A COMPARATIVE STUDY OF THE TOXIC EFFECTS OF PROLONGED INTAKE OF CASSAVA-BORNE ORGANIC CYANIDE AND INORGANIC CYANIDE IN SOME RABBIT TISSUES

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DOI: 10.7897/2277-4572.02457
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Received on: 20/07/13 Revised on: 25/08/13 Accepted on: 31/08/13

ABSTRACT
The study was aimed at comparing the toxic effects of cassava-borne organic cyanide and equivalent level of inorganic cyanide (KCN) in New Zealand White rabbits. Three groups of 3-month old, male weanling rabbits (4 per group) were used. One group received pure growers mash (control), while another was fed isonitrogenous cassava peel flour, CPF containing 702 ppm organic cyanide. A third group was given mash with 702 ppm KCN (inorganic cyanide). Feed intake, body weight gain, and serum SCN were recorded. The animals were fed for 10 weeks prior to sacrifice and isolation of tissues for assays of rhodanese, LDH, ALT, AST and serum urea and creatinine. Feed intake and weight gain were significantly lower in CPF group than in KCN group, but LDH activities in serum, liver, and lung were significantly higher in the CPF group (p < 0.05). There were no significant differences in the tissue and serum levels of AST and ALT between both groups. In addition, serum urea and creatinine did not differ significantly between the CPF and KCN groups. However liver and kidney rhodanese activities and urinary thiocyanate were significantly higher in the KCN group (p < 0.05). More pronounced pathological lesions (tubular and glomerular necrosis) were evident in the kidney from the CPF group, while liver lesions (congestion, necrosis and inflammatory reactions) were more severe in the KCN group. These results suggest that although the metabolic effects of cassava-borne organic cyanide and inorganic cyanide resemble, the severity of their tissue toxicities may differ.

Keywords: Cassava-borne Organic Cyanide, KCN, Toxicity, Rabbits.

INTRODUCTION
The underground tuberous roots of cassava, *Manihot esculenta* Crantz provide an important source of dietary calories for over 500 million people in the tropics. Virtually all parts of the plant are useful. The peel derived from the tubers, which were hitherto discarded during cassava processing, are currently gaining acceptance as possible ingredients such as millet, corn and sorghum, due to increasing cost of these inputs. However one major drawback in the use of cassava either as human food or as animal feed is the presence of cyanogenic glucosides in all its tissues. Studies have shown that cassava peel contains 5-10 times more cyanide than the edible portion of the tuber. Cyanide is present in cassava in the form of the cyanogenic glucosides linamarin and lotaustralin, the latter of which constitutes about 93% of the total glucosides. On hydrolysis, the intact glucosides liberate free cyanide which is toxic. The toxicity of cyanide derives from its potency as a respiratory poison in all aerobic forms of life. In acute doses, death results from respiratory failure due to the high susceptibility of the nerve cells of the respiratory centre to hypoxia. Cyanide toxicity is influenced by the form of cyanide ingested. Thus in humans, free cyanide and intermediate breakdown products of linamarin (metal cyanides and cyanohydrins) are more toxic than intact linamarin. This is so because humans lack beta-glucosidase, the enzyme required to hydrolyse linamarin to free cyanide. The metabolic effect of cyanide in lower animals may also vary with the type of cyanide ingested. Indeed it has been reported that cassava organic cyanide and inorganic cyanide produced different effects on rate of muscle development in dogs. Thus these two forms of cyanide may exert different levels of toxicity in other organs and tissues. However information on the relative toxicities of these two forms of cyanide are few and are based on short-term studies. The present study was therefore carried out to compare the toxic effects of prolonged exposure of rabbits to cassava peel-borne organic cyanide and equivalent amount of inorganic cyanide.

MATERIALS AND METHODS

Animals
12 male weanling New Zealand White rabbits (initial mean weight = 1.52 kg) aged about 16 weeks, were obtained from the Animal House, Department of Microbiology, University of Benin. The animals were housed singly in metal hutches and acclimatized for 2 weeks to growers mash (product of Bendel Feed and Flour Mills, BFFM Ltd, Ewu, Nigeria) prior to the experiment.

Preparation of Cassava Peel Feed
Fresh cassava peels were collected in bulk from a cassava-processing factory in Benin City. After manually removing the thin outer brown layer, the peel was washed in tap water and subsequently ground coarsely in a cassava mill. The grated peel was placed in a clean jute bag and mechanically pressed for 1 h to facilitate de-watering. The resultant coarse cake was then manually broken down to smaller particle sizes and sun-dried for 3 days. The total cyanide content of the flour was determined colorimetrically by the alkaline picrate method.

