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The Effects of Cassava Cyanide on the Antioxidant (Glutathione) Status and Some Clinically Important Enzymes of Rats *

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Abstract: The effects of cassava cyanide on the antioxidant (glutathione) status and some clinically important enzymes were investigated biochemically in male albino wistar rats fed for 28 days with cassava diet containing 54.6 mg CN·kg⁻¹ DM and 10% protein supplement. Analysis of the urinary and serum cyanide and thiocyanate of the test and control rats showed a statistically significant difference ($p < 0.05$) between the test and the control. The mean serum and urinary cyanide were 2.92 ± 0.53 and 8.21 ± 6.32 $\mu\text{g mL}^{-1}$ after 7 days and 3.80 ± 0.67 and 11.08 ± 0.54 $\mu\text{g mL}^{-1}$ after 28 days, respectively. Mean serum and urinary thiocyanate were 16.73 ± 0.42 and 19.90 ± 1.35 $\mu\text{g mL}^{-1}$ after 7 days and 18.14 ± 0.18 and 36.59 ± 1.87 $\mu\text{g mL}^{-1}$ after 28 days, respectively. Depletion in whole blood glutathione level by 47.3 and 89% (after 7 and 28 days, respectively) compared to that of the control was also observed. Increases in plasma activity of aspartate aminotransferase (90%), alanine aminotransferase (88.5%) and alkaline phosphatase (49%) were also measured after 28 days of the feeding experiment. There was elevation in blood glucose of the test animals, while the levels of protein and albumin remain within the normal range for both test and control animals.

Key words: Cassava diet, glutathione status, clinical important enzymes, rats

Introduction

Many plants used for human nutrition contain cyanogenic glycosides and are thus capable of releasing hydrogen cyanide under certain conditions (Kingsbury *et al.*, 1964). Several recent reviews exist on food plants within cyanogenic compounds (Jones, 1998). Cassava (*Manihot esculenta* Crantz) has been considered to be the major human food crop with a high content of cyanogenic glycoside (Osuntokin, 1969). Cassava roots form important staple food for more than 500 million people (Brocas, 1987) mostly in the tropical countries such as Africa, Asia and Latin America.

The toxicity of cyanogenic plants and their products is primarily associated with the free HCN formed in the material when cyanogenic glycosides have been hydrolyzed (Coursey, 1973). The toxic effects of cyanide (HCN) have traditionally been attributed to inhibition of cytochrome C oxidase, the terminal enzyme of respiratory chain, which compromises oxidative phosphorylation leading to cytotoxic hypoxia. However, numerous other biochemical effects of cyanide not directly related to the inhibition of the respiratory chain have been reported in the recent past. Some of these biochemical effects include enhancement of N-methyl-D-aspartate (NMDA) receptor response (Arden *et al.*, 1998;

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Sun *et al.*, 1999), elevation of extracellular glutamate concentration (Patel *et al.*, 1999) and mobilization of calcium from intracellular store (Yang *et al.*, 1996). Furthermore, the involvement of apoptosis in cyanide induced neural death has been identified (Jensen *et al.*, 1999) while elevation in blood aminotransferases and alkaline phosphatases has been reported (Okafor *et al.*, 2002). Also cassava cyanogens have been assumed to be involved in the etiology of tropical pancreatitis (McMillian and Geervarghese, 1979).

The mechanisms by which cyanide exert these effects have not all been fully elucidated. Since cyanide is a neurotoxin that stimulates intracellular generation of Reactive Oxygen Species (ROS), some of its biochemical effects could be mediated through attack of the generated reactive oxygen species on some target organs and cells such as the liver, kidney and mitochondria. Also exposure to high levels of ROS leads to depletion in antioxidant levels in animals. One of these antioxidants in the body is reduced glutathione, a reducing agent in biological cells that provides primary antioxidant defence against reactive intermediates of metabolism, drugs or carcinogens (Meister and Anderson, 1983). Animal experiment using the naturally occurring cruciferous compound 1-cyano-2-hydroxy-3-butane, were among the earliest investigation to disclose the vital role reduced glutathione plays in determining organ toxicity of plant glycosides in general, cyanogenic or non-cyanogenic (Wallig *et al.*, 1988). Acrylonitrile an aliphatic nitrile that manifests its toxicity through cyanide liberation has also been reported (Ahmed and Farooqui, 1982). However, there has not been any previous research on the effect of cassava cyanide on the glutathion status of animals or humans. This forms the basis of this present study. Other biochemical effects of cyanide will also be investigated.

Materials and Methods

This present research was carried out in the Department of Biochemistry, Federal University of Agriculture Umudike, Nigeria.

Animals

Twenty male albino rats of the wistar strain (weighing 120 g on the average) bred in animal house of University of Nigeria Nsukka were used. All animals were kept at room temperature (27-30°C) and had free access to drinking water and their diets. The animals were acclimatized to their environment and diet before experimentation.

