Vol. 4, No. 1, June 2014, ISSN: 2049-4963

www.ijbmsp.org

An investigation of the toxic effects of cooked phosphide-residue contaminated cowpea on wistar rats.

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Abstract – Pesticide related death is common in Nigeria. Many of these deaths have been identified to occur as result of misuse of these chemicals. For example, it was demonstrated in an earlier study that contamination of cowpea with phosphide residue post-fumigation is capable of considerable hepato-renal damage. The aim of this study is to determine if processing of such contaminated cowpea is capable of such degree of hepatic and renal damage. Eighteen female Wistar rats used for the study were randomly divided into 3 groups and fed cooked phosphide-residue contaminated cowpea for a period of 8 hours. After which blood was collected for the estimation of hepato-renal indices; tissues were also processed for histologic examination. None of the indices (alanine and aspartate amino transferases, alkaline phosphatase, γ-glutamyl transferase, total protein, albumin, globulin, urea, creatinine, uric acid) were significantly different ($p>0.05$) compared with control, histology result revealed no visible lesion for kidney section although mild infiltration of mononuclear cells were observed for liver sections. The results of this study suggest that processing of phosphide-residue contaminated cowpea is capable of modulating its hepato-nephrotoxic effects.
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Keywords: liver, kidney, phosphide residue, cowpea.

1. Introduction

Cowpea, *Vigna unguiculata (L.) Walpers*, an ancient crop, widely cultivated all over the world is a good source of vegetable protein for millions of Africans. Because large quantities of cowpea are lost during post-harvest period, a number of chemicals have been tried for its preservation. The history of grain fumigation shows that many of the chemicals that have been used as fumigants, include chloroform, carbon tetrachloride, ethylene dibromide, and phosphide [1]. Reports are also available that confirm the preservative properties of naturally occurring substances such as neem (*Azadirachta indica* A. Juss) and moringa (*Moringa oleifera*) seed oils [2]-[6]. Although phosphine is now the most prominent fumigant used for the preservation of grains both locally and internationally, many grain merchants in Nigeria who utilize this chemical do not use it in accordance with manufacturer's instruction, since among many anomalies, contamination of cowpea with phosphide residue does occur.

The results of an earlier study [7] in which rats were fed phosphide-residue contaminated cowpea revealed both hepato and nephrotoxic nature of phosphide residue, but cowpea is not consumed unprocessed by humans, as such the study could not help to identify if such contamination is a source of many of the medical complaints associated with cowpea consumption in Nigeria. This study in which processed (cooked) contaminated cowpea is fed to female rats may provide an insight into whether processing of phosphide-residue contaminated cowpea is also capable of causing such profound hepato-renal damage.

2. Materials and Methods

2.1. Animals Treatment

Female Wistar rats of average age of 13 weeks were used for the study. The animals were kept in the animal house of the Department of Veterinary Physiology, University of Ibadan and housed in cages. They were given standard rat pellets and supplied with water without any form of restriction. The animals were randomly selected and divided into 3 groups with each group comprising 6 rats. The fumigant used was Protex (manufactured by United Phosphorus Ltd, India). The fumigation period lasted 72 hours at average temperature of 29º C, the phosphide dust obtained was used for the cowpea contamination, and about a quarter of the tablet was mixed with 1 kg of treated cowpea that had been aired for 3 hours. Six rats consumed Protex powder-residue contaminated cowpea; six other rats were given uncontaminated type. The third group served as the control and the rats were fed untreated cowpea. The feeding period lasted 8 hours while the study was terminated 24 hours after the commencement of the procedure. Blood was drawn through retro-orbital bleeding. The study was in conformity with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research; especially as promulgated and adopted by United States Institutes of Health (1985).

2.2. Laboratory assessment of biochemical indices of hepato-renal damage

The following indices of hepato-renal function were estimated in the serum of each rat; activities of hepatic enzymes such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and γ-glutamyl transferase (ALT, AST, ALP & γ-GT), others were serum bilirubin, albumin, total protein, creatinine, urea and uric acid. While alkaline phosphatase (ALP) was estimated by the method of Mc Comb and Bowers [8]; AST & ALT were quantified using Bergmeyer et al. [9] method. Serum bilirubin and albumin were also quantified using modified Jendrassik-Groff [10] & standard bromocresol methods respectively. Serum level of total protein was measured by Biuret's method [11]; creatinine by the Jaffé reaction while the level of urea was also assessed using the diacetyl monoxime oxidase method. Hitachi® 902 automated machines (Roche Diagnostic, Germany) was used for these estimations.

