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The Effects of Short-term Repeated Oral Administration of Potassium Cyanide on Some Haematological Indices and Internal Organs Morphology of Rabbits

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Authors' contributions

This work was carried out in collaboration amongst all authors. Authors ICO and OVU designed the study wrote the protocol and interpreted the data. Authors UC and OVU anchored the field study, gathered the initial data and performed preliminary data analysis. While authors ICO and UC managed the literature searches and produced the initial draft. All authors read and approved the final manuscript

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ABSTRACT

This study investigated the effects of short-term repeated oral administration of sub-toxic dose of potassium cyanide on the haematological indices and the structure of the thyroid, liver, adrenal, and spleen of rabbits. A total of 16 rabbits, weighing 1.2±0.2 kg were randomly divided into two groups. Group 1 was the control, and the animals were treated with 10 mL/kg body weight of distilled water *per os*. Group 2 was treated with 0.3 mg/kg potassium cyanide (KCN) in distilled water *per os*. Results revealed atrophy and distended thyroid follicles with flattened epithelial cells

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only in the cyanide treated group. The liver revealed severe periportal lymphocytic infiltration only in the cyanide treated animals, coupled with focal areas of hepatocellular coagulative necrosis, and cholangitis. The spleen revealed mild congestion of the red pulp in both treated and control groups, while hemosiderosis was seen only in the cyanide treated group. There was no visible lesion in the adrenal gland. The values of parameters evaluated in the KCN- treated animals were as follows: Packed Cell Volume (PCV) (33.25±2.4%), Red blood Cell Count (RBC) (6.93±0.7 x 10^6/µL), TWBC (Total White Blood cell Count) (9.4±1.0 x10^3/µL), Haemoglobin Concentration (HC) (14.7±1.9 g/dL), Aspartate Transaminase (AST) (29.8±5.7 IU/mL), Alanine amino transaminase (ALT) (12.8±1.8 IU/mL) and Alkaline Phosphatase (ALP) (48.0±5.7 IU/mL). Those of controls were PCV (31.0±0.94%), RBC (5.45±O.3 x10^6/µL), TWBC (6.8±0.43 x10^3/µL), HC (11.07±0.94 g/dL), AST (16.33±0.3 IU/mL), ALT (8.33±1.0 IU/mL), ALP (23.7±2.8 IU/mL). There was no significant difference ($p<0.05$) between the haematological indices of the treated and the control group. AST and ALP of the treated group was significantly higher ($p<0.05$) than that of the control.

Keywords: Potassium cyanide; liver; thyroid; sub-toxic dose; hematological indices.

1. INTRODUCTION

Cyanogenic glycosides are substances present in many plants that can produce highly toxic hydrogen cyanide (HCN) and the contents of this substance can be as high as 100 – 800 mg/kg of the plant material [1]. Enzymatic conversion enhanced when plant cells are damaged or stressed, of the glycosides is as it occurs when the plant is chewed, crushed, droughted, wilted, or frozen. A myriad of plant species are known to contain cyanogenic glycosides with the potential to produce HCN poisoning. Some of these plants are grown as food sources for humans and animals, for example, sorghum (*Sorghum* spp.), corn (*Zea mays*), clovers (*Trifolium* spp.), and manihot or cassava (*Manihot esculenta*). Although cyanide most commonly occurs as hydrogen cyanide, and in salt forms, such as sodium and potassium cyanide, it also occurs naturally in cassava (*Manihot esculenta* Cranz) as linamarin, a cyanogenic glycoside [2]. Cassava roots are a major source of calories for over 500 million people in the tropics, and the leaves are also used as vegetable in soups [3,4]. This increasing dependence of both man and animals on cassava and maize-based foods has made further study into the possible adverse effects of cyanide necessary. A relationship has also been suggested between pancreatic diabetes and prolonged exposure to the cassava [5]. While there is substantial information on the effect of cyanide generally [6], not much is known about the specific effects of sub-chronic or repeated short term oral administration of cyanide in rabbits, especially its effects on the haematology and some structures of the internal organs. The objective of this study is therefore to evaluate the effects of short-term repeated oral administration of potassium cyanide on the structure of the thyroid, liver, adrenal, spleen as well as the liver markers (AST, ALP and ALT) and the haematological indices (RBC, WBC and Hb) of rabbits.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Pre-pubertal rabbits, weighing 1.2±0.2 kg were purchased locally. They were acclimatized for 3 weeks and were ascertained to be in good health. The rabbits were housed in standard cages in a room with daily temperature range between 20ºC and 28ºC. All animals had access to feeds (both freshly cut grass and Vital® feeds Ltd, Nigeria) and water *ad libitum*, and were exposed to a 12-hour light-dark cycle. The laboratory animals were handled in accordance with the good laboratory practice regulation as contained in the Helsinki Declaration of 1975, as revised in 2000 and 2008.

