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Altered Serum Micronutrient Levels in Wistar Rats Fed Cooked Phosphide-Residue Powder Contaminated Cowpea

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ABSTRACT

Micronutrients, known for their important physiologic roles in maintenance of health, have been identified to be significantly affected in rats exposed to phosphide residue contaminated cowpea. Processing of such cowpea was carried out prior to their exposure to rats, so as to identify if cooking will modify the micronutrient depletion-characteristic property of such residue. Eighteen female Wistar rats were divided into 3 groups of 6 rats each. While the first group served as the control, the second and third groups were supplied cooked phosphide-residue contaminated and uncontaminated cowpea respectively. Blood obtained through retro-orbital bleeding was used for the estimation of Mg, Zn, Cu, Se, Mn, Fe, Co, Mo, and Cr as well as vitamins (folic acid, vitamins A, C, D, E, niacin, riboflavin). Results revealed non-significant differences ($p>0.05$) in the levels of all vitamins and minerals for the uncontaminated and contaminated groups except Zn, Cu and vitamin D that were significantly reduced ($p<0.05$) compared with control. These results therefore may be an indication that by processing phosphide-residue contaminated cowpea, its ability to alter serum micronutrient levels may be slightly reduced.

Keywords: micronutrients, phosphide residue, cowpea.

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INTRODUCTION

Micronutrients consisting of mainly vitamins and trace elements occur and are required in the body in minute quantities[1]. Despite the uniqueness of the amount required daily, they are known to play significant role physiologically, such that their depletion or deficiencies can have profound effects on an organism, which is the basis of "amplification of action" – one of the known characteristics of trace elements. The toxicity of many agents is usually determined through observation on the degree of tissue damage that occurs as a result of exposure and this usually happens through the use of indices of organ changes/damage; namely AST, ALT, urea, creatinine as in cases of assessment of hepato-renal impact as well as histologic assessment for a variety of organ.

In few cases the possible functions of some antioxidant-micronutrients have been investigated so as to determine their role in many of oxidative stress-induced organ damage in toxicity studies. But apart from their physiologic functions as antioxidants, both vitamins and trace elements are known for their role as cofactors for a number of enzymes. Zinc alone has been reported to be involved in the catalytic activities of more than 300 enzymes [2, 3]. This therefore implies, when the toxicity of an agent is determined, it may be essential to assess the impact of such an agent on micronutrient metabolism. For this reason the impact of phosphide-residue on a mammalian species is being investigated through this study.

Phosphide is the most commonly used grain fumigant world-wide. In Nigeria it is widely used for cowpea fumigation so as to prevent weevil infestation and post-harvest crop loss [4]. Cowpea is commonly consumed in Nigeria, for many households it is an affordable source of plant protein. Although direction for use of phosphide is available upon purchase as it is supplied with phosphide, because many of the grain merchants are illiterate, both its mode of use and the needed precaution to take in the course of application to grain are always largely neglected. Contamination of fumigated cowpea with phosphide residue is a common practice and two earlier studies[5] have demonstrated that it is capable of inducing significant changes in the serum levels of some of the micronutrients (vitamins A, C and E). Since prior processing of cowpea is common before its consumption by humans, cooking of phosphide-residue contaminated cowpea was carried out before it was fed to Wistar rats. It is hoped that this will help to determine the impact of such contamination on serum micronutrient levels, such that one may more accurately postulate the likely negative effect of such contaminations.

MATERIALS AND METHODS

Experimental Animals:

Female Wistar rats weighing of between 12 and 14 weeks were used for the study and kept in the Animal House of the Department of Veterinary Physiology, University of Ibadan. The animals were left to acclimatize for two week prior to commencement of the experiment. All the animals were fed with standard laboratory pellets and supplied water *ad libitum*. They were kept in cages at ambient temperature of 23±3°C and a 12 h light, 12 h dark cycle. A total of eighteen rats were used, and these were randomly divided into 3 groups of 6 rats each. The fumigation of cowpea took place over a period of 72 hours in an airtight container. Protex (aluminum phosphide- 57% inert ingredients- 43%) produced by United Phosphorus Ltd, India was used for this purpose. A ratio of 2 tablets of phosphide per m^3 of space was employed and the fumigation was carried out at the average temperature of 29 ºC.

