Oxid Antioxid Med Sci 2013; 2(1):55-59 **ISSN: 2146-8389**

GESDAV

Oxidants and Antioxidants in Medical Science

available at www.scopemed.org

Original Article

Assessment of oxidative stress status and select heavy metal levels in serum of rats fed cooked phosphide powder residue contamined cowpea

Ayobola A. Iyanda, Oliatan A. Adeomi

Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria.

Received January 3, 2013
Accepted March 6, 2013

Published Online March 28, 2013

DOI 10.5455/oams.060313.or.030

Corresponding Author
Ayobola Iyanda
Department of Chemical Pathology,
College of Health Sciences,
Ladoke Akintola University of Technology, Osogbo, Nigeria.
lapeiyanda@yahoo.com

Key Words
Cowpea; Heavy metal; Phosphide residue; Wistar rat

Abstract

Although it is widely believed that the mechanistic mode of action of the toxic effects of phosphide is inhibition of cytochrome c oxidase enzyme, the involvement of oxidative stress has also been reported when animals were exposed to its post-fumigation residue. The aim of this study is to determine whether prior food processing (cooking) of phosphide residue contaminated cowpea will affect its ability to induce oxidative stress or not. Eighteen Wistar rats were divided into 3 groups of 6 rats each. The twelve rats in groups 1 and 2 were fed with cooked phosphide residue contaminated and uncontaminated cowpea, respectively, while the rats in group 3 were fed cooked untreated cowpea and served as control. Activities or serum levels of indices of oxidative stress as well as serum levels select heavy metals were determined. Results of the study revealed that while reduced glutathione and reduced/oxidized glutathione ratio were significantly reduced compared with control; malondialdehyde, catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione S transferase were not significantly changed. Moreover, none of the heavy metals (Al, Cd, Si, As, Pb) were significantly changed compared with control. The results of the study suggest that when phosphide residue contaminated cowpea was cooked before being fed to Wistar rats its oxidative stress potential was altered.

© 2013 GESDAV

INTRODUCTION

Oxidative stress occurs in living organisms if there is imbalance between the levels of oxidants and antioxidants. Endogenous antioxidant enzymes are the first-line cellular defense against oxidative stress; this is because they decompose oxygen and hydrogen peroxide (H_2O_2) before they interact to form the more reactive hydroxyl radical. Rao and Viswanath [1] have identified that superoxide dismutase (SOD) and catalase (CAT) are important antioxidant enzymes in mitigating free radical-induced cell injury and a reduction in the activity of SOD and CAT can result in the decreased removal of superoxide ion and H_2O_2 radicals that brings about a number of reactions, which are harmful to the body. For this reason it is important to investigate if cooked phosphide residue contaminated cowpea will alter the activity of antioxidant defense system.

Cowpea, an important source of protein for many countries south of the Sahara, is a warm season, annual, herbaceous legume [2]. It is known to be one of the most venerable grains to insect infestation and therefore postharvest losses [2-4]. The use fumigants (*e.g.* phosphide) - most of them xenobiotics - is to prevent post-harvest losses. Xenobiotic induced oxidative stress is a common phenomenon; the mechanisms by which agents generate oxidative stress are varied. For example, while agents such as acetaminophen and aflatoxins are known to cause toxicity through highly reactive free radicals, phosphide induced toxicity occurs as a result of inhibition of cytochrome oxidase, although increase in oxidative stress cannot be ruled out. Data obtained from a past study [5] has shown that exposure of rats to phosphide residue contaminated cowpea resulted in significant decrease in serum antioxidant levels. The aim of this study is to identify whether by cooking such contaminated cowpea, before it is fed to the rats, significant alterations in antioxidant levels will still persist.

MATERIALS AND METHODS

Animals

Eighteen adult female Wistar rats (12 to 14 weeks of age) used for the study were divided equally into three groups. These animals were kept in the animal house of the Department of Veterinary Physiology, University of Ibadan. Without any form of restriction, they were given access to standard laboratory diet and drinking water. This experimental 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).

Cowpea treatment and sample preparation

Unlike an earlier study in which the fumigation of the cowpea lasted only 48 h and the possibility of the unspent phosphide being present in the dust could not be ruled out, this present procedure involves fumigation of the cowpea in an airtight container for a period of 72 h as advised by the manufacturer (United Phosphorus Ltd, India). The fumigant used was Protex (aluminum phosphide 57%, inert ingredients 43%); and the fumigation process involved using a ratio of 2 tablets of phosphide per m^3 of space, at average temperature of 29ºC. 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. The 3 different types of cowpea were cooked differently until tender and were given to the rats for a period over 8 h. Twenty-four hours after the commencement of the study, blood was drawn through retro-orbital bleeding from each rat and dispensed into an anticoagulant-free bottle. Serum was separated by centrifugation at 3000 rpm and stored in a refrigerator at –20 ºC.

