Bio-Research 3(2): 21 - 24

Toxicity Evaluation of the Cyanogens of a Nigeria Local Legume *(Vigna species)* in Rabbits

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Abstract

The toxicity of a Nigerian local edible legume (Vigna sp.) with high cyanogenic potential was monitored in male rabbits over a period of 48 and 72 hours using biochemical indicators of cyanide toxicity. Analysis of the urine and serum of the animals fed the local legume over the periods of 48 and 72 hours indicated the presence of both free and bound cyanide and elevated levels of thiocyanate. The serum levels of free and total cyanide were 6.10 and 6.51 μg/ml after 48hrs and 13.82 and 14.71μg/ml after 72hrs. The values of these compounds measured in the urine were 23.98 and 29.70 μg/ml after 48hrs and 54.06 and 63.60 μg/ml after 72hrs. Serum concentrations of thiocyanate were 13.62 and 16.73μg/ml after 48 and 72hrs respectively. That of urine was 49.38 and 134.53 after 48 and 72hrs respectively. These free radicals were also detected in the walls of the stomach and intestine and their contents. The biochemical and toxicological implications of these findings were discussed.

Keywords: Cyanide, toxicity, Vigna species, rabbits, thiocyanate .

Introduction

The seeds from several species of legumes also known as pulses are important sources of protein diet of millions of people in the tropics where many depend on plant protein as the major source of their dietary protein. Pulses are among the 23 food crop produced in the largest tonnage world wide in 1994 as estimated by UN Food and Agricultural Organization.

Legumes are known to contain antinutritional factors which reduce their protein digestibility and nutritive value and they also contain natural toxicants (Onigbinde and Akinyele, 1983; Okolie and Ugochukwu 1988; Okafor *et al*.2002a) One class of potentially toxic constituent of legumes are the cyanogenic glycosides. In this context, Montgomery (1965) reported that several plants foodstuff in common use, particularly in the tropics contain cyanide either in the form of glycosides or nitriles. Evidence for the cyanogens contents of some Nigerian legumes has been provided (Okolie and Ugochukwu 1988; Okafor *et al* 2002a). Several reports exist on the acute and chronic toxicity of cyanogenic glycosides of plants towards man and animals (Nestel 1973; Ermans *et al* 1980; Ekpechi 1973; Tylleskar *et al* 1992; Akintonwa and Tunwashe 1992; Okafor *et al* 2002b). There is now increasing evidence that thiocyanate, the main metabolite of cyanide in animals may lead to the production of nitrosamines *in vivo* which may enhance carcinogenesis (Maduagwu and Umoh 1988; Okoh 1992).

Although many commonly consumed legumes are reported to be cyanogenic, the amount of cyanide produced by most of these species is quit low and therefore pose little problem if any, to the health of human and domestic animals (Seigler *et al* 1989). However, Okafor *et al* (2002a) have identified a legume of Vigna species (local name. Odudu mbamba) usually consumed in large quantities in eastern-Nigeria that contain considerable amounts of cyanogens in the unprocessed form. Study of the toxic effects of this legume species towards animals is desirable in view of increasing development of pulses as source of dietary protein. This forms the basis of this present study.

Materials and Methods

Collection and treatment of *Vigna sp* (Odudu mbaba): The edible seeds of the Vigna sp (Odudu mbamba) used in this experiment were purchased from a local market in Awgu, Enugu State, Nigeria and ground into flour using a kitchen blender. The treatments feed was prepared by mixing 90% of the ground seeds with 10% commercial feed bought from Umuahia Township market. This was then kept in a plastic container and stored in a cool dry place till required for analysis.

Animal and treatment: Eight male Newzyland rabbits weighing between 900g –1000g bought from Non-ruminant Department of Michael Okpara University of Agriculture were used for this study. The animals were grouped into two of four animals each. Group 1 served as control and the other group served as test. The animals were fed commercial feed for 21days and then allowed to starve for 24 hours so as to eliminate any cyanide from their bodies, followed by feeding them with the *Vigna* sp compounded feed for 48 – 72hrs. The animals were sacrificed after 48 and 72hrs after feeding. The animals were anaesthetized with chloroform and blood removed by heart puncture using a syringe. The blood was allowed to clot and

Table 1: Total cyanide in gastrointestinal tract, serum and urine of rabbits fed the *Vigna sp.* (Odudu mbamba) µg/ml/total tissue weight