At the end of the fumigation process, the grains were separated from the fumigant, and the treated cowpea was divided into two, and 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. Untreated cowpea was set apart for the control animals. Specific cowpea type for each group was cooked differently until tender and served to the rats over a period of 8 hours. The entire experimental procedure lasted exactly 24 hours after which blood for trace elements and vitamins estimation was drawn through retro-orbital bleeding.

Serum Trace Elements and vitamins estimation:

The blood collected from each rat was dispensed into an anticoagulant free bottle and left to clot. Subsequent to this, it was centrifuged at 3000 g for ten minutes; the serum obtained was kept at -20 ºC until required for analysis. The quantification of serum concentration of elements (Mg, Zn, Cu, Se, Mn, Fe, Co, Mo, Cr) were carried out using the atomic absorption spectrometric method as described in an earlier study (6). The serum levels of the following vitamins; folic acid, thiamine, niacin, riboflavin, and vitamins A, C, D and E were determined using the High Performance Liquid Chromatographic technique. While Buck Scientific 205 Atomic Absorption (Buck Scientific, East Norwalk, Connecticut, USA) was employed for the estimation of serum elements, the HPLC equipment supplied by Waters® Corporation Milford, Massachusetts USA was used for that of serum vitamins.

Statistical analysis

The results obtained were subjected to statistical analysis using SPSS version 15. Results were expressed as mean ± standard error of mean (SEM). Student's t test was used to test the level of significant difference between each group and control whereas analysis of variance was employed for inter-group comparison, a value of $P \leq 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Food poisoning cases especially those occurring from chemical preserved grains is a common occurrence in Nigeria. This has lead to a number of interventions not only from the academic environment but from the political class as well as the mass media. The causes are diverse, while clinical evaluations of patients have helped in many cases to identify many of the commonly misused chemicals; market surveys have also aided in identifying many of the wrong practices associated the fumigation process. That the cases of grain preservative-related deaths are frequently encountered in many developing countries is an indication that poverty and its twin-evil, illiteracy are some of the common causes of this problem.

This is because synthetic chemical preservatives that have been deemed acceptable not only for local grain market but international ones as well, have been chosen based on the fact that not only are they effective but they are also characterized with leaving minimum residue on fumigated grains. This has not been the case in Nigeria, studies abound that have raised the possibility, that greater than acceptable limits of preservatives are found on a variety of grains, crops or other food items in not only Nigeria but other parts of the world [7-10] and a source of health concern [11]. Although many reasons/causes can be suggested to be the basis of this high amount of residue on grain, one of them is contamination of grains with phosphide residue post-fumigation. In two of the earlier studies such contamination was identified to be a cause of tissue damage and micronutrient alterations in Wistar rats fed unprocessed phosphide-residue contaminated cowpea [5, 12] although when such contaminated cowpea was processed (cooked), its ability to induce hepato-renal damage was diminished in an acute setting but this may not necessarily rule out abnormal micronutrient presentation.

The present study in which significant decreases ($p < 0.05$) were observed for only vitamin D (Table 1) and two elements, the antioxidant elements- zinc and copper (Table 2) is an indication that damage can still result from such exposure. Although other micronutrients such as vitamins A, C and E were not significantly different ($p > 0.05$) compared with control as against significant decreases in the serum levels of these vitamins in rats fed unprocessed cowpea [5], the significant decrease in the level of only zinc and copper in this case is still an indication that by cooking such contaminated cowpea, the harmful effects of phosphide residue is not completely abolished but slightly reduced. Both zinc and copper are cofactors for the antioxidant enzymes superoxide dismutase; they are also known for their role in the catalytic activities of enzymes taking part in a number of other metabolic processes (13).

Table 1: **Serum vitamin levels of rats fed cooked phosphide –residue contaminated cowpea.**

