Effect of *Manihot esculenta* and *Manihot utilissima* Cyanide Extract on Some Biochemical Parameters of Albino Rats

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**Authors’ contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**ABSTRACT**

Cassava roots (*Manihot esculenta* and *Manihot utilissima*) are largely consumed as staple food in African but contain a toxic compound, cyanogenic glycosides. This study evaluated the effect of *Manihot esculenta* and *Manihot utilissima* cyanide content on biochemical parameters and histological examination of male albino rats. A total of thirty rats were used for the acute toxicity study while thirty-five rats were used to determine the effect of *Manihot esculenta* and *Manihot utilissima* cyanide on biochemical and histological parameters. The results revealed that *Manihot esculenta* and *Manihot utilissima* cyanide were toxic to rats even at a lower dose (10 mg/kg body weight) and cause death. Studies on the cyanide concentration determined using the standard curve revealed a high amount of cyanide in *Manihot esculenta* compared to *Manihot utilissima*. Biochemical parameters determined include Aspartate transaminases (AST), Alanine transaminases (ALT), Urea, Creatinine, Acid Phosphates, and Lactate Dehydrogenase (LDH). The results revealed a significant (p< 0.05) increase of AST, ALP, Creatinine, Acid Phosphates, and LDH on rats administered with *Manihot esculenta* cyanide compared to normal rats and *Manihot utilissima* cyanide while *Manihot utilissima* cyanide showed significant (p< 0.05) increase on the activity of AST, ALT, ALP, Urea, and Creatinine compared with...
normal rats. Histological examination of livers and kidneys of the rats revealed that rats administered with *Manihot esculenta* cyanide showed disruption of the liver and kidney cells with distorted hepatic portal areas and necrosis compared to rats fed with *Manihot utilissima* cyanide and normal rats. The deleterious effect of the hydrogen cyanide is more pronounced on the tissues of the rats treated with *Manihot esculenta* as compared to those treated with *Manihot utilissima* as depicted by the photomicrographs. This study, therefore, concluded that *Manihot esculenta* is toxic causing alteration on some biochemical and histological parameters, this toxicity was correlated to dysfunction of vital organs which are a reconfirmation of the need for adequate processing of certain food crops prior to consumption. Cassava is a rich source of cyanide, highly documented for its adverse effects on the living system.

**Keywords:** Wistar rats; Cyanogenic glycosides; toxicity; *Manihot esculenta*; *Manihot utilissima*.

### 1. INTRODUCTION

Plants synthesize chemical substances that can pose potential risks to consumers, and one of these types of substances is cyanogenic glycosides. Cyanogenic glycosides are chemical compounds contained in foods that release hydrogen cyanide during hydrolysis [1]. Exposure to cyanide from chronic consumption of cyanogenic glycosides may accelerate the onset of many ailments. Cassava roots should not be consumed raw because they contain two cyanogenic glucosides, linamarin and lotaustralin. These are decomposed by linamarase, a naturally occurring enzyme in cassava, liberating hydrogen cyanide (HCN) [2]. Cassava is classified as either sweet (*Manihot utilissima*) or bitter (*Manihot esculenta*), according to the difference in toxicity and palatability of the roots (signifying the absence or presence of toxic levels of cyanogenic glucosides). The sweet varieties produce 20 milligrams of cyanide (CN) per kilogram of fresh roots, whereas bitter ones may produce more than 50 times as much (1 g/kg) [3].

Symptoms of acute cyanide intoxication resulting from ingesting raw or poorly processed cassava include nausea, vomiting, diarrhea, dizziness, weakness, mental confusion, and convulsions. In some cases, death may result within one or two hours whereas chronic, low-level cyanide exposure is associated with the development of goiter and with tropical ataxic neuropathy, a nerve-damaging disorder that renders a person unsteady and uncoordinated [4]. According to Obeten et al. [5], adequate cassava processing techniques may generally lead to substantial cassava detoxification, conditions, such as famine, drought, and failure of other less- well-adapted root crops generally lead to increased demands for cassava roots during which the traditional processing methods may be compromised.