Sample	C 48	T 48	C 72	T 72
Serum	1.78± 0.06[a]	6.51±0.10[a]	3.02 ±0.05[a]	14.71±0.21[a]
Urine	5.95 ±0.06[a]	29.7 ± 7.3[a]	12.01±1.02[a]	63.6 ± 6.15[a]
Stomach wall	1.70±0.02[b]	1.84±0.04[b]	2.8 ±0.03[b]	10.60 ± 1.12[b]
Stomach content	2.99 ±0.07[b]	4.81 ±0.08[b]	3.3 ±0.4[b]	14 ± 22 + 1.37[b]
Small intestinal wall	0.89± 0.011[b]	2.94 ±0.03[b]	1.02 ±0.06[b]	3.77 ±0.08[b]
Small intestinal content	1.4 ±0.02[b]	2.07 ±0.10[b]	1.32 ±0.02[b]	2.07 ±0.07[b]

**The values given are the means ±SD of three determinations from two rabbits. a = Cyanide in µgml[-1] b = Cyanide in µg per total tissue weights. C 48 = 48 hr control T 48 = 48 hr treatment C 72 = 72 hr control T 72 = 72 hr treatment*

Table 2: free cyanide in gastrointestinal tract, serum and urine of rabbits fed the fed the *Vigna sp.* (Odudu mabamba)

Sample	C 48	T 48	C 72	T 72
Serum	1.60± 0.03[a]	6.10 ±0.05[a]	2.80 ±0.02[a]	13.82 ±0.32[a]
Urine	4.88 ±0.06[a]	23.985.4[a]	10.45 ±1.32[a]	54.06 ±7.05[a]
Stomach wall	1.10±0.01[b]	1.16 ±0.07[b]	1.74 ±0.06[b]	6.47 ±0.81[b]
Stomach content	2.12 ±0.09[b]	3.51 ±0.10 [b]	2.41 ±0.08[b]	10.5 2 ±1.21[b]
Small intestinal wall	0.75 ±0.03[b]	2.49 ±005[b]	0.85 ±0.01[b]	3.20 ±0.05[b]
Small intestinal content	1.25 ±002[b]	1.80 ±0.03[b]	1.17 ±0.01[b]	1.86 ±0.05[b]

**The values given are the means ±SD of three determinations from two rabbits. a = Cyanide in µgml[-1] b = Cyanide in µg per total tissue weights. C 48 = 48 hr control T 48 = 48 hr treatment C 72 = 72 hr control T 72 = 72 hr treatment*

then centrifuged rapidly at 2700 x g and serum removed by means of pasture pipette. The gastrointestinal tract (GIT) was carefully dissected out and separated into stomach, small intestine, and large intestine portion. The contents of stomach and small intestine were removed, the weights of the walls and contents were recorded and stored separately. All samples including the collected samples of urine from the bladder were stored frozen in screw-capped bottles until required for analysis.

Cyanide assay in the feed samples, tissues, serum and urine: Approximately 3g of each of the dried commercial and animal treatment feeds were extracted with 15ml orthophosphoric acid (0.1M) The extract was then stored frozen in a tight closed vessel until assayed for cyanide content. In the case of the gastrointestinal walls and contents, the total separated portion was extracted with normal saline after mincing and homogenizing in the same medium. The cyanide in the various extracts was determined by a procedure based on the spectrophotometric method of Esser *et al* (1993), as described below.

For total cyanide, 0.1ml of aliquots of the extracts were pipetted into different test-tube. 0.1ml of linamarase enzymes was added followed by incubation at 40°c for 30 mins. The reaction was stopped using 0.6ml of 0.2M NaOH followed by addition of 2.8ml of phosphate buffer (pH 6.0), 0.2ml chloramineT and 0.8ml color reagent. The test- tube were allowed to stand for 20min and the blue color developed read at 605nm. Free cyanide determination was carried out as for total cyanide except there was no incubation with linamarase enzyme.

Thiocyanate assay: Thiocyanate in serum, urine and GIT tract was estimated using ferric nitrate reagent as described by Sorbo (1953).

Result and Discussion

The results of total and free cyanide in serum, urine, small intestinal walls and contents and stomach wall and contents are shown in Tables 1 and 2. The animals maintained on this local legume (Vigna sp) for 72hrs had higher levels of totals and free cyanide in serum, urine and the GIT followed by those of 48hrs and then the control. The results showed the presence of both glucosidic and non -glucosidic cyanide in serum, urine and along the gastrointestinal tract. The high level of both total and free cyanide in the urine and serum of the experimental rabbits indicated exposure to cyanide resulting from ingestion of the *Vigna sp.* The lower concentrations of both totals and free cyanide in the stomach contents than in both small intestinal walls and contents indicated evidence of cyanide degradation and metabolism along the gastro intestinal tract. The levels of free cyanide in serum, urine and GIT might have resulted in part from hydrolysis of glycosidic (bound) cyanide, as suggested by Okoh *et al* (1988) who reported the hydrolysis of dhurin along the gastrointestinal tract of rats fed sorghum. The blood levels of cyanide reflect the balance between the rate of absorption and the rate of enzymatic detoxification to thiocyanate the main cyanide metabolite.

