

Biochemical and Hematological Evaluation of Cyanide Rich Extracts from *Manihot Utilissima* on Wistar Rats

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Abstract – This study was designed to determine the effect of cyanide rich extracts from *Manihot utilissima* on wistar rats. Biochemical parameters such as total proteins, albumin, total bilirubin, direct bilirubin, AST and ALT activity was determined while hematological parameters determined includes PCV, PLT, WBCs, HGB, neutrophiles, lymphocyte and monocytes were determined. Data were statistically computed using analysis of variance (ANOVA) with level of significance taken at $p < 0.05$ using SPSS version 22. Biochemical results showed significant ($p < 0.05$) increase in levels of AST and ALT activity in group 4 and 5 (treated with 40 mg/kg and 50 mg/kg) respectively. Significant ($p < 0.05$) increase was observed in group 5 (treated 50 mg/kg) in albumin, total bilirubin and direct bilirubin. Hematological results showed significant ($p < 0.05$) in WBCs, HGB and Neutrophiles at groups 4 and 5 (treated with 40 mg/kg and 50 mg/kg) respectively. We concluded that *M. utilissima* (sweet cassava) contained small amount of cyanide glycosides, linamarin a chemical responsible for the toxicity of cassava and if consumed raw and for long period may lead to alteration in both biochemical and hematological changes and the toxicity may lead to vital organ dysfunction.

Keywords – Biochemical parameters, Hematological parameters, Cyanide, *Manihot utilissima*.

I. INTRODUCTION

Manihot utilissima is an annual crop cultivated in the tropical and subtropical regions and reported to be a native of South America. It is a woody shrub belonging to the family of Euphorbiaceae (spurge family). Cassava is an edible tuberous root and serves as a major source of carbohydrates (Phillips, 1984). Cassava plant can withstand draught and mostly grow well in tropical and humid conditions. Cassava tubers are very rich in starch; since it contains about 10 times starch as corn and also twice as much as potatoes. It grows very well on poor soil and requires no or less attention. Due to the linamarin content of cassava (a precursor of cyanide glycosides); the plant is considered poisonous when eaten raw. Cassava can be consume when boiled or ritualized in process such as grating, washing and squeezing out the harmful juices (Jesus *et al.*, 1986). Sweet and bitter varieties of cassava have been identified as the two main species. Sweet cassava contains less amount of linamarin compared to the bitter variety (Okeke *et al.*, 2009).

Maladiyah *et al.*, (2011) reported that cassava has been used in folk medicines for the remedies of various ailments including boils, cancer, conjunctivitis, dysentery, abscesses, diarrhea, marasmus, flu, hernia, snake bite, inflammation, rheumatism, headache, prostatitis, sore,

fever and hemorrhoids. Furthermore, cassava leaves used in the treatment of ringworms, sores, conjunctivitis and abscesses.

M. utilissima leaf have been used ethno-medicinally; scientifically, the plant have been validated and reported to produce dose-related, sustained and significant reduction in fresh egg albumin-induced acute inflammation in rat paw oedema (Adeyemi *et al.*, 2008). Zakaria, (2006) reported that extract of chloroform leaves of bitter cassava were reported to possessed antibacterial activity against *V. cholera*, *S. flexneri*, *S. thyphi* and *P. auruginosa*. This study was designed to evaluate the cyanide rich extracts from *M. Utilissima* on biochemical and hematological parameters on Wistar rats.

II. MATERIALS AND METHODS

2.1 Plant materials

M. utilissima roots (sweet cassava) were purchased from a local market (Bye-Pass), Jimeta/Yola, Adamawa State, Nigeria. They were identified in the Department of Plant Science, Modibbo Adama University of Technology, Yola, Adamawa State. The plants roots were peeled and cut into pieces and further shade-dried.

2.2 Study design

Manihot utilissima was dried at room temperature and made into powder using mortar and piston. One hundred grams of the powdered bulb was extracted by methanol followed and filtered through Whatman filter paper No.1. The extract was concentrated into a semi-solid materials using rotary evaporator at $< 50^{\circ}\text{C}$. Distilled water was used as solvent for the preparation of the doses.

2.3 Experimental animals

Fifty (50) Wistar rats were used for the experiment. They were obtained from the National Veterinary Research Institute Vom, Jos, Plateau State. They were divided into five different groups; each group consists of five Wistar rats. Group 1 receives no dose from the extract and served as control group. Groups 2, 3, 4 and 5 were given methanolic extract of *M. utilissima* roots 20 mg/kg, 30 mg/kg, 40 mg/kg and 50 mg/kg through oral route respectively. The Wistar rats were administered with methanolic extract of *M. utilissima* for the period of twenty-one days after which the blood samples and liver were taken for analysis and histopathology. All animals used for the study were handled in compliance with the guide to the care and use of animals in research and teaching.