*Manihot esculenta* (Cassava) belongs to the family of *Euphorbiaceae*, which is common in West Africa, Nigeria. Cassava is extensively cultivated as an annual crop in the tropic because of its edible starchy tuberous root, which constitute a good source of dietary carbohydrates. Cassava roots are long and tapered, with a firm, homogeneous flesh encased in a detachable rind, about 1 mm thick, rough and brown on the outside. Nigeria is the largest world producer among 100 countries that grow cassava and is responsible for 21% of world production [6]. Cassava roots are high in calories and contains 60% water, 38% carbohydrates, 1% protein, 0.2 fat, 0.3 fiber, calcium (16 mg/100 g), phosphorus (27 mg/100 g), and vitamin C (20.6 mg/100 g) while they are poor in protein and other nutrients [7]. Commercial cultivars can be 5 to 10 cm (2.0 to 3.9 in) in diameter at the top, and around 15 to 30 cm (5.9 to 11.8 in) long. The woody vascular bundle runs along the root’s axis and the flesh can be chalk-white or yellowish. This study illuminates the toxicological potentials of *Manihot esculenta* and *Manihot utilissima* cyanide content on some biochemical parameters of male albino rats.

### 2. MATERIALS AND METHODS

#### 2.1 Chemicals/Reagent Kits

Alanine Transaminase (ALT), Aspartate Transaminase (AST), Total Protein, Albumin, Creatinine, Urea, Direct Bilirubin and Total Bilirubin. Reagents used for all the assays were commercial kits and products of Randox Laboratory, London. Lactate Dehydrogenase (LDH) was bought from AGAPE Diagnostic kits, Switzerland.

#### 2.2 Plant Materials

The *Manihot esculenta* (Bitter cassava) and *Manihot utilissima* (Sweet cassava) were
2.4 Determination and Extraction of Cyanide Content of *Manihot esculenta* and *Manihot utilissima*

Following the method by Cooke [8] cassava roots were washed, peeled, dried and made into powder. The dried powdered samples were thoroughly mixed and 60 grams were extracted with about 150 ml of 0.1 M orthophosphoric acid. Then 0.1 ml of the sample, 0.1 ml of linimarase and 0.4 ml of phosphate buffer pH 7 are incubated in a shaking water bath at 30°C for 15 minutes. Exactly 0.6 ml of 0.2 M NaOH was added to stop the reaction of linimarase at the end of 15 minutes period. Colour was developed by adding 0.2 ml of Chloramine T reagent followed by pyridine/pyrazolone reagent. The sample was left to stand for 90 minutes for the color to develop. All samples were analyzed in triplicates. A series of standards ranging from 50 - 200 µg/ml of HCN were prepared and color developed as above. The absorbance of both standards and sample at 50 - 200 µg/ml were spectrophotometrically read at 620 nm. The cyanide content of the sample was determined using a standard calibration curve. Concentration of hydrogen cyanide in *Manihot esculenta*, *Manihot utilissima* and standard was computed graphically from a standard curve by plotting absorbance versus concentration of known solution using Beer's law. The equation states that:

\[ A = \varepsilon_m C_1 \]  
\[ y = mx + b \]

Where \( A \) = Absorbance, \( \varepsilon_m \) = molar extinction coefficient, \( C \) = Concentration, \( 1 \) = Path length of 1 cm Equation from linear graph; \( y = mx + b \)

2.5 Determination of LD\(_{50}\)

These were done according to the method of Behrens and Karber [9]. A total of thirty (30) male albino rats weighing between 110-120 grams were used for the LD\(_{50}\) test. They were divided into two groups, each containing fifteen (15) rats treated with *Manihot utilissima* and *Manihot esculenta* cyanide extract. The rats were randomly divided into three groups of five (5) rats each. The rats in each group were given graded doses and utilized for the studies.

\[ \text{LD}_{50} = \frac{\text{Sum of (Dose difference X Mean death)}}{\text{Number of animal per group}} \]

2.6 Experimental Design

The total number of thirty-five (35) rats weighing between 100-120 g was used for the experiment. The rats were divided into seven groups with five rats in each group. Group one served as the control while groups two, three and four were treated with *Manihot esculenta* cyanide extract while group five, six and seven where treated with *Manihot utilissima* cyanide extract at doses of 2 mg/kg, 4 mg/kg and 6 mg/kg per body weight of the animals respectively through intubation for the period of twenty-one days after which the blood sample was utilized for biochemical analyses while the liver and kidney samples of rats were utilized for histopathology.

