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The Role of Low-Protein and Cassava-Cyanide Intake in the Aetiology of Tropical Pancreatitis

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Abstract: The contribution of low-protein and cassava-cyanide intake in the aetiology of tropical pancreatitis was investigated in male albino Wistar rats fed for 28 days with cassava diet containing 50 mg CN^{-} kg^{-1} DM and 3% protein supplement, using acceptable biochemical methods. Assay for pancreatic amylase activity (indicator for pancreatic dysfunction) in blood of both control and cassava-cyanide fed groups indicated 9.43% and13.57% rise in activity of this enzyme in the latter above the former after 14 and 28 days respectively. Increases activity of some hepatic enzymes in the blood were also measured. Depletion of whole blood glutathione of the test animals by 57.33 and 84.38% after 14 and 28 days respectively above that of control was also observed. There was non-significant increase ($p>0.05$) in plasma malonaldehyde (lipid peroxidation status) of the cassava-cyanide fed group when compared to the control. Significant decreases in plasma albumin and elation in blood and urine thiocyanate levels were also measured. The results are discussed from toxicological and mechanism of action points of views.

Key words: Cassava-cyanide · low-protein · tropical pancreatitis and rats

INTRODUCTION

Chronic pancreatitis is a relatively progressive disease that causes untold misery in its victims. Cyanogenic glycosides in dietary staples have been postulated as an aetiologic factor in this disease [1, 2]. The good geographical match between the occurrence of tropical chronic pancreatitis and consumption of cassava (Manihot esculenta Crantz) a plant rich in cyanogenic glycosides, linamarin and lotaustralin formed the basis for the cassava cyanide toxicity theory [1] This theory has been discredited by the lack of cassava connection in some tropical areas [3], the apparently innocus nature of cassava in other zones [4] and the declining incidence of chronic pancreatitis in the province of Kerala, South India in the past decades [5] although dietary pattern has not changed in this region where the cassava connection was first postulated [1].

That long term feeding of cassava itself can produce chronic pancreatitis-like morphological changes in rat and rabbit exocrine pancreas has been demonstrated [6, 7]. However no direct link between chronic cyanide exposure per se and pancreatitis has been made in humans or in animal studies.

This paradox suggests that either the non-cyanide moiety is the true pancreatic toxin, or that additional factor most likely low sulfur-amino acid content in diet or tissue contribute to the damage.

The present study is aimed at investigating the latter, 'the possible role of low-protein and cassava cyanide intake in the aetiology of tropical pancreatitis'. This will be carried out by feeding rats on cassava diet containing cyanide and low protein (i.e. 3% protein supplement) and then monitor indices of cyanide toxicity, destruction of exocrine pancreas and pancreatitis. This will be achieved by determining the activity of pancreatic amylase, whole blood glutathione (GSH) and malonaldehyde (lipid peroxidation) product along with cyanide and thiocyanate in blood and urine as well as plasma albumin.

Blood test for amylase is used to diagnose pancreatitis and glutathione (GSH) plays vital role in determining organ toxicity from plant glycosides in general, cyanogenic and non-cyanogenic [8]. Increased lipid peroxidation products (such as malonaldehyde) resulting from oxidant stress has been associated with pancreatitis [9].

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MATERIALS AND METHODS.

Animals and their diet: Twenty male albino rats of the wistar strain (weighing 150-165 g), bred in animal house of University of Nigeria Nsukka were used. All animals were kept at room temperature and had free access to drinking water and their diets. The animals were acclimatized to their environment and their diet for 7days before experiments were commenced. The test rats were fed before and throughout the period of investigation on a high cassava (Manihot esculenta Crantz) starch diet containing 50 mg CN^- equivalent Kg^{-1} DM (Table 1). Control diet prepared as above with corn starch in the place of cassava starch was fed to the control animals.

Treatment of animals: The rats were grouped into three. Groups 1 and 2 comprised 5animals each while group 3 of 10 rats served as the control. Groups 1 and 2 were fed cassava diet containing 50 mg CN^- kg^{-1} DM for 14 and 28 days respectively. Group 3 were fed control diet prepared with corn starch in place of cassava (Table 1 and 2).

