Original Research Article

The effects of short-term repeated oral administration of potassium cyanide on the haematological indices and the morphology, of some internal organs of rabbits

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 distilled water PO. Group 2 was treated with 0.3mg/kg potassium cyanide (KCN) in distilled water PO. Parameters evaluated were erythrocyte count (EC), total while blood cell counts (TWBC), haemoglobin concentration (Hb), aspartatetransaminase (AST), alkaline phosphotase (ALP) and alanineaminotransferase (ALT). Histopathology of the structure of the thyroid, liver, adrenal, and spleen of rabbits were evaluated to assess the damage. Results revealed atrophy and distended thyroid follicles with flattened epithelial cells 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. There was no 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.

Key words: 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 (Conn, 1978). Enzymatic conversion of the glycosides is enhanced when plant cells are damaged or stressed, as 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 (*Manihotesculenta*). Although cyanide occurs most commonly 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 (Kamalu, 1995). 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 (FAO, 2002; Maduagwu and Umoh, 1982). 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 (McMillian and Geevarghese, 1997). While there is substantial information on the effect of cyanide generally (Faust, 1994), 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. MATERIAL AND METHOD

2.1 Experimental animals

Prepubertal rabbits, weighing 1.2 ± 0.2 kg were purchased locally. They were acclimatized for 3 weeks, together with prophylactic administration of Embazin Forte® (an anticoccidial agent). The rabbits were housed in standard cages in a room with daily temperature range between 20℃ and 28℃. 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 used 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 0.3mg/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 both EDTA and non-EDTA bottles. The organs thyroid, liver, adrenal, and spleen were collected for histological analysis as described by Bancroft and Stevens (1977). Other parameters evaluated were erythrocyte count (EC) and total while blood cell counts (TWBC), which were assayed using the method of Schalm et al., 1975; haemoglobin concentration (Hb) which was assayed using the method of Kachmar, 1970. Serum alkaline phosphatise (ALP) was determined using the phenolphthalein monophosphate method as described by Klein et al (1960), serum ALT was estimated colorimetrically by the 2, 4-dinitrophenylhydrazine (DNPH) method of Reitman and Frankel (1957) as described by Bergmeyer (1974) while serum AST was estimated by the Reitman and Frankel (1957) 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 as presented in the table 1. The results revealed 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.

The results of the effect of cyanide treatment on the structures of the liver, thyroid, adrenal and spleen of the rabbits are as presented in figs 1 to 3. The results revealed atrophy and distended thyroid follicles with flattened epithelial cells in the cyanide treated group. The liver revealed severe periportal lymphocytic infiltration 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 controls, while hemosiderosis was seen only in the cyanide treated group. There was no visible lesion in the adrenal gland.

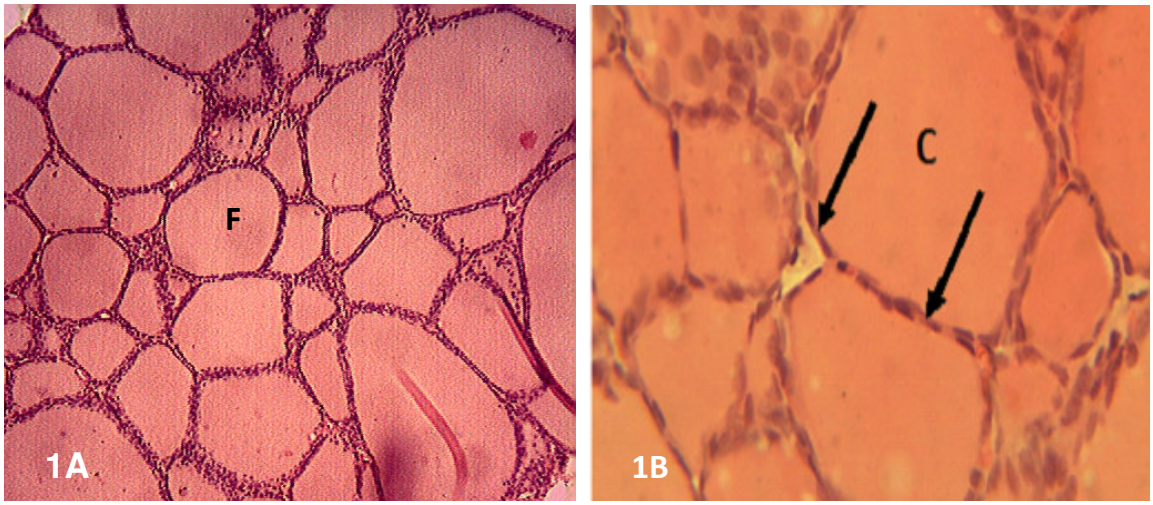

FIG.1A; Thyroid of animal in group 1 (10m/kg distilled water) showing no visible pathological lesion, F=follicle distended with colloid, while in **FIG 1B:** shows thyroid parenchyma of animal in treated group 2 (0.3mg/kg KCN) with atrophy of thyropid epithelium with distended follicles and flattened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