Group I: Served as the control, they received no treatment.

Group II: Administered with 2 mg/kg/day of cyanide extract of *Manihot esculenta*

Group III administrated with 4 mg/kg/day of cyanide extract of *Manihot esculenta*

Group IV: Administered with 6 mg/kg/day of cyanide extract of *Manihot esculenta*

Group V: Administered with 2 mg/kg/day of cyanide extract of *Manihot utilissima*

Group VI: Administered with 4 mg/kg/day of cyanide extract of *Manihot utilissima*

Group VII: Administered with 6 mg/kg/day of cyanide extract of *Manihot utilissima*

2.7 Determination of Biochemical Parameters

At the end of the twenty days, animals in all the groups were sacrificed a day after the end of the administration under chloroform anesthesia. Blood was collected through cardiac puncture...
from the left ventricle into labeled specimen bottles and centrifuged for 5 minutes at 1000 rpm to obtain serum which was used for assay to determine liver enzyme. The methods for enzyme activity of control and tested rats were performed according to the instructions in the manual of assay kits (Randox laboratory, London) and (AGAPE Diagnostic kits, Switzerland). The transaminases (AST and ALT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, total bilirubin, direct bilirubin, albumin, lactate dehydrogenase, urea, and creatinine were measured and calculated.

2.8 Pathological Methods

This was carried out according to the method described by Andrew et al. [10]. The specimens of the liver and kidneys were removed and immediately fixed in 10% neutral buffered formalin. The organs were embedded in paraffin wax, sectioned at 5 mm diameter and stained routinely with hematoxylin and eosin (H and E).

2.9 Statistical Analysis

All values were expressed as Mean ± SEM (Standard Error of Mean) and data were analyzed using Analysis of Variance (ANOVA) with the aid of Statistical Package for the Social Sciences (SPSS) software version 28. The values p< 0.05 were considered significant.

3. RESULTS

Table 1 and Fig. 1 respectively showed cyanide concentration and standard curve for the determination of cyanide concentration in Manihot esculenta and Manihot utilissima compared with standard (Hydrogen cyanide). The results showed the highest cyanide concentration was found in Manihot esculenta compared to Manihot utilissima. Standard (Hydrogen cyanide) however showed significant cyanidin concentration compared to Manihot esculenta and Manihot utilissima as presented in Fig. 1. The concentration of hydrogen cyanide in Manihot esculenta, Manihot utilissima and standard was computed graphically from a standard curve by plotting absorbance versus concentration of known solution using Beer's law equation.

3.1 LD$_{50}$ of Manihot esculenta and Manihot utilissima Cyanide Extract

Tables 2 and 3 showed the results for LD$_{50}$ after administration of Manihot esculenta and Manihot utilissima cyanide extract on rats and the toxicity signs observed. The results revealed that rats administered with Manihot esculenta cyanide extract at doses of 10 mg/kg.bw, 20 mg/kg.bw and 30 mg/kg.bw did not show any survival rate and exhibited toxicity signs such as weakness, convulsion, coma, and death. Rats administered with Manihot utilissima cyanide extract at dose 10 mg/kg.bw showed that three rats survived while administration with 20 mg/kg.bw showed that one rat survived in the group and all rats died after administration with 30 mg/kg/body weight.

3.2 Effect of Cyanide Extract of Manihot esculenta and Manihot utilissima on Some Biochemical Indices of Rats

Tables 4 and 5 showed the effect of Manihot esculenta and Manihot utilissima cyanide extract on biochemical indices including Aspartate transaminases (AST), Alanine transaminases (ALT), Alanine Phosphatase (ALP), Acid Phosphates, and Lactate Dehydrogenase (LDH) and Urea, Creatinine on albino rats. The results showed a significant (p< 0.05) increase in the activity of AST, ALP, Creatinine, Acid Phosphates and LDH on rats administered with Manihot esculenta cyanide extract compared to control rats and Manihot utilissima cyanide extract. The results also showed a significant (p< 0.05) increase in the activity of AST, ALT, ALP, Urea, and Creatinine on rats administered with Manihot utilissima cyanide.