It is interesting to note that reasonable amounts of cyanide were measured in the urine, serum, and tissues for control animals. This is not surprising, since the commercial feed given to the control animals was found to contain appreciable levels of cyanide (Table 4). The results of the cyanide content of both the *Vigna sp* and commercial feed were consistent with earlier report by Montgomery (1965) that several plant foodstuff in common use, particularly in the tropics contain cyanide in the form of glycosides or nitriles. The amount of thiocyanate, the principal cyanide metabolite measured in urine,

Table 3: Thiocyanate in n gastrointestinal tract, serum and urine of rabbits the fed the *Vigna sp.* (Odudu mabamba) (μg per total tissue weight/ μg/ ml

Sample	C 48	T 48	C 72	T 72
Serum	9.73± 1.37[a]	13.62±2.31.05[a]	11.25 ±2.51[a]	16.73 ±2.05[a]
Urine	30.72±5.21[a]	49.38±3.48[a]	29.76 ±3.31[a]	134.53 ±1.34[a]
Stomach wall	12.78±1.55[b]	2193±2.91[b]	17.38 ±1.99[b]	32.04 ±4.42[b]
Stomach content	7.12±2.01[b]	7.92±0.87[b]	12.43±0.77[b]	28.64 ±3.71[b]
Small intestinal wall	7.24±1.05[b]	13.10±10.1[b]	8.23±1.12[b]	7.73 ±0.73[b]
Small intestinal content	9.13±0.179[b]	14.04±1.45[b]	6.07±1.01[b]	7.32 ±0.61[b]

**The values given are the means ±SD of three determinations from two rabbits. a = Cyanide in μgml[-1] b = Cyanide in μg per total tissue weights. C 48 = 48 hr control T 48 = 48 hr treatment C 72 = 72 hr control T 72 = 72 hr treatment*

Table 4: Some chemical components of test and control feeds

Cyanide. Mg HCN/kg= Feed	Total	Free	Thiocyanate mg/kg
Vigna sp	30.55± 2.47	20.77±3.01	N.D
Commercial feed *vigna sp* +10%	17.32 ±3.07	12.16±1.76	N.D
Commercial (Test feed)	27.59±2.03	19.27± 20.3	ND

**the values given are the mean of at least 3 separate determinations. *ND = detected by the method of assay.*

serum, small intestinal walls and contents are shown in Table 3. Highest level of thiocyanate was found in the 72hrs treatment group followed by the 48hrs treatment group. The increases in the levels of cyanide and thiocyanate in serum, urine, small intestinal walls and contents and stomach walls and contents of the treatment groups above the controls is an evidence of cyanide exposure resulting from ingestion of the local legume by the rabbits.

These high amounts of thiocyanate were due to increase in thiocyanate pool (Boxer and Rickard, 1952, Okoh and Pitt, 1982) resulting from the detoxification of cyanide by the enzymes rhodanese and mercaptopyruvate sulfur transferase.

The high levels of thiocyanate founds in the systems of the treatment group suggest that a greater percentage of the ingested cyanide from the legumes has been metabolized into thiocyanate within the period of study. This is in agreement with the elimination half- life of thiocyanate which has been estimated to be 3 days in healthy subjects (Shulz 1984) and the work of Okoh and Pitt (1982) who demonstrated the metabolism of cyanide into thiocyanate and the excretion of this radical in urine.

From the results of this study, acute cyanide poisoning may not likely occur in rabbit as a result of ingestion of this local legume. However the reverse may be the case in the condition of economic stress bearing in mind that cassava (another cyanogenic plant) consumption is very high in Nigeria. Another significant findings from this study is that ingestion of this local legume results in elevation in the body of thiocyanate the main cyanide metabolite. In this context, thiocyanate has been implicated as an aetiologic candidate of goiter (Ermans, 1980) cretinism (Lagasse, 1980), ataxic neuropathy (Osuntokun, 1983) and Konzo an upper motor neuron disease (Banea, 1993, Lantum, 1998). Moreover, in protein deficient subject where sulfur amino acids (SAA) are low, CN may considerably be converted to cyanate (OCN^-) which is known to cause neurodegenerative disease in humans and animals.

Since this local legume is consumed frequently and in large quantities, appropriate processing techniques to detoxify cyanide should be used during its preparation in order to reduce the risk of cyanide intoxication.

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