

SHORT COMMUNICATION**RELATIONSHIP BETWEEN THE INGESTION OF CYANOGENIC GLYCOSIDES AND THEIR EXCRETION IN URINE**P.N. OKAFOR^{1*} and E.N. MADUAGWU²¹ Department of Biochemistry, College of Health Sciences, Ladoko Akintola University of Technology, P.M.B 4000 Ogbomoso, Nigeria.² Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria.

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Abstract: Quantitative relationship between orally administered pure linamarin (cyanogenic glucoside) and crude cyanogenic glucoside preparation from cassava and their excretion in 24 h urine, as a possible means of estimating average daily intake of cyanogenic glucoside of cassava by animals and man was investigated in rats. There was no statistically significant difference ($p < 0.01$) between the mean of the percentage excretion of pure linamarin ($\bar{x} = 20.6 \pm 2.37\%$) and that of crude cyanogenic glucoside ($\bar{x} = 21.19 \pm 3.3\%$). The urinary excretion of those compounds was found to be dose-dependent with good positive correlation ($r = 0.75$ for linamarin and $r = 0.74$ for crude cyanogenic glucoside) between ingestion of the glucosides and their excretion in urine as glucosidic cyanide. These results indicate that average daily intake of cyanogenic glycosides of cassava can be assessed by monitoring urine.

Key words: Cassava, cyanide, cyanogenic glucosides, linamarin, urine.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) roots and leaves are important food sources in most tropical countries of Africa, Asia and Latin America. The cassava plant is known to contain cyanogenic glycosides in its edible roots and leaves. Hydrolysis of cyanogenic glycosides by β -glucosidase² or intestinal bacteria³ yields hydrogen cyanide. Exposure to dietary cyanide from cassava is known to pose a hazard to public health. Cyanide in cassava has also been implicated in the etiology of certain nutritional neuropathies⁴⁻⁶. After ingestion of cassava foods, both glucosidic (bound cyanide) and non-glucosidic (free cyanide) cyanide are known to appear in urine of humans and animals.⁷ Excretion of high levels of glucosidic cyanide in human urine has also been reported by Mlingi *et al*.⁸ However, the relationship between ingestion of glucosides of cassava and their appearance in urine has not been subject to much investigation. This study, is aimed at providing a possible means of assessing or estimating the average daily intake of cyanogenic glycosides of cassava by animals and man by monitoring urine.

* Corresponding author

METHODS AND MATERIALS

Chemicals : Pure Linamarin (m.p. 138.147°, mol/wt 247.2), Linamarase [EC. 3.2.1.21], bispyrazolone (G.P.R grade) 3-methyl-1-phenyl-5-pyrazolone (G.P.R grade), Chloramine T (G.P.R. grade) were all obtained from BDH Chemicals, U.K.

Crude cyanogenic glucoside preparation : An ethanol extract of cyanogenic glucosides of cassava was prepared in our laboratory according to the method of Nartey⁹. Cassava tissues (roots) were homogenised in hot 80% aqueous ethanol and the slurry obtained filtered. The filtrate was washed with hot aqueous ethanol and then evaporated to dryness in a rotary evaporator at 35°C. The residue was extracted with methanol, evaporated to dryness and re-extracted with hot ethylacetate which on working up gave a creamy solid product. The product was then separated into the glucosides and other compounds chromatographically.

Animals: Fifty four male albino rats of the Wistar strain (weighing 100g on the average) bred in our pre-clinical animal house were used. All the 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 diets before experimentation.

Dosing of animals: There were nine groups of rats each comprising six animals. Animals in group 1 were dosed with physiological saline and served as the control. Those in groups 2,3,4 and 5 were dosed with 1.0, 2.5, 5.0 and 7.5 mg linamarin/100g body weight by stomach incubation respectively. The animals in groups 6, 7, 8 and 9 were administered 1.0, 2.5, 5.0 and 7.5 mg of crude cyanogenic glucoside preparation from cassava as above.

Collection of animal urine, blood and faeces for cyanide and thiocyanate determination : Urine, blood samples and faecal matter of the dosed animals were collected after 24h and analysed for the presence of linamarin (bound or glucosidic cyanide), free (non-glucosidic) cyanide and thiocyanate by the methods of Cooke¹⁰, and Sorbo¹¹ respectively. An aliquot of 0.1ml of the urine diluted with ortho-phosphoric acid, or a filtered slurry of faeces in the acid (1:2w/v) was mixed with 0.4ml of 0.2M phosphate buffer pH 7.0 and incubated with 0.1ml of linamarase for 30min to give total cyanide. The enzyme reaction was stopped by adding 0.6ml of 0.2N NaOH and the cyanide present determined spectrophotometrically by making up the solution to 5ml with 2.8ml of 0.1M phosphate buffer pH 6.0 followed by addition of 0.2ml chloramine T and 0.8ml of pyridine/pyrazolone reagent. The blue colour developed was read at 620nm after 60 min. Samples incubated without addition of enzyme preparation gave the non-glucosidic cyanide content.

Before the animals were administered with pure linamarin and crude cyanogenic glucoside preparation from cassava, their urine samples and faeces were collected and analysed for the presence of glucosidic and non-glucosidic cyanide. Moreover, their diets were carefully checked for any ability to release HCN/CN⁻ from linamarin by incubating a portion of the diet along with linamarin with and without an active linamarase preparation from cassava. Only when the active linamarase was included in the incubation mixture preparation was there any detectable production of HCN. Prior to use, linamarin was assayed to ensure that it was free of hydrogen cyanide and is hydrolysable by β -glucosidase of cassava.