Grouping of Animals and Feeding
Following acclimatization, the rabbits were randomly assigned to 3 groups (4 per group). One group was fed the cassava peel feed, made isonitrogenous with growers’ mash (11.2% protein) by addition of soybean meal (32% protein). Rabbits in the control group were given pure mash, while rabbits in the third group received mash containing 702 ppm inorganic cyanide (KCN), equivalent to the level of total cyanide present in the cassava peel. The KCN-mash mixture...
was prepared daily by mixing 1 kg of mash with 1.755 g of KCN dissolved in 50 ml of tap water. The supplementation of cassava feed with soybean meal was also carried out on daily basis, just before feed presentation. Feeding was at the rate of 90 g / kg body weight / day. Both mash and cassava feed were also supplemented with vitamins and minerals using Vitadol™ premix (tucol Products Ltd, Ontario). Prior to feeding, each feed was mixed with water in the ratio of 10:1 (w/v) to achieve a texture acceptable to the animals. The feeds were stored in an airy room with a 200 W electric bulb regularly lit to discourage mould growth. The animals had unrestricted access to clean drinking water. All stale feed were daily weighed and discarded before supply of fresh feed. Rabbits in each group were weighed weekly. Rabbits in each group were weighed weekly.

Determination of Total Cyanide in Cassava Feed
Total cyanide was determined colorimetrically by a modification of the alkaline picrate method15. In essence, 2.0 g of feed was thoroughly ground in a hand mortar and homogenized with 50 ml of 0.1M acetate buffer, pH 5 for 10 minutes. The homogenate was allowed to stand for 10 minutes. To 0.5 ml of the supernatant in a quick-fit tube was added 2.5 ml of 1M acetate buffer, pH 5. After mixing, 0.5 ml of 150 U/ml beta-glucosidase (Sigma) was added. A tube containing 0.5 ml of distilled water in place of extract served as blank. The tubes were vortexed and incubated at room temperature (27°C) for 20 minutes. Then 4.0 ml of alkaline picric acid reagent was added to each tube, and the tubes were placed in near-boiling water (95°C) bath for 5 minutes. On cooling, the absorbance of the resultant orange color was read at 490 nm. Corresponding cyanide levels were extrapolated from a cyanide calibration curve, and converted to ppm (mg / kg).

Enzyme Assays
Rhodanese activity was assayed colorimetrically according to the method of Sorbo17. In this assay, thiocyanate liberated by rhodanese reacts with ferric nitrate (Soro reagent) to yield an orange colored complex which absorbs strongly at 460 nm. Lactate dehydrogenase, LDH was assayed spectrophotometrically by following NADH oxidation at 340 nm according to the procedure described by Bergmeyer18. The activities of alanine transaminase, ALT and aspartate transaminase, AST were determined colorimetrically with 2, 4-dinitrophenyl hydrazine according to the method of Bergmeyer and Bern19.

Estimation of Metabolites
Urea was determined colorimetrically by the Berthelot reaction20, while creatinine was estimated in de-proteinized samples using the Jaffe reaction20. Thiocyanate levels were assayed colorimetrically according to the method of Bowler21.

Histology
Liver and kidney sections fixed in formal-saline were processed for light microscopy at the Department of Morbid Anatomy, University of Benin Teaching Hospital, Benin City. The resultant slides were read and interpreted by a qualified pathologist at the hospital, who also photographed relevant sections using a camera-fitted, binocular Leitz microscope.

Statistics
Values of parameters for the 3 groups were expressed as mean ± SEM and compared for significant differences using Analysis of Variance, ANOVA. P values < 0.05 were taken as significant.

RESULTS
Table 1 shows the level of mean daily feed intake for rabbits in each group as well as their mean weight changes, daily cyanide exposure and serum SCN profiles. Although the cassava peel feed caused a significantly lower weight gain than mash / KCN feed, its feed efficiency was higher. Cyanide exposure from the cassava peel was significantly lower than that from the mash / KCN feed (p < 0.05). However the serum SCN from the cassava peel group was significantly higher than corresponding value from the mash / KCN group.

The effect of the two forms of cyanide on some tissue metabolites are shown on Table 2. Rhodanese activities in the liver, kidney and heart tissues from the rabbits given inorganic cyanide were significantly higher than corresponding values for tissues derived from rabbits fed the cassava-borne organic cyanide (p < 0.05). Although the cassava feed led to increases in tissue rhodanese, these increases were not statistically significant.