Potassium Cyanide (KCN) was procured from BDH Chemicals, UK.

2.2 Experimental Design

A total of 16 rabbits, weighing 1.2±0.2 kg were randomly selected into two groups. Group 1 was the control, and the animals were treated with 10 mls/kg body weight of distilled water. Group 2 was treated with 0.3 mg/kg body weight of potassium cyanide (KCN) reconstituted in distilled water. Both the distilled water and the KCN were administered daily through the oral route using an improvised oro-gastric canula. Animal weights were regularly taken in order to effect any necessary adjustment(s) to the dose of KCN administered. The animals were treated for

30 days, at the end of which the animals were mildly euthanized using chloroform chamber anaesthesia. Blood samples were collected into EDTA bottles. The organs thyroid, liver, adrenal, and spleen were collected for histological examination as described by [7]. Other parameters evaluated were erythrocyte count (EC) and total while blood cell counts (TWBC), which were assayed using the method of [8]. Haemoglobin concentration (Hb) was assayed using the monophosphate method as described by [9], serum ALT was estimated colorimetrically by the 2, 4-dinitrophenylhydrazine (DNPH) method of [10] as further described by [11], while serum AST was estimated by the [10] colorimetric method using a QCA test kit (Quimica Clinica Applicada, Spain).

3. RESULTS

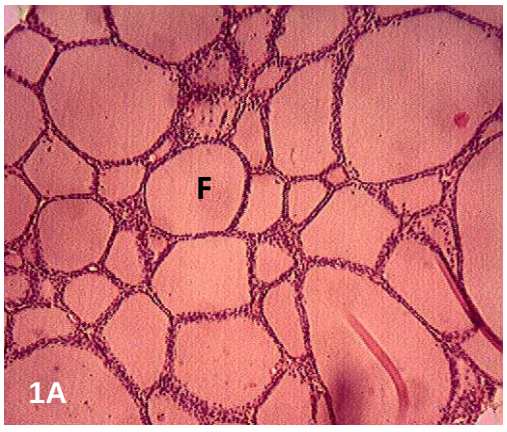
The results on the haematological indices and the three liver enzymes assayed are presented in the Table 1. The values for control groups were as follows: PCV (31.0±0.94%), RBC (5.45±0.3 x10^6µL), TWBC (6.8±0.43 x10^3/µL), HC (11.07±0.94 g/dL), AST (16.33±0.3 IU/mL), ALT (8.33±1.0 IU/mL), ALP (23.7±2.8 IU/mL) and those of KCN-treated animals were as follows: PCV (33.25±2.4%), RBC (6.93±0.7 x 10^6/µL), TWBC (9.4±1.0 x10^3/µL), HC (14.7±1.9 g/dL), AST (29.8±5.7 IU/mL), ALT (12.8±1.8 IU/mL) and ALP (48±5.7 IU/mL). The results showed that there was no significant difference ($p<0.05$) between the haematological indices of the treated and the control group. AST and ALP of the treated group was significantly higher ($p<0.05$) than that of the control.

