RESEARCH ARTICLE

TOXICOLOGICAL EFFECTS OF PHOSPHIDE POWDER RESIDUE IN FEMALE RATS

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DOI: 10.5455/ijmsph.2013.100620131 **Received Date: 26.09.2012** **Accepted Date: 10.06.2013**

ABSTRACT

Background: Fumigation of cowpea (Vigna unguiculata L. Walp.) using aluminium phosphide is a common practice and contact with this chemical has been demonstrated to result in organ damage, but there is dearth of data on the impact of its powder residue on a mammalian species.

Aims & Objective: The aim of this study is to determine the effect of post-fumigation residue on both liver and kidney of female Wistar rats.

Material and Methods: Eighteen rats were randomly divided into each of the three experimental groups. A group was fed with untreated cowpea and served as control; either of the two other groups was fed phosphide-powder residue contaminated or uncontaminated cowpea. Serum obtained from blood that was collected through retro-orbital bleeding was utilized for biochemical analysis.

Results: While contact with treated but uncontaminated cowpea did not result in either hepatic or renal damage, rats fed phosphide-residue contaminated cowpea exhibited both hepatic and renal damage as the indices (alanine & aspartate aminotransferases, γ- glutamyl transferase, alkaline phosphatase, total protein, albumin, urea, uric acid, creatinine) of study were significantly different ($p<0.05$) compared with control.

Conclusion: The results of this study suggest that phosphide residue may both nephro and hepatotoxic to female Wistar rats.

Key-Words: Liver; Kidney; Phosphide Residue; Cowpea

Introduction

Cowpea (Vigna unguiculata L. Walp.), is an annual legume. It probably got the name "cowpea" from its use as an important livestock feed for cows in the United States. Although Africa has been identified as the its origin, where it is widely grown, appreciable quantities are obtained from Latin America, Southeast Asia and in the southern United States. It is mainly used as a grain crop, animal fodder, or as a vegetable. The history of cowpea cultivation especially in association with sorghum and pearl millet dates back to about 5 to 6 thousand years.

Its production has continued to grow especially in some of these countries because its seed is a nutritious component in the human diet, as well as a nutritious livestock feed. Nutrient content of cowpea seed as revealed through a study is as follows; Protein- 24.8%, Fat- 1.9%, Fiber- 6.3%, Carbohydrate- 63.6%, Thiamine- 0.00074%, Riboflavin- 0.00042%, Niacin- 0.00281%. Two amino acids, lysine and tryptophan, are found more richly in cowpea protein than in protein content of cereal grains; but cowpea is deficient in methionine and cysteine when compared to animal proteins. For this reason, cowpea seed is appreciated more as a nutritional supplement to cereals and an extender of animal proteins.

Although cowpea is eaten more as seed, its use at all stages of growth as a vegetable crop has also been recognized. In Africa, its tender green leaves are an important food source being prepared as a pot herb, like spinach. Furthermore, immature snapped pods are prepared as snapbeans, usually when combined with other foods. Boiled, dry mature seeds have been found useful as components of meal as well as green cowpea seeds. While dry mature seeds can be canned, green mature seeds can be both canned and frozen. In many regions of the world (Africa, Asia, Europe, United States and Central and South America), cowpea is a food and animal feed crop. Not only the protein and vitamins level but mineral contents have also been determined.

Although this study is focused on preservation of dry seeds, many other parts of the cowpea crop are useful since they are rich in nutrients and fiber. In many parts of Africa, humans consume not only the young leaves but also immature pods, immature seeds, as well as the mature dried seeds. Apart from its usefulness as an edible grains; other uses include; its stems, leaves, and vines serve as animal feed and are often stored for use during the dry season. With all this usefulness, a good portion of cowpea is wasted each year. Slightly less than fifty-two percent of the quantity produced in Africa is used for food, 13% serve as animal feed, while 10%, 9% and 16% are used as seeds, other uses and wasted respectively. To prevent such wastage a number of preservative techniques have been put into use, most notable of them being the use of phosphide. Human/animal contact with this chemical has resulted in death in some cases, and since contamination of cowpea is possible in the course of fumigation, this study is designed to identify the hepatotoxic and nephrotoxic effect of phosphide powder residue on female Wistar rats.