The percentage excretion of pure linamarin was calculated from the doses administered to the rats and the corresponding amounts that appear in their urine unmetabolised. Averages were taken from the amounts of the glucoside excreted by the animals in each group.

RESULTS AND DISCUSSION

Analysis of urine of test rats showed that, the administered linamarin and crude cyanogenic glucoside preparation were partially metabolized yielding free (non-glucosidic) cyanide and thiocyanate while certain proportions were excreted unchanged. All the rats in the test groups excreted glucosidic (bound) cyanide as shown in Table 1. From the table 0.24 ± 0.01 - 1.365 ± 0.05 mg of the administered pure linamarin (representing 18.2 - 24.3%; $\bar{x} = 20.6 \pm 2.37\%$) appeared in 24h urine of rats unchanged, whereas, 0.25 ± 0.01 - 1.32 ± 0.02 mg of the crude cyanogenic glucoside (representing 17.6-25.2%; $\bar{x} = 21.2 \pm 3.3\%$) given to the rats were excreted unmetabolised. This indicates that urinary excretion of bound or glucosidic cyanide could be a good indicator for monitoring cyanogenic glucoside intake by animals and man within 24h.

The urinary excretion of both pure linamarin and crude cyanogenic glucoside preparation was found to be dose-dependent. There was a good positive correlation ($r=0.75$ for linamarin and $r=0.74$ for crude cyanogenic glucoside) between the amounts of the ingested glucosides and their excretion in urine as glucosidic cyanide. Increase in the total amounts of the glucosides excreted with increasing concentrations of the administered compounds was observed. However, with increasing concentrations of the administered compound, the increase in excretion becomes smaller and smaller. This may reflect the activity of the enzyme that is responsible for hydrolysis of the glucosides. Moreover, diet has been shown to influence the population of bacteria that function in the cyanogenic glucoside hydrolysis and detoxification³. Thiamin status of animals has also been shown to affect the metabolism of linamarin¹² and Fukuba & Mendoza¹³ showed that absorption of linamarin is faster during the first 60 min following incubation of linamarin with rat intestine.

Table 1: Relationship between administered cyanogenic glucosides and the excreted unchanged compounds in 24h urine samples.

| Linamarin dose level mg/100g body wt. | No of animals | Total cyanide (glucosidic + non-glucosidic) excreted (mg) Mean \pm S.D | Bound cyanide (glucosidic) excreted (mg) Mean \pm S.D | Free cyanide (non- glucosidic) excreted (mg) Mean \pm S.D | Percentage excretion of glucosidic cyanide |
|--|---------------|--|--|--|---|
| 1.00 | 6 | 0.53 \pm 0.01 | 0.24 \pm 0.01 | 0.29 \pm 0.01 | 24.3 |
| 2.50 | 6 | 0.91 \pm 0.0.15 | 0.53 \pm 0.02 | 0.39 \pm 0.02 | 21.0 |
| 5.00 | 6 | 1.40 \pm 0.03 | 0.94 \pm 0.05 | 0.46 \pm 0.01 | 18.9 |
| 7.50 | 6 | 1.97 \pm 0.02 | 1.37 \pm 0.05 | 0.60 \pm 0.03 | 18.2 |
| Cyanogenic glucoside dose level mg/100mg body wt (mg) | | | | | |
| 1.00 | 6 | 0.48 \pm 0.02 | 0.25 \pm 0.01 | 0.23 \pm 0.01 | 25.2 |
| 2.50 | 6 | 1.07 \pm 0.03 | 0.59 \pm 0.02 | 0.48 \pm 0.03 | 23.7 |
| 5.00 | 6 | 1.52 \pm 0.02 | 0.91 \pm 0.01 | 0.61 \pm 0.02 | 18.3 |
| 7.50 | 6 | 1.96 \pm 0.03 | 1.32 \pm 0.02 | 0.64 \pm 0.03 | 17.6 |
| Control | 6 | NS | ND | NS | NS |

*NS = Not significant

*ND = Not detectable

Elevation in the amounts of urinary thiocyanate (cyanide metabolite) excretion by the test animals was observed. Separate administration of 1.0, 2.5, 5.0 and 7.5mg/100g body.wt of pure linamarin and the crude cyanogenic glucoside preparation resulted in the urinary thiocyanate output of 0.37 ± 0.02 , 1.14 ± 0.03 , 2.49 ± 0.05 and $2.59 \pm 0.03 \mu\text{g}$ for linamarin and 0.41 ± 0.02 , 1.08 ± 0.03 , 2.51 ± 0.03 , and $2.58 \pm 0.05 \mu\text{g}$ for the crude cyanogenic glucoside preparation above the base level in urine.

No detectable amounts of the glucosides were found in the blood or faeces of these rats. Non detection of glucosidic cyanide in the blood and faeces could be that their concentrations in blood and faeces are below the detection limit of the analytical method used. Again, linamarin may be bound to some other compounds making it to be in forms not available for analysis.

Normal urine neither interferes with the hydrolysis of cyanogenic glycosides and the release of HCN formed, nor does it contain substances which in themselves give rise to positive reaction¹⁵. Thus assaying the extent of cyanogenic glycoside excretion in urine could be a good analytical method for assessing or estimating the intake of cyanogenic glucosides.

The relevance of this study is that it could be applied in estimating the average daily intake of cyanogenic glycosides by animals and man. However, such quantitative relationships have to be established for each species. This is because there are often wide species differences (especially between the rodents and primates) in the metabolism of ingested chemicals.

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