Table 1. Mean hematological indices and three liver enzyme levels of rabbits administered short-term repeated sub-lethal dose of potassium cyanide (KCN)

Parameters	Groups	
	1	2
PCV (%)	31.0±0.94	33.25±2.4
RBC (×10^6/ µL)	5.45±0.3	6.93±0.7
TWBC (×10^3/µL)	6.8±0.43	9.4±1.0
HC (g/dL)	11.07±0.94	14.7±1.9
AST (IU/mL)	16.33±0.3	29.8±5.7*
ALT (IU/mL)	8.33±1.0	12.8±1.8
ALP (IU/mL)	23.7±2.8	48.0±5.7*

** Significance at p ≤ 0.05; Group 1 was administered 10 mL/kg body weight distilled water; Group 2 was administered 0.3 mg/kg bw KCN*

The results of the effect of cyanide treatment on the structures of the liver, thyroid, adrenal and spleen of the rabbits are presented in Figs. 1 to 3. The results revealed atrophy and distended thyroid follicles with flattened epithelial cells in the cyanide treated group (Figs. 1A and 1B). The liver revealed severe periportal lymphocytic infiltration in the cyanide treated animals, coupled with focal areas of hepatocellular coagulative necrosis, and cholangitis. (Figs. 2A and 2B), The spleen revealed mild congestion of the red pulp in both treated and controls, while hemosiderosis was seen only in the cyanide treated group (Figs. 3A and 3B). There was no visible lesion in the adrenal gland.



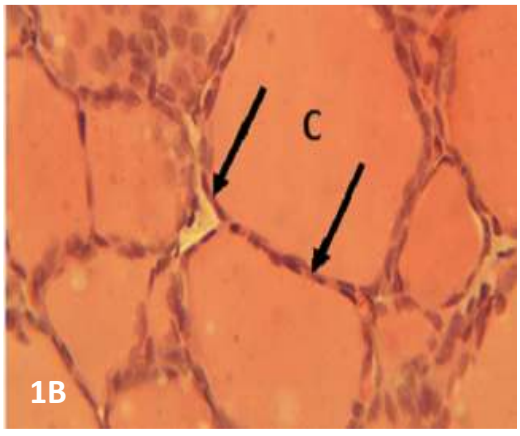


Fig. 1A. Thyroid of animal in group 1 (10 mL/kg distilled water) showing no visible pathological lesion, F= Follicle distended with colloid, while in 1B. Shows thyroid parenchyma of animal in treated group 2 (0.3 mg/kg KCN) with atrophy of thyropid epithelium with distended follicles and flattened epithelial cells (arrows), C= Colloid. H & E stain, x40; x200)

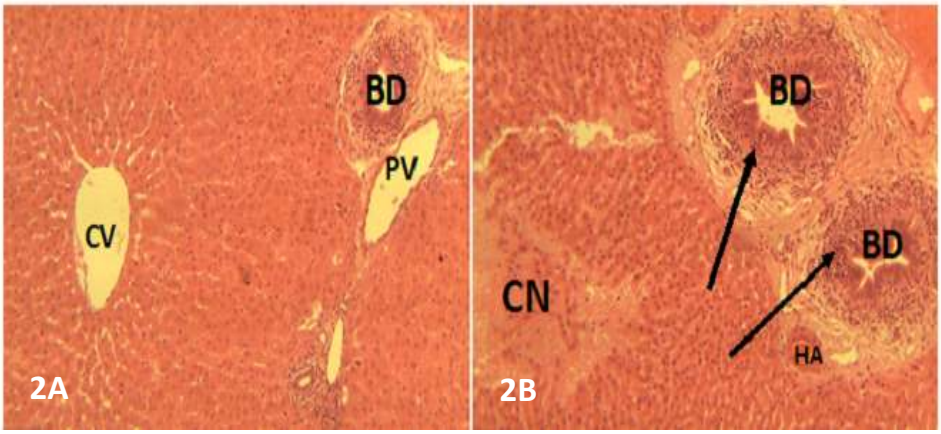


Fig. 2A. Histology of the liver of group 1 (10 mL/kg distilled water) showing mild periportal lymphocytic infiltration around bile duct (BD), PV=portal vein, CV=central vein.
2B. The histology of the liver in treated group 2 (0.3 mg/kg KCN), showing the liver with hepatocellular coagulative necrosis (N) and peri-portal lymphocytic infiltration with marked cholangitis (arrows), HA=hepatic artery. H & E stain x40