Sacrificing of the animals: After 14days, Group 1 animals together with five from control group were sacrificed and their blood collected with 5.0 ml syringe intravenously from the heart. The group 2 animals and the remaining five in control were sacrificed at the end of 28 days and their blood collected as above

Determination of Whole Blood Glutathione (GSH): Glutathione in blood is well established as an accurate indicator of whole body glutathione status [10]. Whole blood glutathione was determined spectrophotometrically as described by Duron and Kelly [11].

Malonaldehyde determination: Malonaldehyde was determined by the modified thiobarbituric acid (TBA) method of Gutteridge andWhilkin [12].

Cyanide and thiocyanate: Cyanide was determined by the method of Esser *et al.* [13] and thiocyanate by ferric nitrate reagent [14].

Serum protein and albumin: Total protein was determined by the Biuret method as described by Layne [15] and albumin by the dye-binding (Bromo-cresol green) method [16].

Table 1: Composition of cassava diet for the feeding experiment

Ingredient	Quantity g Kg^{-1}
Cassava flour	830
Fat-free soybean	30
Vitam mixture	40
Salt mixture	20
Banana flavour	40
Groundnut oil	40

Bassir (Personnel communication, 1979)

Table 2: Some chemical components of the test and control diets

	cyanide, mg CN^- kg^{-1} equivalent		
Diet	Free	Bound	Total
Cassava based diet	3.45±0.11	46.55±0.21	50.00±0.3
Control diet	ND	ND	ND

The values given are the mean of three determinations. ND = not detected by the method of assay

Determination of serum amylase activity and some other serum enzymes: The assay of some serum enzymes as indicator of damages to some organs such as the pancreas, liver and kidney was carried out. The pancreatic amylase activity was determined using a kit from Quimca Clinica Aplicada S.A. Amylase-BPS. Kinetic enzymatic test with benzylidene-G_7 PNP as substrate for *in vitro* determination of amylase in serum or plasma. Aspartate aminotransferases (AST) and alanine aminotransferases (ALT) were determined as recommended by Reitman and Frankel [17] and alkaline phosphatase (ALP) as described by Klein *et al.* [18].

Statistics: Students' t-test was used for statistical analysis.

RESULTS

Physical examination: The physical activities of the rats during the period of the experiment were closely monitored. It was observed that all the rats were very active.

The rate of feed consumption before and during the feeding experiment did not change in any of the groups. However, there was decrease in body weight of the cassava diet rats and the decrease was more pronounced at the end of the experiment (Table 5). On the other hand there was weight gain in the control rats. Increase in relative organ weight was also observed among the cassava diet animals compared to the control (Table 5).

Table 3: Concentrations of total cyanide, thiocyanate, glutathione and malonaldehyde in blood and urine of rats red the cassava cyanide diet

	BLOOD (mg mL^{-1})				URINE (mg mL^{-1})	
	CN	SCN	GSH	MDA	CN	SCN
Group 1 (after 14 days)	2.61±0.42	14.41±1.03	23.00±2.11	10.31±0.42	6.73±0.82	17.82±2.03
Group 2 (after 28 days)	3.21±0.33	19.66±1.14	8.42±0.53	10.98±0.37	6.33±0.45	30.63±1.51
Group 3 (Control)	ND	4.03±0.32	53.91±4.01	9.97±0.17	0.79±0.11	6.60±0.32

ND: Non detectable, Each is an average of three determinations

Table 4: Levels of some serum enzymes, protein and albumin in rats fed cassava diet

Groups	Total protein (g dL^{-1})	Albumin (g dL^{-1})	ALP (U L^{-1})	AST (U L^{-1})	ALT (U L^{-1})	Pancreatic amylase (U L^{-1})
Group 1	3.04	2.21	120	14.00	12.00	101.00
Group 2	2.61	1.32	147	18.00	15.00	105.00
Group 3	4.82	3.30	101	10.00	8.00	92.00

ALP: Alkaline phosphatase, AST: Aspartate aminotransferases, ALT: Alanine aminotransferases

Table 5: Relative organ weight and % weight gain/loss of the various animal groups