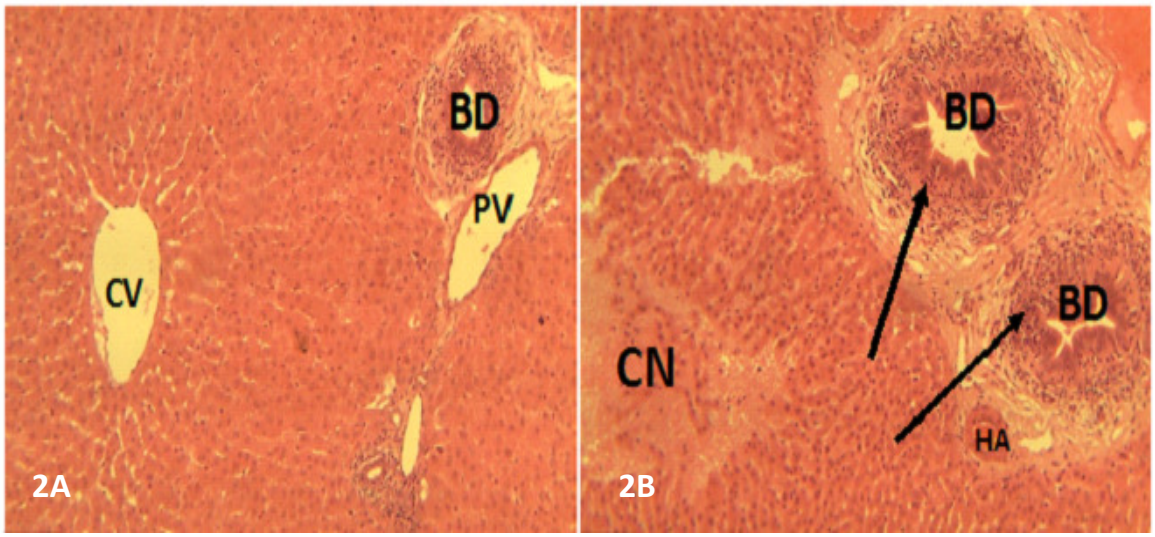

FIG. 2A: Histology of the liver of group 1 (10ml/kg distilled water) showing mild periportal lymphocytic infiltration around bile duct (BD), PV=portal vein, CV=central vein. **FIG.2B:** The histology of the liver in treated group 2 (0.3mg/kg KCN), showing the liver with hepatocellular coagulative necrosis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

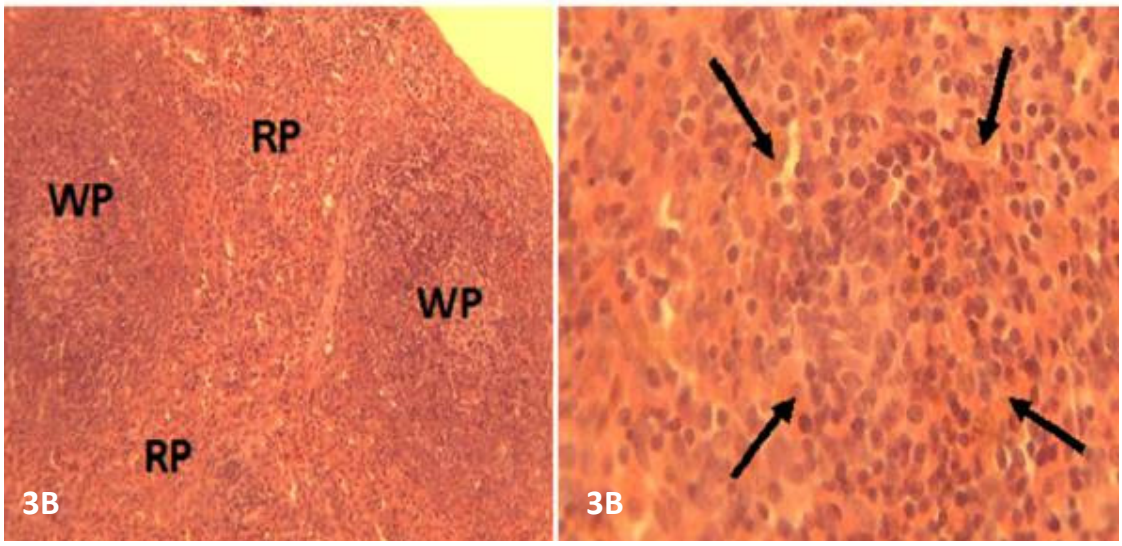

FIG.3A: Histologic changes of the spleen of group one (10ml/kg distilled water) showing mild congestion of red pulp (RP) and **3B:** showing that in group 2 (0.3mg/kg KCN) showing severe congestion of red pulp with hemosiderosis (arrows), white pulp (WP). H & E (X40).

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 (X 10^6/ µL)	5.45 ± 0.3	6.93 ± 0.7
TLC (×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 $p \leq 0.05$.

Group 1 was administered 10 mL/kg body weight, bw distilled water.

Ggroup 2 was administered 0.3 mg/kg bw KCN.

4. DISCUSSION

The hepatic effects observed in this study which includes mild-severe periportal 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 (Okolie and Osagie, 1999, 2000; Kamalu, 1993; Sousa et al., 2002; Soto-Blanco and Gorniak, 2003). 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 (Sousa et al., 2002). The periportal 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 (gari), which may be consumed at least once a day in many homes, is reported to release 128µmol of cyanide per 150g of diet. 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 strongly suggest that cyanide poisoning may not lead to anaemia.

CONCLUSION

It could be concluded from the results of this study that repeated administration of sub-toxic dose of cyanide could lead to damage of the liver and thyroid glands.

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).**

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