2.4 Biochemical analysis

Blood samples from the Wistar rats were collected and plasma separated by centrifugation at 3000 rpm for 5 minutes. Plasma parameters were measured using Randox Laboratories UK reagent kits. Aspartate amino transferase (AST), and alanine amino transferase (ALT) were evaluated colorimetrically at 546 nm using a standard method. Total plasma protein (TP) values, plasma albumin (ALB), total bilirubin (T.Bil) and direct bilirubin (D.Bil) were measured colorimetrically at 546 nm using the biuret method.

2.5 Hematological analysis

Blood samples was collected in EDTA bottles for the estimation of hemoglobin concentration (HGB), packed cell volume (PCV), white blood cells (WBCs), neutrophils (N), Lymphocytes (L) and monocytes (M) using automated hematology analyzer (Sysmex KX-21, Japan,1999).

2.6 Statistical analysis

Data are expressed as mean \pm SEM. Statistical differences between groups were computed using the SPSS software, version 22. Results were analyzed using Student 't' test and significance between group was taken at $p < 0.05$.

III RESULTS

Table 1: Effect of cyanide from *Manihot utilissima* (sweet cassava) on some biochemical parameters in rats

Treatment Group	T.P (dl)	ALB (U/L)	T.Bil (U/L)	D. Bil (U/L)	AST (U/L)	ALT (U/L)
Group 1: (Control)	42.15 \pm 4.15	34.38 \pm 1.83	0.93 \pm 0.37	0.98 \pm 0.26	126.95 \pm 3.95	46.10 \pm 8.30
Group 2: 20 mg/kg	42.85 \pm 1.85	35.24 \pm 3.49	1.04 \pm 0.12	0.99 \pm 0.46	127.3 \pm 32.50	47.6 \pm 10.80
Group 3: 30 mg/kg	43.20 \pm 7	36.47 \pm 3.84	1.06 \pm 0.09	1.60 \pm 7.30	128.1 \pm 12.30	48.05 \pm 13.7
Group 4: 40 mg/kg	44.15 \pm 1.5	36.62 \pm 3.84	1.39 \pm 0.65	1.09 \pm 1.35	131.05 \pm 4.70 ^a	51.1 \pm 10.50 ^a
Group 5: 50 mg/kg	44.96 \pm 0.40	38.35 \pm 3.80 ^a	2.45 \pm 3.89 ^a	2.35 \pm 0.13 ^a	133 \pm 13.10 ^a	53.45 \pm 3.95 ^a

Mean \pm SEM; (n = 5). ^aSignificantly ($P < 0.05$) increased compared to control

TP: Total Protein, ALB: Albumin, D.Bil: Direct Albumin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

Table 2: Effect of crude extracts from cyanide from *Manihot utilissima* (sweet cassava) on Hematological parameters in rats

Treatment Group	PCV (dl)	PLT (U/L)	WBC (U/L)	HGB (U/L)	N (U/L)	L(U/L)	M (U/L)
Group 1: (Control)	39 \pm 1.0	198 \pm 12	7.3 \pm 2.40	10.38 \pm 1.48	32.00 \pm 20.	65.00 \pm 19	3.00 \pm 1.00
Group 2: 20 mg/kg	38 \pm 0.00	199.55 \pm 55.6	8.85 \pm 2.95	11.7 \pm 0.80	32.08 \pm 1.10	60 \pm 3.00	4.00 \pm 0.30
Group 3: 30 mg/kg	39.5 \pm 2.50	199.76 \pm 14.5	9.35 \pm 0.05	12.20 \pm 0.30	30.01 \pm 19.20	60.01 \pm 3.20	4.03 \pm 1.68
Group 4: 40 mg/kg	40.5 \pm 0.50	201 \pm 41.02	13.05 \pm 3.75 ^a	13.58 \pm 0.42 ^a	29.81 \pm 8.00 ^a	65.00 \pm 3.00	4.14 \pm 0.46
Group 5: 50 mg/kg	40.9 \pm 2.00	201.87 \pm 35.00	14.75 \pm 0.25 ^a	15.82 \pm 0.16 ^a	27.69 \pm 2014 ^a	66.00 \pm 9.00	5.96 \pm 0.00