Treatment of Animals

The rats were grouped into three. Groups 1 and 2 comprised 5 animals each while Group 3 of 10 rats served as control. Groups 1 and 2 were fed cassava diet (Table 1) containing 54.6 mg CN·kg⁻¹DM for 7 and 28 days, respectively. Group 3 were fed commercial rat pellets purchased from a retail outlet near Michael Okpara University of Agriculture, Umudike, Nigeria.

Table 1: Composition of cassava (*Manihot esculanta* Crants) diet for the feeding experiment

Ingredients	Quality g kg ⁻¹
Cassava flour	770
Vitamin-free caesin	90
Vitamin mixture	40
Salt mixture	20
Banana flour	40
Groundnut oil	40

Bassir (personal communication)

Sacrificing the Animals

After seven days, Group 1 animals together with five rats from control group were sacrificed and blood collected with 10 mL syringe intravenously from the heart. The Group 2 animals and the remaining 5 animals in control group were sacrificed at the end of 28 days and their blood collected as above.

Animal Diet

The composition of cassava diet for the experimental animals is shown on Table 1, while control feed is rat pellets purchased from livestock feed shop near Michael Okpara University of Agriculture, Umudike in Nigeria.

Determination of Whole Blood Glutathione (GSH)

Glutathione determination in blood is well established as an accurate indicator of whole body GSH Status (Richie, 1996). 0.2 mL of whole blood from each of the animals was added to 1.8 mL of distilled water immediately after collection followed by addition of 3 mL of precipitating reagent. The mixture was allowed to stand for 10 min and then filtered. To 2 mL of filtrate was added 8 mL of 0.1 M phosphate buffer (pH 7.4) followed by 1 mL of Ellman's reagent (DTNB). The yellow color solution obtained was read spectrophotometrically immediately at 430 nm. This method is as described by Duron and Kelly (1963).

Cyanide (CN⁻) and Thiocyanate (SCN⁻) Determination

The blood and urine collected from each group of animals were pooled together (for each group) and used for analysis. Urinary and serum thiocyanate was determined according to Haque and Bradbury (1999) while the cyanide content of these samples was estimated by the method of Esser *et al.* (1993).

Enzyme Assay

The assay of some plasma enzymes as indicator of damages to some organs such as the liver and kidney was carried out. The plasma enzymes were assayed as follows: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as recommended by Reitman and Frankel (1957) and alkaline phosphatase (Alk. Phos) as described by Klein *et al.* (1960).

Other Biochemical Determinations

Glucose was determined using glucose oxidase method (Passey *et al.*, 1977), total protein by the Biuret method as described by Layne (1957), serum albumin by the dye-binding (bromocresol green) method (Dumas *et al.*, 1971).

Statistics

Students' t-test was used for statistical analysis.

Results

Cyanide was at a mean concentration of 2.92 ± 0.53 and $3.80 \pm 0.67 \mu\text{g mL}^{-1}$ in serum after 7 and 28 days respectively, while the urine concentration was 8.21 ± 0.32 and $11.08 \pm 0.54 \mu\text{g mL}^{-1}$ following the order above. There was a statistically significant increase ($p < 0.05$) in the cyanide levels of serum

Table 2: Concentration of total cyanides thiocyanate and glutathione in blood and urine of rats fed cassava cyanide

	BLOOD ($\mu\text{g mL}^{-1}$)			URINE ($\mu\text{g mL}^{-1}$)	
	C N	SCN	GSH	CN	SCN
Group 1 (after 7 days)	2.92 $\pm$ 0.53	16.73 $\pm$ 0.42	25.00 $\pm$ 3.08	8.21 $\pm$ 0.32	19.90 $\pm$ 1.35
Group 2 (after 28 days)	3.80 $\pm$ 0.67	18.14 $\pm$ 0.18	6.33 $\pm$ 0.15	11.08 $\pm$ 0.54	36.59 $\pm$ 1.87
Group 3 Control	ND	5.23 $\pm$ 0.01	52.83 $\pm$ 3.65	1.03 $\pm$ 0.07	7.60 $\pm$ 0.05

*ND-Non-detectable, Each is an average of three determinations

and urine of the test animals compared to that of the control with the period of investigation (Table 2). The concentration of compound followed the cyanide trend above. There was also a statistically significant increase ($p < 0.05$) in thiocyanate of the test animal above that of the control.

Whole blood glutathione concentration was 25.00 $\pm$ 3.08 and 6.33 $\pm$ 0.15 $\mu\text{g mL}^{-1}$ after 7 and 28 days respectively while that of the control was 52.83 $\pm$ 3.65 $\mu\text{g mL}^{-1}$. These levels represent 47.3 and 89% depletion after 7 and 28 days when compared with the blood glutathione of the control rats (Table 2).