Materials and Methods

Female Wistar rats (250 g) used for this study were purchased from the Department of Veterinary Physiology, University of Ibadan and housed in cages. They were given standard rat pellets and given water without any form of restriction. They were divided into 3 groups, with each group comprising 6 rats. The rats in 1st and 2nd groups were fed with cowpea that had been previously treated with Protex (manufactured by United Phosphorus Ltd, India), while rats in the 1st group consumed Protex powder-residue contaminated cowpea, the ones in the second group were fed uncontaminated type. The fumigation period which lasted 48 hours at average temperature of 29° C resulted in the conversion of Protex tablet into powder. After the period of fumigation the derived powder of about a quarter of the tablet was mixed with 1 kg of treated cowpea that had been aired for 3 hours. The third group served as the control and rats were fed untreated cowpea. The study was terminated exactly 16 hours after the end of the feeding period; the feeding period itself lasted 8 hours after which blood was drawn through retro-orbital bleeding. The study was executed 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).

Clinical Chemistry assay

Activities of hepatic enzymes such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and γ-glutamyl transferase (ALT, AST, ALP & γ-GT) were determined in the serum of each rat. While Bergmeyer et al[1] method was used for the estimation of AST & ALT, alkaline phosphatase (ALP) was determined using the method of Mc Comb and Bowers[2]. Serum bilirubin and albumin on the other hand were quantified using modified Jendrassik-Groff[3] & standard bromocresol methods respectively. Serum level of total protein was measured by Biuret's method.[4] Creatinine was estimated by the Jaffé reaction while the level of urea was also measured by the diacetyl monoxime oxidase method. Hitachi® 902 automated machines (Roche Diagnostic, Germany) was used for these estimations.

Statistical Analysis

Results obtained from blood biochemistry estimations are expressed as mean ± SD (standard deviation). By using Student 't' test the degree of significant difference between each of the treatment group and the control was established. Analysis of variance was employed to establish inter-group comparison. SPSS package version 15 was used for this purpose. $P \leq 0.05$ was considered significant.

Results

The results of this study are presented in Tables 1-3 below. In Table 1 serum activities of hepatic enzymes; namely ALT, AST, ALP and γ-GT of rats fed phosphide powder residue contaminated cowpea were significantly increased ($p<0.05$) compared with control. The activities of these enzymes were not significantly different ($p>0.05$) in rats fed phosphide residue uncontaminated cowpea. On the other hand, as shown in Table 2 while bilirubin, total protein, albumin were significantly reduced in rats fed contaminated

cowpea compared with control, rats fed uncontaminated cowpea exhibited non-significant differences (p>0.05) in the serum levels of bilirubin, total protein, albumin and globulin. Investigation of nephrotoxic effect of this residue also revealed significant increases in the indices of renal indices, urea, creatinine and uric acid in rats phosphide residue exposed rats compared with control but those of rats fed uncontaminated cowpea presented with non-significant differences in the levels of these indices as shown in Table 3

Table-1: Serum Activities of Hepatic Enzymes of Rats Fed Phosphide Powder Residue Contaminated and Uncontaminated Cowpea

Groups	AST (IU\L)	ALT (IU\L)	GGT (IU\L)	ALP (IU\L)
Controls	35.96 ± 8.91	32.08 ± 11.04	39.12 ± 4.36	50.74 ± 18.13
Contaminated	406.75 ± 80.06*	317 ± 58.78*	183.00 ± 29.96*	188.41 ± 30.04
Uncontaminated	34.72 ± 10.59	34.09 ± 9.79	32.02 ± 4.66	49.09 ± 14.24
F-value	72.50	79.92	190.66	134.07
P-value	0.001**	0.006**	0.004**	0.002**

AST: Aspartate Amino Transferase; ALT: Alanine Amino Transferase; GGT: γ- Glutamyl Transferase; ALP: Alkaline Phosphatase. Results are expressed as mean ± standard deviation. *P < 0.05 is significant when compared with control using Student 't' test. **P < 0.05 using ANOVA, n=6

Table-2: Serum Levels of Select Hepatic Indices of Rats Fed Phosphide Powder Residue Contaminated and Uncontaminated Cowpea