3.3 Histological Examination of Manihot esculenta Cyanide Extract on Liver of Rats

Figs. 2 - 4 showed histological examination of Manihot esculenta cyanide extract on the liver of rats. Photomicrograph A as indicated by the black arrow showed control hepatic lobules displaying control hepatic plates with moderate sinusoidal spaces. The nuclei of the hepatocytes are oval and vesicular, with fine granular acidophilic cytoplasm. The hepatic portal areas are well organized with active blood circulation. Photomicrograph B (Fig. 2) showed liver rats administered with 2 mg/kg.bw of Manihot esculenta cyanide extract. The black arrow on the liver section showed control hepatic lobules displaying hepatic plates with slight shrinkage around the central vein area. The nuclei of the hepatocytes are oval and vesicular, with fine granular acidophilic cytoplasm. The hepatic portal areas are infiltrated by numerous presences of polymorphs. Photomicrograph C
(Fig. 3) showed a section of rat’s liver administered with 4 mg/kg.bw of *Manihot esculenta* cyanide extract. The black arrow showed the liver section with control hepatic lobules displaying hepatic plates with slight shrinkage around the central vein area. The nuclei of the hepatocytes are oval and vesicular, with fine granular acidophilic cytoplasm. The hepatic portal areas are infiltrated by numerous presences of polymorphs. Photomicrograph D (Fig. 4) section of rat’s liver administered with 6 mg/kg.bw of *Manihot esculenta* cyanide extract. The liver section as indicated by the black arrow showed hepatic lobules displaying hepatic plates with widespread shrinkage of the hepatocytes and marked dilation of the sinusoidal spaces. The nuclei of the hepatocytes are oval and vesicular, with fine granular acidophilic cytoplasm. The hepatic portal areas are distorted and necrotic.

**Table 1. Cyanide concentration in *Manihot esculenta* (M. E), *Manihot utilissima* (M. U) and standard (Hydrogen cyanide)**

<table>
<thead>
<tr>
<th>HCN (µg/ml)</th>
<th><em>Manihot utilissima</em></th>
<th>Absorbance (nm)</th>
<th><em>Manihot esculenta</em></th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.07 ± 0.004</td>
<td>0.09 ± 0.002</td>
<td>0.11 ± 0.002</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.11 ± 0.001</td>
<td>0.17 ± 0.001</td>
<td>0.21 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>0.14 ± 0.01</td>
<td>0.21 ± 0.02</td>
<td>0.25 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>0.20 ± 0.02</td>
<td>0.24 ± 0.05</td>
<td>0.28 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

Key: Values are Mean ± SEM

*a* Significantly higher compared to *Manihot utilissima* and *Manihot esculenta*

*b* Significantly higher compared to *Manihot utilissima*

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![Graph](image_url)

**Fig. 1. Standard curve showing the cyanide extract in *Manihot esculenta*, *Manihot utilissima* and standard (Hydrogen cyanide)**

**Table 2. LD<sub>50</sub> of the *Manihot utilissima* and *Manihot esculenta* calculated by arithmetical method of Behrens and Karber (1983)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg.bw)</th>
<th>Dose difference = a</th>
<th>Dead</th>
<th>Mean Death = b</th>
<th>Product = axb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>10</td>
<td>2</td>
<td>0.4</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>10</td>
<td>4</td>
<td>0.8</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

**LD<sub>50</sub> of *Manihot esculenta* cyanide extract**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg.bw)</th>
<th>Dose difference = a</th>
<th>Dead</th>
<th>Mean Death = b</th>
<th>Product = axb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

(n = 5)
Table 3. Toxicity signs observed in rats treated with *Manihot utilissima* and *Manihot esculenta*

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg.bw)</th>
<th>Observations</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>Weakness and raised tail</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>Weakness, convulsion and coma</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>Weakness, convulsion and coma</td>
<td>5</td>
</tr>
</tbody>
</table>