Mean \pm SEM; (n = 5). ^aSignificantly ($P < 0.05$) increase compared to control PCV: Packed cell volume, PLT: Platelet, HGB: Hemoglobin, WBC: White blood cell, N: Neutrophils, L: Lymphocytes, M: monocytes

3.1 Biochemical changes

Biochemical changes for rats given the daily oral doses of cyanide rich extracts from *M. utilissima* (sweet cassava) are presented in table 1. The results showed that after treatment for twenty one days, the activity of ALT and AST group 4 and 5 (treated with 40 mg/kg and 50 mg/kg) significantly ($p < 0.05$) increased compared to the control (group 1). Total protein, Albumin, Total Bilirubin and Direct Bilirubin at ($p < 0.05$) were elevated in (group 2, 3, 4 and 5) compared to the control (group 1).

3.2 Hematological changes

Hematological changes for rats given daily oral doses of cyanide rich extracts from *M. utilissima* (sweet cassava) are presented in table 2. The results showed that WBCs and Lymphocytes in treatment groups 4 and 5 (treated with 40 mg/kg and 50 mg/kg) showed significant ($p < 0.05$) increase compared to the control (group 1) while

Neutrophils showed significant ($p < 0.05$) decrease compared to the control group. Elevated levels of PCV, PLT, WBCs, HGB and Monocytes were shown at ($p < 0.05$) compared to the control (group 1).

3.3 Pathological changes

The pathological changes showed the effects of the cyanide rich extract on liver sections of the treated rats treated with cyanide rich extract of *M. utilissima* for the period of twenty one days. Figure 1 showed normal liver section of rats with no lesion while figures 2 (treated with 20 mg/kg) of cyanide rich extract of *M. utilissima* showed mild liver section with no lesion. Figure 3 (treated with 30 mg/kg) of cyanide rich extract of *M. utilissima* showed mild hepatic degeneration (mostly cytoplasmic) while figure 4 (treated with 40 and 50 mg/kg) of cyanide rich extract of *M. utilissima* showing very mild hepatic degeneration with infiltrations of the cell.

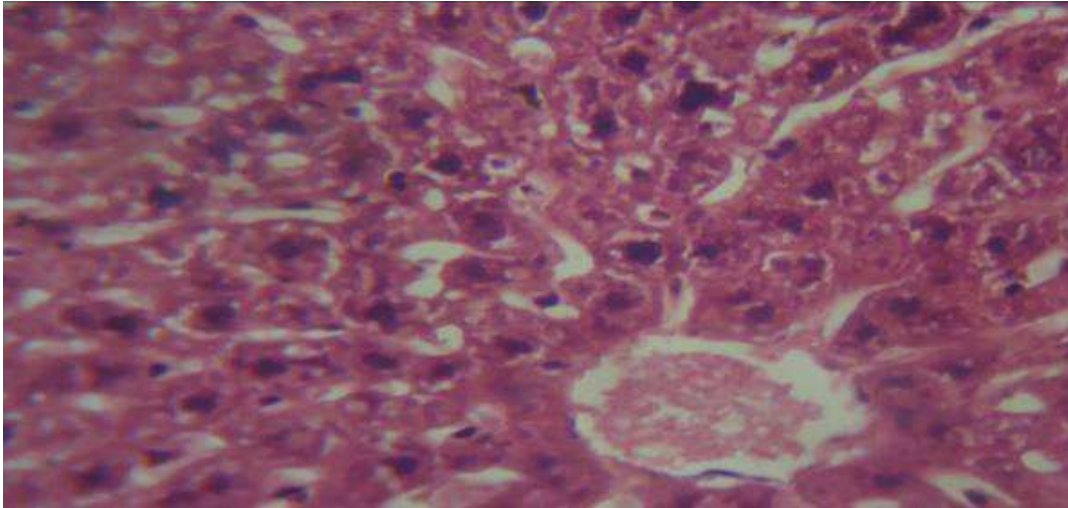


Fig. 1. Photomicrograph of Normal liver section showing no lesion (x 400)