Groups	Bilirubin (µmol\L)	Total Protein (g\dl)	Albumin (g\dl)	Globulin (g\dl)
Controls	8.61 ± 2.14	5.96 ± 0.17	3.48 ± 0.42	2.80 ± 0.19
Contaminated	13.57 ± 3.39*	5.39 ± 0.08*	2.71 ± 0.31*	2.68 ± 0.29
Uncontaminated	8.73 ± 3.56	6.01 ± 0.22	3.32 ± 0.14	2.71 ± 0.31
F-value	4.98	3.17	3.56	0.984
P-value	0.019**	0.024**	0.018**	0.0.319

Results are expressed as mean ± standard deviation. *P < 0.05 is significant when compared with control using Student 't' test. **P < 0.05 using ANOVA, n=6

Table-3: Serum Levels of Urea, Creatinine and Uric Acid of Rats Fed Phosphide Powder Residue Contaminated and Uncontaminated Cowpea

Groups	Urea (mmol\L)	Creatinine (µmol\L)	Uric Acid (mmol\L)
Controls	3.44 ± 0.59	57.79 ± 8.94	211.38 ± 87.56
Contaminated	16.57 ± 4.11*	223.41 ± 44.16*	463.16 ± 60.51*
Uncontaminated	3.60 ± 1.24	59.74 ± 11.04	230.786 ± 80.07
F-value	70.09	177.90	26.04
P-value	0.010**	0.001**	0.006**

Results are expressed as mean ± standard deviation. *P < 0.05 is significant when compared with control using Student 't' test. **P < 0.05 using ANOVA, n=6

Discussion

Phosphide toxicity in man and experimental animals has been widely reported[5-9] and has been linked to phosphine gas which it releases when it reacts with acid or water. When sufficient quantity is inhaled, phosphine affects the liver, kidneys, lungs, nervous system, and circulatory system. Effects on liver usually translate to elevated serum GOT, LDH and alkaline phosphatase, reduced prothrombin, haemorrhage and jaundice while those on the kidney are kidney haematuria and anuria with characteristic feature of abnormal serum renal biochemistry i.e. an elevation in urea and creatinine levels. Ingestion of phosphide though causes lung and brain symptoms but damage to the viscera seems to be more common.

Currently, phosphine fumigation of cowpea is the major means of controlling weevil infestation and it seems that reliance on phosphine will continue because of international regulatory and market acceptance of this chemical and absence of viable alternatives.[10,11] The general acceptance of phosphine as a fumigant started in the 1980s when public concern over chemical residues in foods became more pronounced in the United States, at that time potentially hazardous amounts of ethylene dibromide (EDB) were detected in several finished grain-based products by governmental food-monitoring laboratories.[12] Consequently, the use of EDB was banned by the U.S. Environmental Protection Agency in 1983 and commercial fumigators resorted more to the use of highly volatile chemicals e.g. methyl bromide and phosphine, EDB is relatively non-volatile. Highly volatile chemicals such as methyl bromide and phosphine are less likely to leave residues on stored crops than the other fumigants that had been used in the past (e.g. EDB, chloroform, and carbon tetrachloride).

Our market survey showed that phosphide is the most commonly used material for the fumigation of cowpeas in Nigeria and literature has also confirmed this for many other parts of the world. Increase outcry against the use of chemical fumigants for the preservation of this crop though has occurred repeatedly in the Nigerian environment because their use has not been

without some negative effects.

Exposure to phosphide has been associated with a number of tissue damage, in animals[13,14] and in man, when it was either accidentally ingested or employed for suicidal reasons[15]. The results of this study, of rats fed with phosphide treated but uncontaminated cowpea revealed non-significant differences in the levels or activities of the indices of hepatic and renal function. Specifically, biochemical parameters e.g. ALT, AST, GGT, bilirubin, urea, creatinine etc. employed to assess both renal and hepatic damage revealed non-significant increases ($p \geq 0.05$) in these rats when compared with the control.

Ingestion of phosphide residue powder by rats though caused significantly higher activities of ALT & AST. Moreover, the membrane-bound enzymes; ALP & GGT were also significantly different ($p \leq 0.05$) when the rats in contaminated cowpea group were compared with control. That both ALT & AST were higher may be an indication that there was involvement of necroinflamatory process; Dafour[16] has indicated that both ALT & AST are elevated in necroinflamatory liver diseases. Urea, creatinine and uric acid were also significantly different ($p \leq 0.05$) in rats in contaminated cowpea group compared with control, which portrays the presence of renal damage.