Groups	Liver	Kidney	Pancreas	Weight gain/loss (%)
1. After 14days	4.42×10^{-2}	7.41×10^{-3}	5.92×10^{-3}	11.66↓
2. After 28days	4.89×10^{-2}	7.50×10^{-3}	6.55×10^{-3}	23.26↓
3. Control	3.40×10^{-2}	7.26×10^{-3}	5.17×10^{-3}	9.01↑

Relative organ weight was determined as the ratio the organ to that of the body weight of the animal, ↑Increase, ↓Decrease

Table 3 shows the blood and urine cyanide levels, whole blood reduced glutathione (GSH) and serum malondialdehyde. There was statistically significant ($p< 0.05$) elevation in blood and urine thiocyanate of the test rats compared to the control. Depletion of Whole Blood Glutathione (GSH) of the test rats by 57.33% and 84.38% after 14 and 28 days respectively. There was statistically non-significant ($p>0.05$) rise in serum malondialdehyde of the cassava diet animals compared to the control.

Table 4 shows the levels total protein, serum albumin and activities of some hepatic enzymes and pancreatic amylase in the blood of both the control and test animals. There was 60% decrease in the serum albumin of the cassava diet rats compared to the control. Some hepatic enzymes and pancreatic amylase were elevated in the blood of the test groups.

DISCUSSION

In this study, we cold not establish conclusively a direct link between cassava cyanide exposure and low-protein intake in the aetiology of tropical pancreatitis. However, our results strongly indicate diverse manifestations of dietary cyanide toxicity in rats. The toxic manifestations strongly suggest that high dose of cyanide in diet in association with low protein intake could precipitate pancreatic dysfunction akin to tropical pancreatitis.

The appearance of significant amounts of both cyanide and thiocyanate in the blood of the test animals clearly indicate exposure to cyanide through the diet.

That the concentrations of blood cyanide of the test animals (2.61±0.42 and 3.2±0.31 after 14 and 28 days respectively) were higher than that reported to cause toxic manifestations in rat [19] lends support to the exposure of the animals to cassava cyanide and the resultant toxic manifestations. Among the toxic manifestations were decreased body weights and increases in relative organ weight of the cassava diet groups (Table 5).

The deceased body weights is as a result of the animals using the sulfur-containing amino acids of their body to detoxify the cyanide, since their dietary sulfur-containing amino acids, being insufficient could not support the detoxification of such high cyanide dose.

Statistically significant ($p<0.05$) elevation in blood and urine thiocyanate is an indication of the attempt by the animals to detoxify the ingested cyanide. Thiocyanate (SCN) remains the most useful biomarker for cyanide exposure since it is a stable metabolite [20]. One of the

obvious implications of utilization of the sulfur amino acids for detoxification of dietary cyanide under low protein intake is that protein synthesis is compromised. This is clearly seen in decreased body weight (Table 5) and very low serum albumin level (Table 4) of the cassava diet group compared to the control.

Depletion of whole blood glutathione (an important antioxidant of the body) of the rats fed cassava diet by 57.33 and 84.36% after 14 and 28 days respectively (Table 3) is a significant finding in this work. The decreased concentrations of whole blood glutathione could be attributed in part to reduced synthesis of this important biological compound as a result of cyanide detoxification as well as its consumption in the course of scavenging for reactive intermediates generated from metabolism of glucosidic and non-glucosidic cyanide as well other chemical species from the diet. In this connection, cysteine, a sulfur-containing amino acid needed for cyanide detoxification in the body [21] is the limiting amino acid in glutathione synthesis.

Depletion in glutathione (GSH) levels to the extent observed in this study could further lead to drastic decrease in the total antioxidant status of the body of the animals. This is because glutathione (GSH) helps to recycle vitamins C and E (cellular antioxidants), blocks free radical damage, enhances the antioxidant activity of vitamin C and plays a critical role in the detoxification of harmful compounds [22]. It is important to note at this point that both 'oxidant stress' and depletion of antioxidant system has been reported in pancreatitis [23, 24]. Drastic decrease in antioxidant status of the body could then precipitate "oxidant stress" with concomitant attack of reactive intermediates or free radicals on cells of some target organs and tissues of the body.