The serum aspartate aminotransferase, alaine aminotransferase and blood glucose levels were significantly ($p < 0.05$) higher in test animals after 28 days than in the control. There was no significant differences in serum total protein and albumin levels of these animals (Table 3).

Discussion

The results from this study clearly show that exposure to cassava cyanide has effect on the antioxidant status of animals ingesting cassava-based foods as evidenced by glutathione (antioxidant) depletion in whole blood of rats fed cassava diet containing 54.6 mg HCN equivalent kg^{-1} at 9% protein supplementation. That ingestion of cassava cyanide depletes blood glutathione (an important antioxidant) is a significant finding from this study. This depletion in glutathione status could be one of the mechanisms by which cyanide exerts its numerous toxicities such as its assumed role in the etiology of tropical pancreatitis (Mcmillian and Geervarghese, 1979). In this context, depletion of antioxidant systems in pancreatitis has been reported (Dabrowski *et al.*, 1991; Luthen and Greendell, 1994; Pitchumoni *et al.*, 1988).

The appearance of cyanide in blood of the test animal after 7 and 28 days following ingestion of the above diet indicated exposure to cassava cyanide and is consistent with the bio-availability of this free radical. Cyanide is known to be absorbed in the gastrointestinal tract of animals (Seigler, 1992). That the concentration of blood cyanide of the test animals (2.92 $\pm$ 0.53 and 3.80 $\pm$ 0.67 $\mu\text{g mL}^{-1}$ after 7 and 28 days, respectively) were higher than the reported toxic level in rats (Egekege and Oehme, 1979) lends support to the exposure of the animals to cassava cyanide resulting in the toxic manifestation as seen in whole blood glutathione depletion. Cyanide is known to be a neurotoxin that stimulates intracellular generation of ROS. Significant elevation ($p < 0.05$) of urine cyanide of the test animals above that of the control further support the exposure of these animal to dietary cyanide.

Significant increase ($p < 0.05$) in blood and urine thiocyanate (Table 2) and serum glucose level (Table 3) were also observed. Cyanide alters glucose metabolism resulting in 100% increase in conversion of glucose by pentose-phosphate pathway (Isom *et al.*, 1975). Thiocyanate on the other hand is known to be the most reliable biomarker for cyanide exposure being a stable metabolite of this free radical (Haque and Bradbury, 1999; Rosling 1994). The plasma half-life of thinocyanate has been

Table 3: Level of some serum enzymes, glucose, protein and albumin in rats fed cassava cyanide

Groups	Glucose (mg dL ⁻¹)	Total protein (g dL ⁻¹)	Albumin (g dL ⁻¹)	ALP (U L ⁻¹)	AST (U L ⁻¹)	ALT (U L ⁻¹)
Group 1 (after 7 days)	101.00	5.10	3.80	116	16.00	13.00
Group 2 (after 28 days)	132.30	5.20	3.80	150	19.00	15.00
Group 3 Control	92.13	5.20	4.30	101	10.00	8.00

reported to be 3 days (Rosling, 1994) and the levels therefore reflect the mean daily load during the last days. The high levels of SCN measured in this research is an indication of the attempt of the animals to detoxify the ingested cyanide. It has been well established that rhodanese enzyme and Mercaptopyruvate Sulphur Transferees (MPST) enzyme utilize sulphur-containing amino acid for cyanide detoxification (Nagahara *et al.*, 1999). From this study, another implication of cyanide detoxification is decreased concentration of the glutathione of the body. This could be in part due to reduced synthesis of this important biological compound. In this connection cysteine a sulphur-containing amino acid needed for cyanide detoxification in the body (Nagahara *et al.*, 1999) is the limiting amino acid in glutathione synthesis. Moreover, decrease in the concentration of blood glutathione could result from its consumption in the course of scavenging for the reactive intermediates generated from the metabolism of glucosidic and non-glucosidic cyanide as well as other chemical species associated with ingestion of cassava. Depletion in glutathione levels depending on the degree could further lead to oxidative stress with concomitant attack of ROS on the cell of some target organs and tissues in the body. This is because GSH helps to recycle vitamins C and E (intracellular antioxidants), blocks free radical damage and enlases the antioxidant of vitamin C and plays a critical role in the detoxification of harmful compounds (Meister and Anderson, 1983).

Another implication of cyanide exposure from diet, detoxification and glutathione depletion is seen in the statistically significant ($p < 0.05$) increases in the serum aminotransferases (aspartate and alanine aminotransferases) and alkaline phosphates above that of control (Table 3). Increase in the serum concentration of these enzymes indicate damage to the cell membranes of some organs such as the liver (Joan *et al.*, 1987). Thus some of the numerous biochemical activities of cyanide either from dietary source or otherwise could be mediated through depletion in the antioxidant status (especially glutathione) of the body.

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