2.3. Histopathological studies of rat kidneys

The liver and kidney of each rat was carefully removed, fixed in 10% buffered formalin and embedded in paraffin. Sections of five µm thickness were stained with hematoxylin-eosin (H and E) and observed under a microscope. Magnification was ×400.

2.4. Statistical analysis

Results of the indices of hepato-renal function are expressed as mean ± standard deviation. Significant difference between each of the treatment group and the control was established by using Student's t test. Analysis of variance was employed to observe inter-group comparison. SPSS package version 15 was used for this purpose. $P \leq 0.05$ was considered significant.

3. Results

Results of this study showed that by feeding Wistar rats with phosphide residue contaminated cowpea and phosphide residue free cowpea, there were no significant differences ($p>0.05$) in the serum levels of the hepatic enzymes: AST, ALT, ALP and γ-GT using Student's t test and ANOVA (**Table 1**). In **Table 2**, non-significant differences ($p>0.05$) were observed for other indices of hepatic function like bilirubin, total protein, albumin and globulin for the contaminated and uncontaminated groups when each was compared with control or when inter- group comparison were made among the three categories of rats. Moreover in **Table 3**, data presented showed that renal indices urea, creatinine and uric acid were not significantly different compared with control when Student's t test and ANOVA were employed to detect level of differences. Photomicrographs of liver sections of rats showing mild cellular infiltration by mononuclear cells for rats fed cooked phosphide-residue contaminated cowpea; no visible lesion for rats fed fumigated but uncontaminated cowpea and no visible lesion for control rats (**Fig. 1**). **Fig. 2** shows photomicrographs of kidney sections of rats fed cooked phosphide-residue contaminated cowpea; fumigated but uncontaminated cowpea and control (un-fumigated) featuring no visible lesion.

Table 1: Serum activities of hepatic enzymes of rats fed phosphide powder residue contaminated and uncontaminated cowpea

	AST (IU\L)	ALT (IU\L)	GGT (IU\L)	ALP (IU\L)
X ± SD (controls)	24.85±4.92	22.50±6.33	47.03±5.17	43.92±18.13
Contaminated	28.79±5.11	26.01±6.95	44.33±5.09	47.54±11.05
Uncontaminated	26.22±5.71	24.65±5.02	45.16±6.68	43.44±10.65

Abbreviations: AST- aspartate amino transferase; ALT- alanine amino transferase; GGT-γ- glutamyl transferase; ALP- alkaline phosphatase. Results are expressed as mean ± standard deviation. n=6

Table 2: Serum levels of bilirubin, total protein, albumin and globulin of rats fed phosphide powder residue contaminated and uncontaminated cowpea

	Bilirubin (µmol\L)	Total protein (g\dl)	Albumin (g\dl)	Globulin (g\dl)
X ± SD (controls)	6.03±0.94	7.08±1.09	3.29±0.62	3.79±0.31
Contaminated	7.43±0.75	6.99±1.25	3.32±0.53	3.67±0.59
Uncontaminated	5.06±0.66	7.21±0.99	3.40±0.60	3.81±0.45

Results are expressed as mean ± standard deviation. n=6

Table 3: Serum levels of urea, creatinine and uric acid of rats fed phosphide powder residue contaminated and uncontaminated cowpea

	Urea (mmol\L)	Creatinine (µmol\L)	uric acid (mmol\L)
Control X ± SD	4.48±0.61	49.06±6.78	197.14±25.09
Contaminated	4.59±0.70	53.06±5.59	189.00±26.98
Uncontaminated	3.99±0.65	50.06±5.05	190.00±34.81

Results are expressed as mean ± standard deviation, n=6.

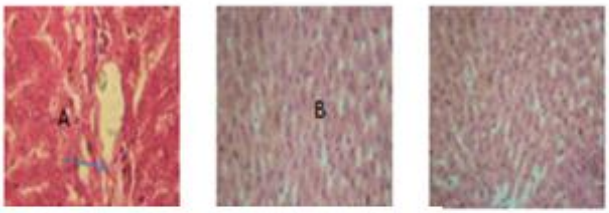

Fig. 1: Photomicrographs of liver sections of rats fed cooked phosphide-residue contaminated cowpea (A); fumigated but uncontaminated cowpea (B) and control (un-fumigated) (C). A- There is mild cellular infiltration by mononuclear cells; B- no visible lesion and C- no visible lesion. Mag. x400