**Manihot esculenta** (Bitter cassava) cyanide

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg.bw)</th>
<th>Observations</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>Weakness, convulsion and coma</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>Weakness, convulsion and coma</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>Weakness, convulsion and coma</td>
<td>5</td>
</tr>
</tbody>
</table>

*(n = 5)*

Table 4. Effect of cyanide extract of *Manihot esculenta* and *Manihot utilissima* on biochemical indices of rats

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>AST (iu/l)</th>
<th>ALT (iu/l)</th>
<th>ALP (iu/l)</th>
<th>Acid Phosphatase (iu/l)</th>
<th>LDH (iu/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>134.00 ± 5.65</td>
<td>35.00 ± 1.00</td>
<td>133.00 ± 3.00</td>
<td>55.50 ± 1.25</td>
<td>201.93 ± 3.30</td>
</tr>
<tr>
<td>2 mg/kg.bw M. E cyanide extract</td>
<td>152.00 ± 9.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.67 ± 1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>151.33 ± 1.53&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>61.50 ± 1.34</td>
<td>302.00 ± 3.75&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 mg/kg.bw M. E cyanide extract</td>
<td>185.33 ± 2.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>41.33 ± 1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>156.00 ± 2.65&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>68.23 ± 0.93</td>
<td>235.98 ± 2.68&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 mg/kg.bw M. E cyanide extract</td>
<td>191.00 ± 2.00</td>
<td>92.33 ± 5.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>167.00 ± 1.73&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>80.70 ± 6.58</td>
<td>441.67 ± 9.14&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 mg/kg.bw M. U cyanide extract</td>
<td>130.67 ± 2.08</td>
<td>36.67 ± 2.52</td>
<td>141.33 ± 1.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.74 ± 0.76</td>
<td>151.05 ± 7.78</td>
</tr>
<tr>
<td>4 mg/kg.bw M. U cyanide extract</td>
<td>177.30 ± 1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.67 ± 2.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>145.33 ± 1.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.44 ± 1.29</td>
<td>162.81 ± 3.44</td>
</tr>
<tr>
<td>6 mg/kg.bw M. U cyanide extract</td>
<td>191.33 ± 1.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.33 ± 4.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>148.00 ± 3.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.25 ± 1.74</td>
<td>180.99 ± 1.30</td>
</tr>
</tbody>
</table>

*Key: Values are Mean ± SEM (n = 5)*

<sup>a</sup>Significantly higher (*p* < 0.05) compared to control

<sup>b</sup>Significantly higher (*p* < 0.05) compared to other extract at the same concentration

*M. E cyanide: Manihot esculenta
M. U cyanide: Manihot utilissima

AST: Aspartate transaminases, ALT: Alanine transaminases, ALP: Alanine Phosphatase, LDH: Lactate Dehydrogenase

Table 5. Effect of cyanide extract of *Manihot esculenta* and *Manihot utilissima* on urea and creatinine of rats

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Urea (mmol/l)</th>
<th>Creatinine (ummol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.63 ± 0.81</td>
<td>24.00 ± 4.00</td>
</tr>
<tr>
<td>2 mg/kg.bw M. E cyanide extract</td>
<td>8.63 ± 1.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.83 ± 3.01&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 mg/kg.bw M. E cyanide extract</td>
<td>9.63 ± 1.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.50 ± 1.32&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 mg/kg.bw M. E cyanide extract</td>
<td>11.68 ± 1.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.75 ± 1.56&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 mg/kg.bw M. U cyanide extract</td>
<td>9.82 ± 0.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.53 ± 0.55</td>
</tr>
<tr>
<td>4 mg/kg.bw M. U cyanide extract</td>
<td>9.33 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.02 ± 0.53</td>
</tr>
<tr>
<td>6 mg/kg.bw M. U cyanide extract</td>
<td>11.96 ± 0.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.32 ± 1.24</td>
</tr>
</tbody>
</table>

*Key: Values are Mean ± SEM (n = 5)*

<sup>a</sup>Significantly higher (*p* < 0.05) compared to control

<sup>b</sup>Significantly higher (*p* < 0.05) compared to other extract at the same concentration