Assessment of levels and activities of antioxidant indices/heavy metals

The serum samples obtained were used for the estimation of indices of oxidative stress. These estimations were carried out using Hitachi 902 Automated machines (Roche Diagnostic®, Germany). Serum activities of glutathione reductase (GR); glutathione S-transferase (GST); superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) as well as levels of malondialdehyde (MDA) and reduced/oxidized glutathione (GSH/GSSG) were assayed. While activity of GST was determined by the method of Habig *et al* [6], GR activity was measured using the method of Zhou and Freed [7]. Reduced and oxidized glutathione on the other hand were estimated using the methods of Prins *et al* [8] and Owen and Butterfield [9], respectively. Serum activities of SOD, GPx, CAT and MDA were quantified by the methods of Kakkar *et al* [10], Rotruck *et al* [11], Sinha [12] and Ohkawa *et al* [13], respectively.

The serum concentrations of the elements Al, Si, Cd, Pb, As and Ni were estimated using the atomic absorption spectrometry. Buck Scientific 205 Atomic Absorption (Buck Scientific, East Norwalk, CO, USA) was used for the estimation of these elements.

Statistical analysis

Statistical differences between the control and the each of the two treatment groups were determined using Student's t-test; this was followed by one-way analysis of variance (ANOVA); P values less than 0.05 were considered as an indication of statistical significance. Results are expressed as mean ± standard error of the mean (SEM) for $n = 6$.

RESULTS

The serum level of Al in rats fed cooked phosphide-residue contaminated cowpea was not significantly different from control (0.005 ± 0.005 μg/dl) in contaminated group (0.004 ± 0.001 μg/dl). On the other hand, fumigation of grain that excluded contamination of phosphide residue also did not result in significant difference in Al level (0.003 ± 0.001 μg/dl, $P > 0.05$). In addition to this, not only Pb but also Ni and Cd were not significantly different either in rats fed cooked phosphide-residue contaminated cowpea or fumigated but uncontaminated rats compared rats fed un-fumigated cowpea; the values were 0.032 ± 0.007 μg/dl for Pb, 0.015 ± 0.003 μg/dl for Ni, 0.010 ± 0.003 μg/dl for Cd in rats fed cooked phosphide-residue contaminated cowpea; control values were 0.033 ± 0.006, 0.018 ± 0.002, and 0.009 ± 0.002 μg/dl, respectively. Treated but uncontaminated rats on the other hand, had values of 0.036 ± 0.006, 0.016 ± 0.004 and 0.008 ± 0.002 μg/dl for Pb, Ni and Cd, respectively. Serum Si levels were 0.006 ± 0.001 μg/dl for rats fed with residue, (0.006 ± 0.002 μg/dl for rats in uncontaminated group and 0.008 ± 0.002 μg/dl in control rats ($P > 0.05$). Finally, As occurred in trace amount in all the three groups.

DOI 10.5455/oams.060313.or.030

Table 1. Levels of malondialdehyde, reduced glutathione, oxidized glutathione and reduced/oxidized glutathione ratio

	GSH (mol/ml)[ǂ]	**GSSG** (mol/ml)	**GSH/GSSG Ratio**[ǂ]	**MDA** (nmol/ml)
Controls	2.1 ± 0.16	0.14 ± 0.004	15 ± 1.77	18.07 ± 4.99
Contaminated	1.63 ± 0.13*	0.14 ± 0.006	11.64 ± 0.73*	17.97 ± 2.97
Uncontaminated	2 ± 0.3	0.13 ± 0.003	15.38 ± 2.26	19.04 ± 3.66

Results are expressed as mean ± SEM. [ǂ]P < 0.05 using ANOVA, *P < 0.05 vs control group using Student's t-test. GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde.

Table 2. Serum activities of antioxidant enzymes of rats fed phosphide residue contaminated and uncontaminated cowpea

	SOD (U/mg protein)	**CAT** (µmol H_2O_2 consumed/min·mg protein)	**GPx** (µmol GSH consumed/(min·mg protein)	**GR** (U/mg protein)	**GST** (U/mg protein)
Controls	12.04 ± 0.97	2.19 ± 0.32	10.68 ± 0.61	63.77 ± 4.95	0.84 ± 0.07
Contaminated	11.51 ± 0.72	2.11 ± 0.29	8.84 ± 0.84	65.16 ± 3.94	0.9 ± 0.06
Uncontaminated	12.59 ± 0.88	2.15 ± 0.34	11.06 ± 0.72	64.36 ± 4.01	0.87 ± 0.04

Results are expressed as mean ± SEM. SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione S-transferase.