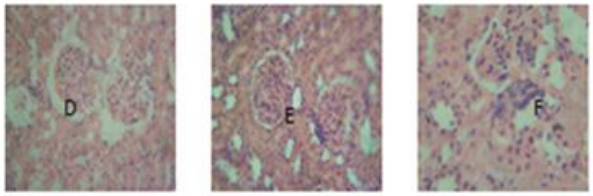

Fig. 2 Photomicrographs of kidney sections of rats fed cooked phosphide-residue contaminated cowpea (D); fumigated but uncontaminated cowpea (E) and control (un-fumigated) (F). D- no visible lesion; B- no visible lesion and C- no visible lesion. Mag. x400

4. Discussion

The liver an organ of great importance to the survival of an organism is made up an array of cell types that are both morphologically and physiologically distinct [12]. Because of these unique differences, each cell type react to chemical insult in its characteristic way, but overall, the liver is very useful in assessing the toxic nature of a substance. This organ is usually a target of study to determine the toxic response of an animal to xenobiotics; this is because of its rich source of the phase 1 enzymes of xenobiotic metabolism, which in most cases generate the reactive species responsible for the degree of organ damage [13]. Moreover, for orally administered agents, subsequent to absorption, the liver is the first point of call and therefore is exposed to a large quantity of an administered agent [14].

Several experimental reports have established that inhibition of cytochrome c oxidase enzyme plays an important role in phosphide toxicity. This understanding therefore makes all oxidative cells target organs of toxic response. Although studies abound that have described the response of different organs to phosphide exposure, this study has its focus on the effect of spent/unspent phosphide residue on rats. Earlier study carried out by Iyanda [7] revealed that when rats were fed phosphide-residue contaminated cowpea, damage to both the renal and hepatic cells occurred. That study was embarked upon to identify some of the causes of cowpea poisoning in Nigerian subjects but the data obtained from it could not be extrapolated to human subjects, because humans hardly take unprocessed cowpea. Among other processing techniques, cowpea is frequently cooked until tender, the results of that study revealed significant increases in the levels of the hepato-renal indices but results of the present study have revealed that by cooking contaminated cowpea, it was found to be uncapable of causing hepatotoxicity in this mammalian species. As such it can be deduced that food processing probably altered the toxicity of this residue. This is possible even when the residue is unspent (i.e. contained small quantities of phosphide); phosphine the active ingredient of phosphide is released upon contact with water, this means post cooking of cowpea only the component of inert material not altered by high temperature of cooking will be able to cause any toxic response in rats, hence the non-toxic response of both liver and kidney.

Hepatic intra-cellular enzymes ALT and AST were not significantly different ($p>0.05$) in phosphide residue exposed rats compared with control. In addition, the membrane-bound enzymes; GGT and ALP were also not significantly different ($p>0.05$) when compared with control. Histology result on the other hand, that revealed slight infiltration by mononuclear cells without significant biochemical hepatic changes can be linked to acute influence of Al on liver architecture. The slight infiltration by mononuclear cells without significant biochemical changes raises the possibility that continuous exposure to such contaminated cowpea even if consumed after cooking may cumulate into profound hepatotoxicity. This is because necroinflammatory process and elevated serum ALT and AST sometimes occur together in hepatotoxic animals [12]. Other hepatic indices such bilirubin, protein, albumin, globulin were not significantly different when compared with both control and fumigated but uncontaminated groups. The non-significant difference in the levels of renal indices (urea, uric acid, creatinine) as well as no visible lesion observed in the kidney sections of all three categories of rats further suggests that cooking modified the earlier observation in which renal damage was evident when rats were fed unprocessed phosphide residue contaminated cowpea.

5. Conclusion

Although most of the studies that have been carried out in the past in relation to phosphide and organ damage in experimental animal have always involved the use of the unspent compound; the results obtained from this study which involves the use of spent phosphide residue suggests that food processing can modify the hepato-nephrotoxic effects of phosphide, but the presence of mononuclear cells is a confirmation that exposure to the residue overtime may be equally dangerous and should be discouraged. Therefore, a study to probe the effects of chronic exposure is being recommended, but not only chronic study but the impact of this residue on levels of micronutrients will help in determining the overall possible harmful effects of phosphide residue contamination of cowpea. Micronutrients are known for their antioxidant activities the effects of their significant alterations may not be immediately reflect as significant tissue damage.

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