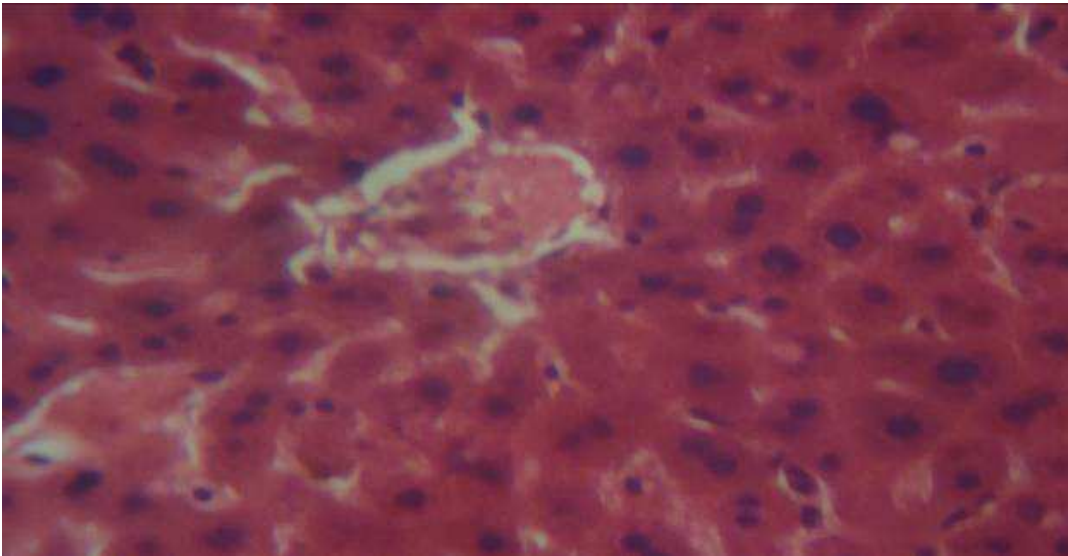


Fig. 2. Photomicrograph of group 2 (treated with 20 mg/kg) of cyanide rich extract of *M. utilissima* showing mild liver section with no lesion (x 400).

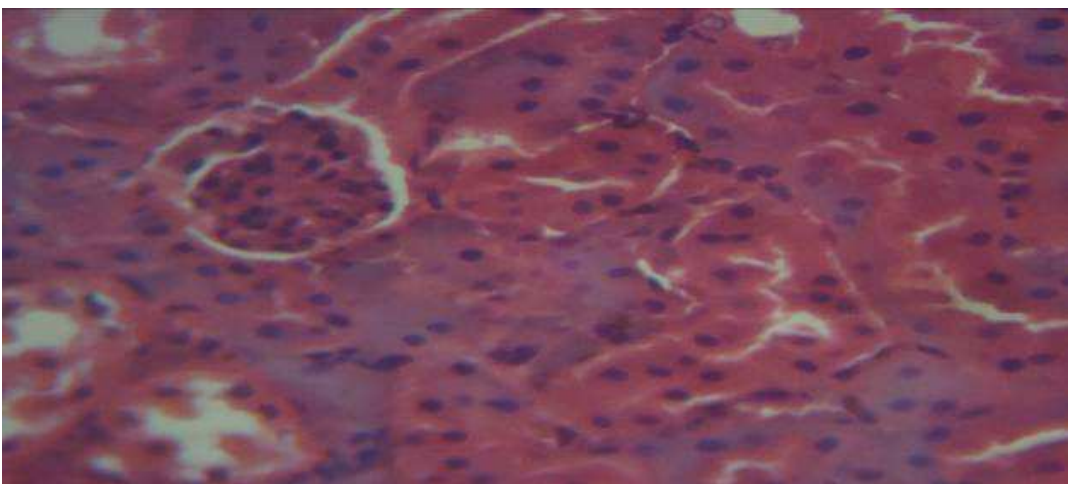


Fig. 3. Photomicrograph of group 3 (treated with 30 mg/kg) of cyanide rich extract of *M. utilissima* showing mild hepatic degeneration (mostly cytoplasmic) (x 400).