The results of GSH, GSSG, GSH/GSSG ratio, MDA are presented in Table 1 while serum activities of GR, GST, SOD, GPx and CAT are shown in Table 2. Significant differences were only recorded for GSH level and GSH/GSSG ratio of the contaminated group compared to controls (P < 0.05 for both).

DISCUSSION

Cowpea is known to suffer significant postharvest losses and because of this, the use of fumigants as grain preservatives is common. Although synthetic chemical preservatives such as methyl bromide and phosphine [14] have gained recognition because of their effectiveness and recent studies have raised the possibility of using natural products such as neem (*Azadirachta indica* A. Juss) and moringa (*Moringa oleifera*) seed oils [15], phosphide is the most widely accepted fumigant in both local and international grain market. Yet this fumigant is not only toxic to infestating weevils but also to all other organisms that derive energy through the oxidative phosphorylation pathway. Ingestion of an appreciable quantity of this fumingant is known to be capable of causing 80%-90% mortality. When subjects have survived exposure, it is usually due to a very small amount had been taken or the tablet had been exposed to air, rendering it less toxic. However, tissue damage is still present in such patients [16].

Even with our knowledge of the toxic nature of this fumigant, in Nigeria users of phosphide are still largely untrained with the possible contamination of cowpea with phosphide residue being a common occurrence. Our previous study in which rats were fed raw phosphide residue contaminated cowpea resulted in significant increase in oxidative stress [5], but food processing is also known to have an impact on digestion and absorption of ingested items. Moreover, phosphide exposure to air or water liberates its toxic component, phosphine. The present results in which rats fed cooked phosphide residue contaminated cowpea exhibited non-significant changes in most of the indices of oxidative stress (MDA, GST, CAT, SOD, GPx, GR) is an indication that food processing like cooking as in this case is capable of modulating presentations commonly associated with chemical contaminated food items. This is because a past study showed that when rats were fed uncooked phosphide-residue contaminated cowpea, considerable oxidative stress were observed since the activities of antioxidant enzymes were significantly decreased in phosphide-residue group compared with either control or uncontaminated group [5]. The significant decrease in the levels of GSH observed in serum of rats in the contaminated group used for this study may be associated with decrease in synthesis rather than profound oxidative stress, since the level of GSSH was not significantly different compared with control.

Aluminum (Al) dust a component of this residue is an element that is widely distributed in the environment and enters the human body through the air, water, food and drugs. The phosphide used for this study is its Al salt. Although evidence exists to suggest that Al toxicity elevates the rate of lipid peroxidation, the result of this study did not recognize Al-induced oxidative stress in rats fed cooked phosphide-residue contaminated cowpea. Moreover, non-significant difference in the level of Al was observed but this does not preclude absorption of an appreciable level of Al from the cooked beans, but it may be suggested that the absorbed Al must have been sequestered in bone. Apart from this, even though increase in Al level was observed in rats fed uncooked phosphide-residue contaminated cowpea [17], the non-significant difference in serum Al level observed in this study may be an indication that cooking may have altered the form in which Al was presented to the enterocytes. Differences in Al compound composition is known to affect its oxidative stress potential. For example, although Al phosphate caused increase in lipid peroxidation rate, leading to a rise in the levels of MDA and a decrease in GSH [18-20]; lipid peroxidation rate and levels of its markers did not change in Al sulfate administered rats [21].

Pesticides such as herbicides and fumigants are used all over the world for the preservation of food. Reports by the World Health Organization (WHO) have shown that annually, not less than three million cases of acute poisoning occurring from food exposed to pesticides are reported, with as many as 20,000 of such cases resulting in death. But the results of this study seem to suggest that phosphide residue contamination of cowpea may not be one of the causes of cowpea-associated deaths in Nigeria. The same report identified that in most instances, presentation at hospitals seems to be important for cases of acute poisoning but gradual build up of the effect of chronic exposure of small amount of these agents can occur and cannot be overruled in such subjects.

Although one of the criteria that were used for the adoption of a standard fumigant was lack of residue on stored grains post-fumigation, other factors such as ease of application, low cost and its effectiveness were given considerable attention. When ethylene dibromide (EDB) was adjudged unsuitable as a grain fumigant in the early 1980s with potentially hazardous levels of this chemical being detected in several finished grain-based products by governmental food-monitoring laboratories, other compounds -more volatile than EDB- such as phosphide replaced it, since post-fumigation examination of grains showed undetectable levels of phosphide. Recent studies have identified that many of these pesticides can still be found in processed food that originated from not only the developing world but even from developed countries where standard procedure for the use of these preservatives exist and are practiced, and phosphide residue contamination of grain is non-existent. This is because newer, more sophisticated techniques now exist that are able to detect even minute amount of residual pesticide, therefore additional hazard of phosphide residue contamination may compound many of the problems associated with cowpea consumption. Moreover, it cannot be ruled out that with continuous exposure to this residue in cooked cowpea form, its toxic effect may begin to manifest. Therefore to rule out phosphide-residue as a cause of many of the clinical conditions associated cowpea consumption in Nigeria, there is need for this study to be carried out in chronic setting. If in Western countries where trained personnel use these pesticides, detectable amounts are known to exist in grains, in Nigeria where grain merchants are untrained and the fumigation process un-regularized, a higher than normal residue is likely to be found on grains and this has been confirmed from results of many studies [22, 23].