Our findings also include increases in the serum aminotransferases (aspartate and alanine aminotransferases) and alkaline phosphatase as well as serum pancreatic amylase activity of the cassava diet animals above that of the control (Table 4). Similar findings have been reported in humans exposed to cassava cyanide through large scale cassava processing [25]. Increases in the serum concentrations of these enzymes indicate damage to cell membrane of some organs [26, 27] such as the liver, kidneys and exocrine pancreas. That exposure to cyanide either in its organic form or as dietary cyanide induces damage to some organs and tissues of animals have been demonstrated [28, 29].The rise in pancreatic amylase activity in blood of the test animals by 9.43 and 13.57% above that of control after 14 and 28 days respectively is a suggestive evidence of destruction of the exocrine pancreas as elevation in serum amylase activity above normal range is an indication of leakage of this enzyme from exocrine pancreas.

The damaging effects of cyanide on these tissues and organs could be mediated either by cyanide ion (being a nucleophile) or free radicals generated in the course of its metabolism or the metabolism of other chemical species within the system. The free radical damaging effect is further suggested by the increase (though not statistically significant, $p>0.05$) in the blood malondialdehyde (a by product of lipid peroxidation) concentration of the cassava diet rats above the control (Table 3). Free radicals are known to induce lipid peroxidation and to generate of malonaldehyde and other lipid peroxidation products. Pancreatitis is a condition in which there is premature activation of the pancreatic enzymes resulting in self-destruction of the gland. It appears from our findings that the destruction of the pancreas could be attributed more to the attack of free radicals or reactive intermediates on the organ than from premature activation of the pancreatic enzymes. This is because other organs such as the liver and the kidney were affected as indicated in rises in aminotransferases and alkaline phosphatase as earlier discussed.

Thus, the divers effects of cyanide on the organs and tissues including exocrine pancreas of experimental animals as reported in this work and by other authors are more of direct attack of reactive intermediates or free radicals on these tissues and organs. This holds true and more pronounced when the antioxidant status is affected as in the case of high cassava-cyanide and low protein intake over a considerable period of time. The body then tries to detoxify the ingested cyanide using sulfur-containing amino acids of the body. Protein synthesis is compromised and as well as the synthesis of glutathione that has cysteine (a sulfur-containing amino acid) as the limiting amino acid for its synthesis. This important biological compound (glutathione) plays central role in the antioxidant activities of the body.

REFERENCES

1. McMillan, D.E. and P.H. Geervarghese, 1979. Dietary cyanide and tropical malnutrition diabetes. Diabetes Care, 2: 202-208.
2. Pitchmunoni, C.S., N.K. Jain, A.B. Lowenfles and E.P. Dimagno, 1988. Chronic cyanide poisoning: Unifying concept or for alcoholic and tropical pancreatitis. Pancreas, 3: 220-223.