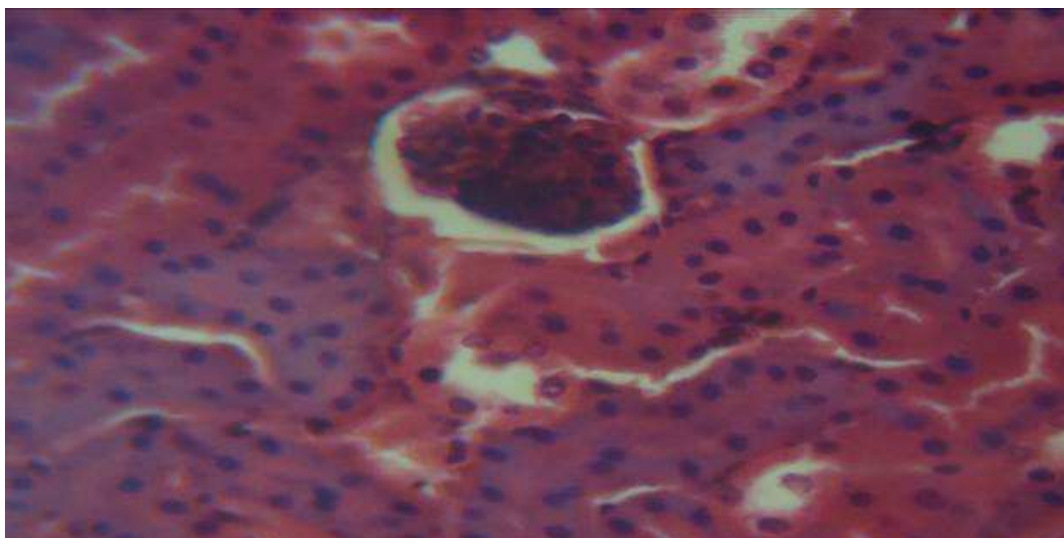


Fig. 4. Photomicrograph of group 4 and 5 (treated with 40 and 50 mg/kg) of cyanide rich extract of *M. utilissima* showing very mild hepatic degeneration with infiltrations of the cell (x 400).

IV. DISCUSSION

Cassava tuber contains small but significant amounts of cyanogenic glucosides, linamarin and lotaustralin. Linamarin in cassava is stored in the roots but manufactured in the leaves and translocated as diglucoside Linustatin. The concentration of cyanogenic glucosides in cassava makes its consumption both for nutritional purposes in humans and animals limited. The hydrolysis of the cyanogenic glucosides by bringing the enzyme linamarase in contact with the cyanogenic glucoside or via acid hydrolysis yields hydrogen cyanide (Rosling *et al.*, 1992).

Studies have shown that consumption of cassava and cassava products with high cyanogens contains may cause acute intoxications (Mlingi *et al.*, 1992), aggravate goiter (Bourdoux *et al.*, 1982) and in severe circumstances, induce paralytic diseases (Tylleskar *et al.*, 1992).

Biochemical parameters such as enzymes activities in tissues and body fluids play an important role in diseases investigation, diagnosis and liver toxicity Larrey, (2002). The biochemical parameters (Table 1) showed an increase ($p < 0.05$) of Total protein, Albumin, Direct Bilirubin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in a dose-dependent manner compared to the control group (untreated) at concentrations of the extracts (20 mg/kg, 30 mg/kg, 40 mg/kg and 50 mg/kg). Significant change in plasma AST and ALT activities studied indicate that the extract caused mild changes in the liver. ALT is a cytoplasmic enzyme and an increase in plasma is an indication of mild injuries caused by chemicals to the liver. Liver injury is characterized as hepatocellular when there is predominant elevation of the ALT, while AST is a mitochondria enzyme whose increased activity in plasma reflects severe tissue injuries (Martins, 2006).

Further studies as reported by Vermeulen, (1992) have shown that subtle membrane changes are sufficient to allow passage of intracellular enzyme to extracellular space. Therefore the extract-induced elevation in serum

ALT and AST levels in this study may be attributed to damaged structural integrity of the liver, because these enzymes are normally located in the cytoplasm of hepatocytes and are released into circulation after cellular damage.

Hematological parameters (Table 2) showed that there was increase ($p < 0.05$) in platelet (PLT), white blood cell (WBCs), hemoglobin (HGB), neutrophil (N), lymphocyte (L) and monocyte (M) while a decrease in packed cell volumes was observed. It speculates that the extracts induce toxicity and may cause anaemic condition in rats. Toxicity effect observed might be due to the presence of cyanogenic glycosides. This could be attributed to the less concentration of the toxic constituent of sweet *M. utilissima*. Consumption of raw cassava and its products that contain large amounts of cyanogens may cause cyanide poisoning with related symptoms of headache, weakness, nausea, vomiting, dizziness, stomach pains, exacerbates goiter, diarrhea and death (Cardoso *et al.*, 2005).

V. CONCLUSION

We conclude that *M. utilissima* (sweet cassava) when eaten raw may have effect on both biochemical and hematological parameters due the cyanide contents as documented when rats were continuously fed for twenty one days. Cassava processing should be done in order to reduce the cyanide content to avoid damages done to organs such as liver and kidney.

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