<table>
<thead>
<tr>
<th>Group / Parameter</th>
<th>Control (Mash only)</th>
<th>Cassava peel (CP)</th>
<th>Mash + KCN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean feed intake (g / rabbit / day)</td>
<td>44.10 ± 2.10a</td>
<td>20.20 ± 1.40b</td>
<td>38.00 ± 1.20b</td>
</tr>
<tr>
<td>Mean weight gain (g / rabbit)</td>
<td>719 ± 183</td>
<td>458 ± 173</td>
<td>612 ± 133</td>
</tr>
<tr>
<td>Feed efficiency (g weight gain / g of feed)</td>
<td>16.30</td>
<td>22.60</td>
<td>16.10</td>
</tr>
<tr>
<td>Total cyanide intake (mg / day / rabbit)</td>
<td>0.50 ± 0.02c</td>
<td>14.20 ± 1.55c</td>
<td>23.20 ± 1.80b</td>
</tr>
<tr>
<td>Serum SCN (µmole / dl)</td>
<td>3.70 ± 0.42c</td>
<td>8.69 ± 0.76c</td>
<td>4.44 ± 0.10c</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± SEM (n = 4). For each parameter, values having different superscripts across differ significantly (p < 0.05)

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LDH activity was significantly increased in the liver, kidney and serum of the cassava-fed group, while in the KCN group, LDH increases were seen only in the liver and kidney (p < 0.05, Table 3). Liver LDH activity was significantly higher in the cassava group than in the KCN group.

Table 3: Effect of Cassava Peel Organic Cyanide and Inorganic Cyanide on Serum and Some Tissue LDH Activities

<table>
<thead>
<tr>
<th>Group / tissue</th>
<th>Control</th>
<th>CP</th>
<th>Mash + KCN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (U/g)</td>
<td>417 ± 50°</td>
<td>995 ± 67°</td>
<td>785 ± 69°</td>
</tr>
<tr>
<td>Kidney (U/g)</td>
<td>302 ± 19°</td>
<td>418 ± 38°</td>
<td>405 ± 48°</td>
</tr>
<tr>
<td>Heart (U/g)</td>
<td>91 ± 3°</td>
<td>116 ± 21°</td>
<td>82 ± 16°</td>
</tr>
<tr>
<td>Serum (U/L)</td>
<td>14 ± 1</td>
<td>15 ± 1</td>
<td>15 ± 2</td>
</tr>
</tbody>
</table>

Each value is Mean ± SEM (n = 4). For each tissue, those with different superscripts across differ significantly ((p < 0.05).

There were no significant differences in the activities of AST in the serum, liver and heart between the cassava feed and the KCN-mash feed (Table 4). In addition, the differences in the serum, liver and heart activities of ALT between both groups were not significant.

Table 4: Effect of Cassava Peel Organic Cyanide and Inorganic Cyanide on AST Activity in Serum, Liver and Heart

<table>
<thead>
<tr>
<th>Group / tissue</th>
<th>Control</th>
<th>CP</th>
<th>Mash + KCN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (U/g)</td>
<td>4.1 ± 0.2</td>
<td>4.3 ± 0.4</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>Heart (U/g)</td>
<td>4.3 ± 0.2</td>
<td>3.9 ± 0.4</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Serum (U/L)</td>
<td>14 ± 1</td>
<td>15 ± 1</td>
<td>15 ± 2</td>
</tr>
</tbody>
</table>

Each value is Mean ± SEM (n = 4). For each parameter, vertical values with different superscripts differ significantly (p < 0.05).

Table 5: Effect of Cassava Peel Feed and KCN-mash Feed on Serum Levels of Urea and Creatinine

<table>
<thead>
<tr>
<th>Group / parameter</th>
<th>Urea (mmole / L)</th>
<th>Creatinine (mg / dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.3 ± 0.5°</td>
<td>0.7 ± 0.1°</td>
</tr>
<tr>
<td>CP</td>
<td>9.3 ± 0.8°</td>
<td>1.2 ± 0.1°</td>
</tr>
<tr>
<td>Mash + KCN</td>
<td>9.8 ± 0.5°</td>
<td>1.2 ± 0.1°</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± SEM (n = 4). For each parameter, vertical values with different superscripts differ significantly (p < 0.05).
DISCUSSION