*M. E cyanide: Manihot esculenta, M. U cyanide: Manihot utilissima*
3.4 Histological Examination of *Manihot utilissima* Cyanide Extract on Liver of Rats

Figs. 5-7 showed histological examination of *Manihot utilissima* cyanide extract on the liver of rats. Photomicrograph A as indicated by the black arrow showed control hepatic lobules displaying control hepatic plates with moderate sinusoidal spaces. The nuclei of the hepatocytes are oval and vesicular, with fine granular acidophilic cytoplasm. The hepatic portal areas...
are well organized with active blood circulation. Photomicrograph F (Fig. 5) showed liver rats administered with 2 mg/kg.bw of *Manihot utilissima* cyanide extract. The liver section as indicated by the black arrow showed hepatic lobules displaying hepatic plates with moderate shrinkage around the central vein area. The nuclei of the hepatocytes are oval and vesicular, with fine granular acidophilic cytoplasm. The hepatic portal areas are unremarkable. Photomicrograph G (Fig. 6) showed the liver section of rats administered with 4 mg/kg.bw of *Manihot utilissima* cyanide extract. The liver section as indicated by the arrow showed hepatic lobules displaying hepatic plates with slight shrinkage around the central vein area. The nuclei of the hepatocytes are oval and vesicular, with fine granular acidophilic cytoplasm. The hepatic portal areas are infiltrated by few polymorphs. Photomicrograph H (Fig. 7) showed a section of rat's liver administered with 6 mg/kg.bw of *Manihot utilissima* cyanide extract. The liver section as indicated by the black arrow showed hepatic lobules displaying hepatic plates with widespread shrinkage around the central vein area. The nuclei of the hepatocytes are oval and vesicular, with fine granular acidophilic cytoplasm fatty changes. The hepatic portal areas are infiltrated by few presences of polymorphs.

### 3.5 Histological Examination of *Manihot esculenta* Cyanide Extract on Kidney of Rats

Figs. 8-10 showed the histological examination of *Manihot esculenta* cyanide extract effect on the kidney of rats. The black arrow in photomicrograph A showed control rat's renal tissue displaying numerous tubules that are lined by simple cuboidal cells with oval nuclei and pale cytoplasm. The lumen of the renal tubules is moderate; the glomeruli are controlled with moderate bowman's capsular spaces. Photomicrograph L (Fig. 8) showed a section of rats kidney administered with 2 mg/kg.bw of *Manihot esculenta* cyanide extract. The black arrow showed control of renal tissue displaying numerous tubules that are lined by simple cuboidal cells with oval nuclei and pale cytoplasm. The lumen of the renal tubules showed mild tubular necrosis with slight tubular distortion, some of the glomeruli are atrophic with moderate infiltration of the portal area by polymorphs. Photomicrograph M (Fig. 9) showed a photomicrograph section of rats kidney administered with 4 mg/kg.bw of *Manihot esculenta* cyanide extract. The black arrow the histological examination showed control renal tissue displaying numerous tubules that are lined by simple cuboidal cells with oval nuclei and pale cytoplasm.
cytoplasm. The lumen of the renal tubules showed marked tubular necrosis with moderate tubular distortion, some of the glomeruli are necrotic and atrophic with moderate congestion of blood vessels. Photomicrograph N (Fig. 10) showed a photomicrograph section of rats kidney administered with 6 mg/kg.bw of *Manihot esculenta* cyanide extract. The black arrow in the photomicrograph showed renal tissue displaying numerous tubules that are lined by simple cuboidal cells with oval nuclei and pale cytoplasm. The lumen of the renal tubules showed marked tubular necrosis with moderate tubular distortion and slight edema, some of the glomeruli are necrotic and atrophic with moderate congestion of blood vessels.