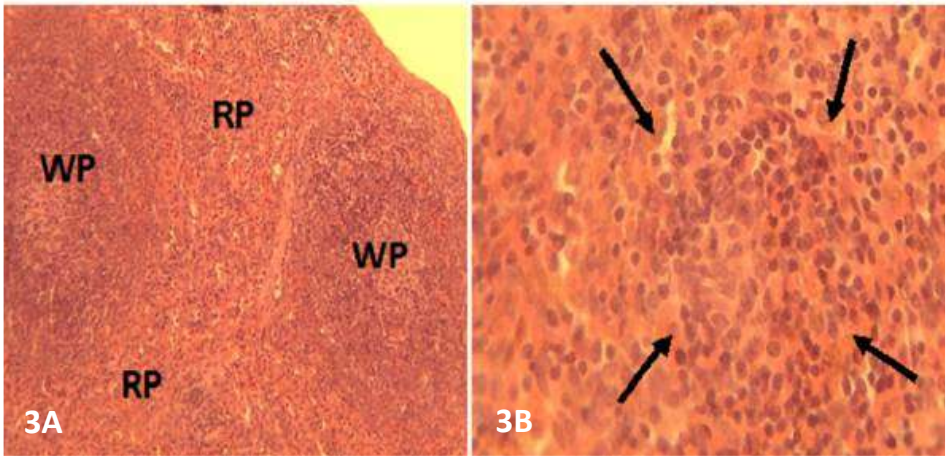


Fig. 3A. Histologic changes of the spleen of group one (10 mL/kg distilled water) showing mild congestion of red pulp (RP) and 3B. showing that in group 2 (0.3 mg/kg KCN) showing severe congestion of red pulp with hemosiderosis (arrows), white pulp (WP). H & E (X40)

4. DISCUSSION

The hepatic effects observed in this study which includes mild-severe peri-portal infiltration of lymphocytes, cholangitis and focal areas of hepatocellular coagulative necrosis indicates the toxic effects of cyanide even at low doses. This likely explains the increase in the levels of the serum enzymes assayed especially AST and ALP. Focal necrosis, congestion, fatty degeneration, hydropic degeneration, and severe cytoplasmic vacuolization of hepatocytes have been reported as hepatotoxic effects of KCN in both man and animal [12,2,13,14]. Severe cytoplasmic vacuolization of hepatocytes was observed in male rats that ingested 3.6 mg/kg/day of KCN in drinking water for 15 days, however hepatic lesion were minimal at 0.36 - 1.2 mg/kg/day, and absent at 0.12 mg/kg/day [13]. The peri-portal inflammatory response observed in this study however appears to be due to factors order than cyanide, as similar picture was seen in control animals though to a lesser extent. In Nigeria, a popular cassava meal (garri), which may be consumed at least once a day in many homes, is reported to release 128 µmol of cyanide per 150 g of diet [4,15]. This value is relatively small when compared to a

minimum of 5.76 *mmol* daily cyanide ingestion in the present protocol, which consequently would have been expected to produce more toxic effects. However, the fact that there is continuous ingestion of this and related food products that contain cyanogenic glycosides in both man and animals calls for worry especially to consumers of products that contain cyanogenic materials even at low doses. The fact that there was no significant difference in the haematological profile between the control and the treated groups suggest that cyanide poisoning may not lead to anaemia.

5. CONCLUSION

In conclusion, the study demonstrated that repeated administration of sub-toxic dose of cyanide may not have caused anaemia but could lead to liver and thyroid damage in the rabbits.

ETHICAL APPROVAL

The authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and followed the appropriate guidelines of Ethics and Research committee of University of Nigeria (2005 Revision).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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