8.0±1.0 | 98.3±9.5 | 104.4±1.9 | 1.79±0.1 | 2.2±0.4 | 1.9±0.1 | 0.13±0.0 |
| p-value | 0.709 | 0.530 | 0.019* | 0.703 | 0.461 | 0.422 | 0.328 | 0.001* | 0.319 |
| F-value | 45.09‡ | 98.16‡ | 145.52‡ | 0.059 | 51.18‡ | 89.33‡ | 19.17‡ | 61.04‡ | 0.556 |

*Results are expressed as mean ± standard error of mean. *p is significant when compared with control. Abbreviations: A-controls; B- contaminated; C- uncontaminated. ‡p is significant with inter-group comparison using ANOVA.*

## 4. DISCUSSION

Consumption of cowpea is a means of improving the nutritional content of meals in many household in some developing countries and studies have shown that when phosphide is used for cowpea fumigation according to the manufacturer's instruction, no or few negative effects have been reported since it is a fumigant that leaves very little residue on fumigated products. This study in which deliberate contamination of the treated cowpea with phosphide powder residue was carried out shows significant alterations in the levels of some of the trace elements in serum of exposed Wistar rats. Many of these trace elements have been recognized to play significant role; zinc, a metal moiety of many enzymes, plays an important role in normal cellular function, cell-mediated immunity, general immunologic defense of the host and tissue growth. Moreover, its role in maintaining structural integrity of enzymes and/or in taking part directly in catalysis has been recognized. Other elements like Cu, Mn, Se, Co e.t.c. are known to play significant role in a number of metabolic processes.

Cowpea is known to be a source of proteins and some minerals (e.g. Na, K, Zn, Cu, Mg), with its Mg, Zn, Cu and Mn levels earlier determined to be: Cu- 6.4+1.5; Mn- 21.7+1.0; Zn- 1030.0+211.2; Mg- 2477.0+111.5 (µ*g/g cowpea*). These results of significant alterations in the levels of many serum trace elements in rats fed phosphide powder residue contaminated cowpea compared with rats feed uncontaminated but also treated cowpea suggest that incorrect handling of the fumigation process may be a source of serious health hazard. The probable causes of these depletions are varied, one of which may be linked to their roles as important cofactors for numerous antioxidant enzymes, Zn and Cu are known catalytic components of superoxide dismutase. Phosphine gas a derivative of phosphide has been recognized to generate free radical in exposed animals and this it does by inhibiting cytochrome c oxidase [9]. Another study has identified that glycerophosphate dehydrogenase may have a very significant part to play in phosphine toxicity [10] via its oxidative role. Furthermore, results findings have shown that inhibition of the electron transport chain (ETC) in Drosophila melanogaster, resulted in significant superoxide generation from glycerophosphate dehydrogenase [11].

The findings of Price & Dance [12] and Bolter & Chefurka [13] though have revealed enhanced activities of superoxide dismutase instead of significant decreases, but a depression in the activities of peroxidase and catalase was still evident. An earlier observation [13] of significant decreases in the activities of catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase, glutathione S transferase as well as the significant elevation in the levels of oxidized glutathione and MDA is an indication that oxidative stress is also involved in the toxicity of phosphide powder residue contamination of cowpea. That is, a high level of free radical generation beyond the capability of the endogenous antioxidant system.

Trace elements and Mg have been identified as elements which play significant role in many cases of poisoning; in acetaminophen induced toxicity, the roles of Zn, and Cu as important cofactors for superoxide dismutase have been recognized [14]. Even in some cases of pesticide poisoning, in which free radical generation has been identified, trace element involvement which was evident by significant decreases has been suggested. For example, according to Monteiro et al. [15] methyl parathion (MP), an organophosphate that is widely applied in agriculture and aquaculture, induces oxidative stress as a result of free radical generation and changes in the antioxidant defense system.

The roles of selenium, one of the elements which though we observed not to be depleted were evaluated in Brycon cephalus exposed to 2 mg L(-1) and their findings suggest that dietary Se supplementation protects cells against MPc-induced oxidative stress. Zinc treatment has also been found to cause significant improvement in the levels of reduced glutathione, and enzyme activities of GST in both cerebrum as well as cerebellum in chlorpyrifos-treated animals. That the drastic reduction in the levels of some of these elements (Zinc, Cu, Mn, Se, Fe) may be due to their role as cofactors for antioxidant enzymes can also be deduced from the fact that Mg has been used with some measure of success to treat phosphide poisoning even in human subjects.

The study conducted by Baeeri et al. [16] to assess the impact of a new (25)Mg(2+)-carrying nanoparticle (25)MgPMC16) on energy depletion, oxidative stress, and electrocardiographic (ECG) parameters on heart tissue of the rats poisoned by aluminum phosphide (AlP), using (25)MgPMC16 doses of 0.025, 0.05, and 0.1 median lethal dose (LD50 = 896 mg/kg) and administered intravenously (iv) revealed that while AlP administration caused blood pressure (BP) and heart rate (HR) to be decreased. (25)MgPMC16 on the other hand significantly elevated the BP and HR at all doses used. Moreover, significant increase in antioxidant power, Mg level in the plasma and the heart as well as a decrease in lipid peroxidation and ADP/ATP ratio at various doses of (25)MgPMC16 occurred. Their observation therefore supports that magnesium in the form of (25)MgPMC16 can increase heart energy by active transport of Mg inside the cardiac cells. This is an indication that (25)MgPMC16 can play the role of an ameliorating agent in AlP-induced toxicity and cardiac failure, although the need for further study was highlighted.

Despite the fact that there is no specific antidote for phosphide toxicity, intravenous magnesium sulphate, has been reported to help in reducing mortality from 90% to 50% and this was linked to its membrane stabilizing action and antioxidant effect. It has also been suggested that hypomagnesemia might be responsible for the high mortality associated with this poison [17,18]. Other results findings that may also support that these trace element alterations may be oxidative stress-induced include the following; there are indications that toxicity of phosphine is known to be directly related to oxidant free radicals and associated inhibition of enzymes of metabolism, such as cytochrome c oxidase. Supportive treatment which sometimes has been indicated to involve the use of N-acetylcysteine suggests that cellular glutathione (an antioxidant) accompanies phosphide toxicity. Magnesium has also been suggested in the management of AlP toxicity [17,18]. Possible interaction between magnesium and any of the components of the residue used to contaminate cowpea might have hindered its absorption in the gastro-intestinal tract. The significant decreases in some of these elements might have been a cause of hepatic and renal damage that had earlier being associated with this residue [19].

## 5. CONCLUSION

Although there is scarcity of data on the effects of phosphide on serum elements, this study carried out in Wistar rats suggest that phosphide residue is capable of inducing alteration in element levels. Although many of these significant changes are being ascribed to toxic effects of phosphide residue; these alterations might have been caused to some extent mainly by aluminum, an important component of the residue. Therefore, further study is required to clearly identify the cause of alteration of many of these serum elements. This may be archived by feeding rats a diet containing the same content of aluminum without phosphide.

## COMPETING INTERESTS

Author has declared that no competing interests exist.

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