	A	B	C	F-value	P-value
Vitamin A (µg/dL)	30.06±2.75	27.58±2.06	29.74±1.98	0.456	0.691
Vitamin C (mmol/L)	34.17±1.97	32.00±3.06	33.91±1.33	0.573	0.664
Vitamin E(mg/dL)	21.88±0.70	22.01±1.42	20.99±1.34	0.329	0.168
Niacin (ng/mL)	10.53±0.64	9.97±1.33	10.07±1.28	0.714	0.336
Riboflavin (µg/dL)	16.23±0.46	15.48±0.66	16.01±0.62	4.096	0.485
Folic acid (nmol/L)	22.01±0.69	23.03±0.92	24.06±2.61	0.419	0.540
Thiamine(nmol/L)	174.50±13.11	166.05±11.10	170.00±13.49	1.179	0.338
Pyridoxine(nmol/L)	109.09±18.00	100.69±10.16	107.19±19.62	0.059	0.874
Pantothenic acid(µmol/L)	1.86±0.17	1.70±0.21	1.88±0.09	1.139	0.336
Vitamin D(nmol/L)	63.03±3.49	50.07±3.68*	61.58±2.94	18.080	0.011*

Results are expressed as mean ± standard error of mean. *p is significant at $p \leq 0.05$. Abbreviations: A- control; B- phosphide-residue contaminated group; C- phosphide-residue uncontaminated group.

Table 2: Serum levels of magnesium and trace elements of rats fed cooked phosphide –residue contaminated cowpea.

	A	B	C	F-value	P-value
Zn (µg/dl)	128.33±19.05	97.63±12.12*	126.94±16.69	5.076	0.032*
Cu (µg/dL)	119.76±12.44	94.44±18.84	116.42±11.72	26.114	0.019*
Mn (µg/dL)	14.00±2.98	13.91±2.81	13.87±1.74	0.684	0.402
Se (µg/dL)	106.02±14.08	101.47±13.99	105.03±15.58	0.099	0.971
Fe (µg/dL)	119.70±5.28	116.33±4.96	115.06±8.02	1.056	0.653
Mg (mg/dL)	1.82±0.06	1.77±0.16	1.89±0.11	2.628	0.202
Cr (µg/L)	1.96±0.18	1.88±0.25	2.00±0.24	3.786	0.421
Mo (µg/L)	2.46±0.30	2.51±0.39	2.40±0.29	1.752	0.226
Co (µg/L)	0.14±0.03	0.14±0.05	0.13±0.03	0.076	0.883

Results are expressed as mean ± standard error of mean. *p is significant when compared with control. Abbreviations: A- control; B- phosphide-residue contaminated group; C- phosphide-residue uncontaminated group.

The decrease in levels of these two elements in the absence of significant alterations ($p>0.05$) in the levels of other antioxidant elements (Table 2) suggests decrease in absorption of Zn and Cu rather than extensive oxidative stress. Interaction between elements is one of the characteristic of trace elements; this is a situation in which the presence of an element can affect the level and therefore, the absorption and bioavailability of another. Both Zn and Cu are known to interact; zinc ions have the ability to block copper absorption by formation of intestinal metallothionein that strongly binds copper. Other food components that are not necessarily elements such as organic phosphate (phytic acid) and excess dietary calcium have been reported to form highly insoluble Ca-Zn-phytate complex (14). The aluminum phosphide residue that was used to contaminate the cowpea is known to contain aluminum and some inert materials. Therefore, these might have interacted with both Zn and Cu to prevent their absorption, consequently leading to low serum levels. Moreover, their significantly low levels may be ascribed to a probable distortion of some of the physiological processes that maintain the levels of these two elements e.g. release of Zn and Cu ions from intracellular compartment.

While the objective of this study is to verify if phosphide residue contamination of cowpea is one of the causes of cowpea-related food poisoning in Nigeria, the results obtained through the study and the past ones [5, 7] seem to suggest that. In addition to that is the fact that a number of banned chemicals may be in use by Nigerian grain merchants. That this is a possibility can be deduced from the report of Awofadeji[15]. He reported an incident of food poisoning resulting from consumption of a meal of beans that was suspected to have been preserved with chemicals occurred, an incidence which resulted in hospitalization of the many of the people involved with 10 of them being in critical condition.

The analysis of samples of the cooked beans consumed, samples of uncooked beans, maggi, palm oil, onions, salt and other condiments used in cooking the beans were tested in NAFDAC laboratories to investigate the cause of the problem. The result of the analysis revealed that samples of the cooked beans and the uncooked beans contained outrageously high levels of lindane, an organ chlorinated pesticide that was banned under the 1989 Rotterdam convention. Data obtained from this study that have revealed significant decreases for Zn, Cu and vitamin D suggests that consumption of cooked phosphide-residue contaminated cowpea may not be safe.

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