The feed intake of the rabbits given cassava peel feed was significantly lower than those of the control and the KCN group, although the cassava-fed rabbits did not manifest weight loss. The absence of weight loss is a clear indication that the rabbits in the cassava peel fed group were not malnourished during the 10-month period of investigation. This is further corroborated by the superior feed efficiency of the cassava feed over pure mash and the mash + KCN feeds. Serum thiocyanate is a reliable index of dietary cyanide exposure. The serum SCN of the cassava organic cyanide group was almost double that of the KCN group, notwithstanding that the difference in their predicted cyanide exposure levels (calculated from daily feed intake). The obvious implication of these results is that the group fed cassava organic cyanide was exposed to higher levels of cyanide than the KCN group which received pure inorganic cyanide. This is consistent with the observation that liver and kidney rhodanese activities in the cassava group did not change with cyanide exposure, while for the KCN group; rhodanese activity was significantly increased in liver and kidney. Rhodanese (thiosulphate: cyanide sulphur transferase) is responsible for the detoxification of cyanide to thiocyanate, a less toxic form of cyanide. The higher exposure of the cassava group to cyanide is evidenced by the higher significant elevation of LDH activities in the serum and tissues of the CP group when compared to the KCN group. One metabolic effect of cyanide exposure is inhibition of the mitochondrial electron transport chain, thereby provoking lactic acidosis in tissues. The inhibition of oxidative metabolism as a consequence of decreased utilization of oxygen leads to a shift from aerobic to anaerobic metabolism, thereby provoking lactic acidosis.

Lactic acidosis arises from the need for the cells to sustain glycolysis as the only viable energy source through LDH-catalysed re-oxidation of NADH produced in the glyceraldehyde-3-phosphate dehydrogenase reaction. The result is accumulation of lactate in the blood and tissues. Thus a block of the respiratory chain triggers on increased activity of LDH. The more pronounced lesions seen in the liver and kidney of the rabbits fed cassava peel lends additional support to the fact that these rabbits were exposed to higher level of cyanide. These results suggest that the metabolic effects of cassava peel organic cyanide may be different from those of free, inorganic cyanide. In contrast, the toxic effects produced in goats after prolonged exposure to KCN were similar to those produced in another group of goats fed chokecherry (Prunus virginiana), a cyanophoric plant which contains the cyanogenic glycoside prunacin. However, our findings are in agreement with the report of Kemah, who observed liver lesions in one group of dogs fed cassava-born cyanide diet for 14 weeks, while in the same study, no liver lesions were observed in another group of dogs given rice supplemented with an equivalent level of inorganic cyanide, although both groups manifested kidney and testicular degenerative changes. Similarly, Ibebujo et al. reported that feeding gari (cassava) organic cyanide for 14 weeks depressed muscle development in dogs, while inorganic cyanide had no such effect. The present study is crucial in that it reports for the first time, differences between the two forms of cyanide based on data from a prolonged exposure period (10 months) and may serve as confirmation that these differences are not transient. Since the two experimental feeds contained the same levels of total cyanide, the observed differences in their metabolic effects must be related to the differences in the form of cyanide in each feed.

CONCLUSION

This study has obtained experimental evidence from prolonged exposure that the metabolic effect of cassava-born organic cyanide may be indeed different from those produced by an equivalent level of inorganic cyanide. The results further emphasize the need for proper processing of cyanophoric food staples, since humans are more likely to consume organic cyanide from these foods.

REFERENCES


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Figure 1 shows photomicrograph of the cortical area of kidney from the rabbits fed cassava peel. The cells lining the proximal and distal convoluted tubules are swollen, causing tubule lumen occlusion in some areas. There were more pronounced degenerative changes in the kidney photomicrographs taken from the cassava peel-fed rabbits. These include focal areas of necrosis in glomerulus, proximal convoluted tubule and distal convoluted tubule (Figure 2). Photomicrograph of liver sections from the cassava and KCN-fed rabbits are depicted in Figures 3 and 4 respectively. While the lesions in the cassava group include congestion of central vein, focal areas of necrosis and pronounced inflammatory reaction, the only lesions seen in the KCN group were focal areas of congestion and necrosis.

Figure 3: Liver section of rabbits fed cassava peel, showing congestion (A) inflammatory reaction (B) & focal areas of necrosis (C). (H & E, x 40)

Figure 4: Liver Section from the KCN-fed Rabbits depicting Focal Areas of Congestion (A), and Necrosis (B). (H & E, x 40)
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Source of support: NII, Conflict of interest: None Declared