In phosphide residue exposed rats, total protein and albumin levels were not significantly different compared with control, which is a confirmation of past findings that treatment of cowpea with phosphine gas does not alter the protein and fat contents of cowpea. Moreover, this also suggests that by treating cowpea with phosphide, digestion and absorption of protein contents as well as the metabolism of derived amino acids were not affected in rats, but both the serum levels of albumin and total protein were significantly different in rats in phosphide-powder residue contaminated cowpea group.

In most toxicology studies, indices of both hepatic and renal damage are used to assess the toxicity of substances. This is because these two tissues are vital to the survival of an organism. Moreover, the liver has been identified as an organ that plays both central and critical biochemical roles in the metabolism, digestion, detoxification and elimination of substances from the body. Moreover, it is also the site where exogenous compounds are bio-transformed and converted to excretable substances. Both the liver and kidney are rich in the cytochrome P450 which are necessary for this biotransformation, but phosphine the active metabolite of phosphide is made up of one molecule of phosphorus and three molecules of hydrogen. Both elements occur abundantly in the body, phosphorus in the form of hydroxyapatite, but when both occur in form of phosphine, they are toxic to the body. Toxicity of phosphine is known to be directly related to inhibition of enzymes of metabolism, such as cytochrome c oxidase[17], this can take place in all oxidative cells. Which means its toxic effect is not limited to these two organs- liver and kidney. By inference, it may be suggested that effect of toxicity may be observed in other tissues. This is likely since many of the symptoms which were identified in human consumers of treated beans are usually more gastrointestinal tract (GIT) related[18] and the GIT is another rich source of CYP and there is dearth of data on the possible modulatory role of phosphide residue on the activities of different isoforms of CYP.

What will make these results a source of major concern is the fact that although phosphide is the predominant fumigant used for the preservation of cowpea world-wide, in many cultures in Africa, cowpea is rarely consumed in isolation, rather it is combined with some food types such as grains (maize, guinea corn, millet) and cassava product, many of which have been preserved on the field as well as post harvesting period, with other pesticides. Aside this, the use of pesticides on the cowpea plant itself does occur. This is because pest attack on cowpea is common during every stage of its life cycle. While as early as the seedling period aphids extract juice from its leaves and stems and spread also the cowpea mosaic virus. Flower thrips feed on it during flowering, pod borers attack its pods during pod growth, and bruchid weevils attack the postharvested seeds. In addition to this, cowpea plants can also be attacked by diseases caused by fungi, bacteria and viruses. Striga and Alectra (parasitic weeds)—may adversely affect the plants growth at all

stages while nematodes prevent the roots from absorbing nutrients and water from the soil. All these may require the use of pesticides. That such is a possibility has been confirmed by a government agency (Oyo State Agricultural development programme) set up to among other things oversee the general agricultural activity in the state, which has confirmed the usage of not only herbicides but insecticide in the course of cowpea life cycle. Even in Europe where a more stringent measure of pesticide use is in place, approximately 300 different pesticides have been reported as contaminants of food products of European origin with as much as 50 percent of fruits, vegetables and cereals grown in the European Union known to contain pesticide residues.[19]

With this understanding, interaction among these various pesticides may aggravate the degree of toxicity observed above and may be a cause of many of the negative effects associated with grain fumigant use in the Nigerian environment. This is probable because NAFDAC has revealed that laboratory analysis reports of the moi-moi (a meal derived from cowpea) and beans (cowpea) from the homes of the victims and beans from the open market in both Benue and Taraba State, Nigeria (places where incidence of cowpea poisoning has been reported) contained outrageously high organophosphates, carbamates, fenithrothion and chloropyrifos which are highly toxic pesticides.[18] Some of these are used in the field.

Conclusion

The results of both the hepatic and renal indices suggest that proper handling of the fumigation process excluded significant toxicity to both liver and kidney whereas contamination of cowpea with partially spent phosphide caused toxicity in these two organs. Therefore there is the need for public awareness among those that carry out grain fumigation using phosphide that exposure of an animal to the residue may lead to significant health hazards. Moreover, because many other tissues have been identified to be susceptible to phosphine gas, a more comprehensive study is required to investigate if this residue is capable of causing damage to organs such as the brain, lung, heart, intestine, etc.

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Cite this article as: Iyanda A. Toxicological effects of phosphide powder residue in female rats. Int J Med Sci Public Health 2013; 2:806-810.
Source of Support: Nil
Conflict of interest: None declared