![Fig. 5. Photomicrograph of normal rats' liver and liver of rats treated with 2 mg/kg.bw of *Manihot utilissima* cyanide (H&E X40). A: Normal rat liver, F: Liver of rat treated with 2 mg/kg.bw of *Manihot utilissima* cyanide extract](image)

![Fig. 6. Photomicrograph of normal rats' liver and liver of rats treated with 4 mg/kg.bw of *Manihot utilissima* cyanide (H&E X40). A: Normal rat liver, G: Liver of rat treated with 4 mg/kg.bw of *Manihot utilissima* cyanide extract](image)
3.6 Histological Examination of *Manihot utilissima* Cyanide Extract on Kidney of Rats

Figs. 11-13 showed histological examination of *Manihot utilissima* cyanide extract on the kidney of rats. Photomicrograph A showed a control section of rat's renal tissue displaying numerous tubules that are lined by simple cuboidal cells with oval nuclei and pale cytoplasm. The black arrow showed that the lumen of the renal tubules is moderate; the glomeruli are controlled with moderate bowman's capsular spaces. Photomicrograph Q (Fig. 11) showed a section of rat's kidney administered with 2 mg/kg.bw of *Manihot utilissima* cyanide extract. Histological examination as indicated by the arrow showed control renal tissue displaying numerous tubules that are lined by simple cuboidal cells with oval nuclei and pale cytoplasm. The lumen of the renal tubules showed mild tubular edema with mild tubular distortion, few of the glomeruli are atrophic and necrotic. Photomicrograph R (Fig. 12) showed the section of rat's kidney administered with 4 mg/kg.bw of *Manihot utilissima* cyanide extract. Histological examination as indicated by the black arrow showed control renal tissue displaying numerous tubules that are lined by simple cuboidal cells with oval nuclei and pale cytoplasm. The lumen of the renal tubules showed mild tubular distortion, few of the glomeruli are atrophic. Photomicrograph S (Fig. 13) showed section rats kidney administered with 6 mg/kg.bw of *Manihot utilissima* cyanide extract. Histological examination as indicated by the black arrow showed renal tissue displaying numerous tubules that are lined by simple cuboidal cells with oval nuclei and pale cytoplasm. The lumen of the renal tubules showed moderate tubular necrosis with focal areas of tubular edema, some of the glomeruli are oedemic and inconspicuous.

4. DISCUSSION

Effect of cyanide extract concentration of *Manihot esculenta* and *Manihot utilissima* have revealed an increase in biochemical markers. Some biochemical parameters such as ALT, AST and ALP activity, which is correlated with hepatic necrosis in rats [11]; indicates an alteration in hepatic functions, were higher in the groups that received *M. esculenta* and *M. utilissima* cyanide extract compared to the control group. Hydrogen cyanide inactivates the enzyme cytochrome oxidase in the mitochondria of cells by binding to the Fe$^{3+}$/Fe$^{2+}$ contained in the enzyme which causes a decrease in the availability of oxygen in the tissues [12]. According to EPA [13], cyanide causes an increase in blood glucose and lactic acid levels and a decrease in the ATP/ADP ratio indicating a shift from aerobic to anaerobic metabolism.

Fig. 7. Photomicrograph of normal rats’ liver and liver of rats treated with 6 mg/kg.bw of *Manihot utilissima* cyanide (H&E X40). A: Normal rat liver, H: Liver of rat treated with 6 mg/kg.bw of *Manihot utilissima* cyanide extract
Cyanide activates glycogenolysis and shunts glucose to the pentose phosphate pathway decreasing the rate of glycolysis and inhibiting the tricarboxylic acid cycle [13]. Cyanide has also been reported to inhibit several other metalloenzymes most of which contain iron, copper or molybdenum (e.g. alkaline phosphatase) as well as enzymes containing Schiff base intermediates (e.g. 2-keto-4-hydroxyglutarate aldolase) [14]. The findings are in agreement with the report by Adam and Ahmed [15] for a significant increase in the
biochemical markers of rats administered with *Manihot esculenta* after traditional Sudanese processing. The measurement of some biochemical parameters such as the activities of enzymes in tissues and body fluids plays a major role in disease investigation, diagnosis and liver toxicity Larrey [16]. The study is also in agreement with findings by Awe and Kolawole [17] for the elevated levels of biochemical indices of rats administered with *Manihot esculenta* Crantz extract.