3. Narendranathan, M. and A. Cheriyan, 1994. Lack of association between cassava consumption and tropical pancreatitis syndrome. J. Gastroenterol Hepatol., 9: 282-285.
4. Abubakare, A., R. Taylor, G.V. Gill and K.G.M.M. Alberti, 1986. Tropical or malnutrition-related diabetes. A real Syndrome. Lancet: pp: 1135-1138.
5. Geevarghese, P.G., 1986. Calcific pancreatitis. Bombay, Varghese Publishing House, 1986.
6. Geldof, A.A., J.L.F. Beching, C.D. De Vries, E. Van Der Ven, 1992. Histopathological changes in rat pancreas after fasting and cassava feeding. *In vivo,* 6: 545-552.
7. Shenoy, K.T., K.B. Leena and R.B. Nair, 1996. Pancreatic changes (enzymes and histological) in experimental model fed with cassava based diet. In: Tropical Tuber Crops: Problems, Prospects and Future strategies. Lebanon, Science Publisher, Chap., 65: 458-466.
8. Wallig, M.A., D.H. Gould and M.J. Fettman, 1988. Comparative toxicities of the naturally occurring nitrile 1-cyano-3, 4-epithiobutene and synthetic nitrile n-valeronitrile in rats: differences in target organs. Metabolism and Toxicol., 26: 149-157.
9. Ssnfey, H., G.B. Bulkley and J.L. Cameron, 1985. The pathogenesis of acute pancreatiris: The source and role of oxygen-derived free radicals in three different experimental models. Ann. Surg., 20: 633-639.
10. Richie, J.P., I. Skowronski, P. Abraham and Y. Leutzinger, 1996. Bloodglutathione concentration in large scale human study. Clin. Chem., 42: 64-70.
11. Duron, O. and B. Kelly, 1963. Improved method for determination of blood Glutathione. J. Lab. Clin. Med., 61: 882-887.
12. Gutterridge, J.M.C. and C. Wilkins, 1982. Copper-dependent hydroxyl radical damage to ascorbic acid. Formation of thiobarbituric acid reactive product. FEBS Letl., 137: 327-340.
13. Esser, A.J.A., M. Bosveld, R.M. van der Grift and A.G. Voragem., 1993. Studies on cassava products and introduction of a new chromogen. J. Sci. Food Agric., 63: 287-296.
14. Sorbo, B.H., 1953. Crystalline rhodanese. Purification and physicochemical examination. Acta. Chem. Scand., 7: 1129-1136.
15. Layne, E., 1975. Spectrophotometric and Turbidimetric methods for measuring proteins. In: Colowick, S.P and Kaplan (Eds.). Methods in Erizymology, New York, Academic Press, Vol: 3.
16. Doumas, B.T., W.A. Watson and H.G. Biggs, 1971. Albumin standards and measurements of serum albumin with Bromo-cresol Green. Clin. Chim. Acta., 31: 87.
17. Reitman, S. and S. Frankel, 1957. Determination of aspartate and alanine amino-transferase activities in blood serum and tissues. Am. J. Clin. Pathol., 28: 56.
18. Klein, B., P.A. Read and L.A. Babson, 1960. Rapid method for the quantitative determination of serum alkaline phosphatase. Clin. Chem., 6: 269-275.
19. Egekege, J.O. and F.W. Oehme, 1979. Blood and liver cyanide concentration in rats poisoned oral doses of potassium cyanide. Toxicol. Lett., 31: 243-247.
20. Haque, R. and J.H. Bradbuny, 1999. Simple kit method for determination of thiocyanate in urine. Clin. Chem., 45: 1459-1464.
21. Nagahara, N., T. Ito and M. Minami, 1999. Mercaptopyruvate sulphur transferase as a defense against cyanide toxication. Molecular properties and mode of toxication. Histol. Histopathol., 14: 1277-1286.
22. Meister, A. and Anderson, 1983. Glutathione. Ann. Rev. Biochem., 52: 711-760.
23. Dabrowski, A.A., A. Gabrylewiez and M. Chwieko, 1991. Products of lipid peroxidation and changes in sulfuhydryl compounds in pancreatic tissues of rats with caerulein-induced acute pancreatitis. Biochem. Metab. Biol., 46: 10-1625.
24. Luthen, R. and J.H. Greendell, 1994. Thiol metabolism and acute pancreatitis: Trying to make the pieces fit. Gastroenterol., 107: 888-1192.
25. Okafor, P.N., C.O. Okoronkwo, E.N. Maduagwu, 2002. Occupational and dietary exposures of humans to cyanide poisoning from large-scale cassava processing and ingestion of cassava foods. Food and Chem. Toxicol., 40: 1001-1005.
26. Joan, F.Z., R.P. Peter and D.M. Philip, 1987. Biochemical test for liver disease. In: Clinical Chemistry in Diagnosis and Treatment. 5th Edn. pp: 291-292.
27. Bogusz, M., 1975. The usefulness of the enzymatic tests in acute poisoning. Archives of Toxicology, 34: 159-167.
28. Okolie, N.P. and A.U. Osagie, 1999. Liver and Kidney lesions and associated changes induced in rabbits by chronic cyanide exposure. Food and Chem. Toxicol., 37: 747-750.
29. Okafor, P.N. and E.N. Maduagwu, 1999. Toxicity evaluation of novel cyanide containing-containing compounds of cassava tissue towards chicken embryo. West Afr. J. Biol. Applied Chem., 44: 24-27.