In conclusion, these results only seem to suggest that in acute state pronounced oxidative stress does not accompany feeding of Wistar rats with cooked phosphide residue contaminated cowpea.

REFERENCES

1. Rao PR, Viswanath RK. Cardioprotective activity of silymarin in ischemia-reperfusion-induced myocardial infarction in albino rats. Exp Clin Cardiol 2007;12:179-87.
2. Carlos G. Cowpea: Post Harvest Operation. Food and Agriculture Organisation of United Nations, Rome, Italy, 2000.
3. Profit M. Bruchid Research at Royal Holloway. University of London, London, UK, 1997.
4. Ntoukam G, Murdock LL, Shade RE, Kitch LW, Endondo C, Ousmane B, Wolfson J. Managing insect pests of cowpea in storage. Proceedings of the Midcourse 2000 Researchers Meeting of the Bean/Cowpea Collaborative Research Support Program, 9-14 April 2000, Senegal, pp 3-4.
5. Iyanda AA. Assessment of lipid peroxidation and activities of antioxidant enzymes in phosphide- powder residue exposed rats. J Drug Metab Toxicol 2012; 3:1000132.
6. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249:7130-9.
7. Zhou W, Freed CR. DJ-1 up-regulates glutathione synthesis during oxidative stress and inhibits A53T α-synuclein toxicity. J Biol Chem 2005; 280:43150-8.
8. Prins HK, Loos JA. Glutathione. In: Yunis JJ (ed) Biochemical Methods in Red Cell Genetics, Academic Press, New York, NY, USA, pp 127-129, 1969.
9. Owen JB, Butterfield DA.. Measurement of oxidized/reduced glutathione ratio. Methods Mol Biol 2010; 648:269-77.
10. Kakkar P, Das B, Viswanathan PN. A modified spectroscopic assay of superoxide dismutase. Ind J Biochem Biophys 1984; 21:130-2.
11. Rotruck JT, Rope AL, Ganther HF, Swason AB. Selenium: biochemical role as a component of glutathione peroxide. Science 1973; 179:588-90.
12. Sinha KA. Colorimetric assay of catalase. Anal Biochem 1972; 47:389-94.
13. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95:351-8.
14. Daft JL. Fumigants and related chemicals in foods: review of residue findings, contamination sources, and analytical methods. Sci Total Environ 1991; 100:501-18.
15. Ilesanmi JO, Gungula DT. Preservation of cowpea (*Vigna unguiculata* (L.) Walp) grains against cowpea bruchids (*Callosobruchus maculatus*) using neem and moringa seed oils. Inter J Agron 2010; 2010:235280.

DOI 10.5455/oams.060313.or.030

16. Darbari A, Tandon S, Chaudhary S, Bharadwaj M, Kumar A, Singh GP. Esophageal injuries due to aluminum phosphide Tablet Poisoning in India. Asian Cardiovasc Thorac Ann 2008; 16:298-300.

17. Iyanda AA. A study of serum levels of aluminium and select heavy metals in female rats fed phosphide-powder residue contaminated cowpea. Int J Pharm Biomed Res 2012; 3:172-4.

18. Chugh SN, Arora V, Sharma A, Chugh K. Free radical scavengers and lipid peroxidation in acute aluminium phosphide poising. Indian J Med Res 1996; 104:190-3.

19. Chugh SN, Kolley T, Kakkar R, Chugh K, Sharma A. A critical evaluation of antiperoxidant effect of intravenous magnesium in acute aluminium phosphide poisoning. Magnes Res 1997; 10:225-230.

20. Hsu C, Han B, Liu M, Yeh C, Casida JE. Phosphine- induced oxidative damage in rats: attenuation by melatonin. Free Radic Biol Med 2000; 28:636-42.

21. Farina M, Lara FS, Brandao R, Jacques R, Rocha JB. Effects of aluminum sulfate on erythropoiesis in rats. Toxicol Lett 2002; 132:131-9.

22. Ogah CO, Coker HA, Adepoju-Bello AA. Pesticide residue levels in maize samples from markets in Lagos State, Nigeria. Nig Q J Hosp Med 2011; 21:169-74.

23. Adeyeye A, Osibanjo O. Residues of organochlorine pesticides in fruits, vegetables and tubers from Nigerian markets. Sci Total Environ 1999; 231:227-33.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided that the work is properly cited.