Normally, AST and ALT are present in high concentrations in the liver cells and are released from the cells due to hepatocyte necrosis, resulting in their increase in the blood. ALT is a sensitive indicator of acute liver damage and elevation of this enzyme is a common feature in hepatic damage. ALT is more selectively a liver parenchymal enzyme than AST [18]. Liver function is assessed by estimating the activities of plasma ALT, AST, ALP, bilirubin, albumin, and total protein. In liver damage, these enzymes leak into the bloodstream in conformity with the extent of damage [19].

Results on urea and creatinine showed a significant increase in groups treated with *Manihot esculenta* and *Manihot utilisima* cyanide extract compared to control. The creatinine synthesized in the liver passes into the circulation where it is taken up almost entirely by the skeletal muscles. According to Wurochekke et al. [20], its retention in the blood is evidence of kidney impairment. In this study, the reduced levels of creatinine in the serum may imply that the cyanide extract has interfered with creatinine metabolism and its eventual excretion from the blood since urea is the main product of protein catabolism. The increase in serum urea level suggests impairment in the control kidney function of the rats in the treatment group as the mechanism of removing it from the blood might have been affected. It may also be an indication of dysfunction at the glomerular and tubular levels of the kidney.

Studies on the effect of *Manihot esculenta* and *Manihot utilisima* cyanide extract compared to control showed a significant increase in groups treated with *Manihot esculenta* cyanide and a decrease in groups treated with *Manihot utilisima* cyanide extract. LDH is a tetrameric enzyme recognized as a potential marker for assessing the toxicity of a chemical [21]. In the present study, *Manihot esculenta* cyanide extract-treated groups exhibited a maximum increase in LDH activity compared to *Manihot utilisima* cyanide extract-treated groups and control. This may be due to the direct effect

Fig. 10. Photomicrograph of normal kidney of rats and rat’s kidney treated with 6 mg/kg.bw of *Manihot utilisima* cyanide (H&E X40). A: Normal rat kidney, N: Kidney of rat treated with 6 mg/kg.bw of *Manihot esculenta* cyanide extract
of the cyanide resulting in the inhibition of cytochrome oxidase activity [22]. Similar observations were made by David et al. [23] in the fish *C. carpio*, exposed to sodium cyanide. Further, the conversion of pyruvate to lactate at the expense of NAD contributed to an increase in LDH activity. Resultantly, to fulfill the energy demands, there may be an increase in the operation of glycolysis under cyanide stress [24].

![Fig. 11. Photomicrograph of normal kidney of rats and rat’s kidney treated with 2 mg/kg.bw of *Manihot utilissima* cyanide (H&E X40). A: Normal rat kidney, Q: Kidney of rat treated with 2 mg/kg.bw of *Manihot utilissima* cyanide extract.](image)

![Fig. 12. Photomicrograph of normal kidney of rats and rat’s kidney treated with 4 mg/kg.bw of *Manihot utilissima* cyanide (H&E X40). A: Normal rat kidney, R: Kidney of rat treated with 4 mg/kg.bw of *Manihot utilissima* cyanide extract.](image)
Results on acid phosphatase showed a significant increase in *Manihot esculenta* cyanide extract-treated group compared to *Manihot utilissima* cyanide treated groups and control. Acid phosphatase is a lysosomal enzyme that hydrolyzes the phosphorous esters in acidic medium. It is hydrolytic and acts as one of the acids hydrolyzes in the autolysis process of the cell after its death whereas alkaline phosphatase splits various phosphorous esters at alkaline pH, its activity is related to the cellular damage or injury. The study contradicts a report by Ogundele et al. [25] for the inhibition of ALP and ACP activity in the adult Wistar rats, administered with the cassava.

The findings of the study further revealed that *Manihot esculenta* extract contains high cyanide concentration compared to *Manihot utilissima* extract. The high concentration of cyanide in the study are in accordance with claims by Sundaresan et al. [26] for the distribution of cyanogens in root of *Manihot esculenta* extract against *Manihot utilissima*. The results of this finding are in agreement with the report by FSA [14] which report for values from 15-400 mg/kg fresh weight of hydrogen cyanide in bitter cassava roots while sweet varieties of cassava (low cyanide content) typically contain approximately 15-50 mg/kg hydrogen cyanide on a fresh weight basis.

Histological examination of the liver revealed injury to the liver cells of rats administered with cyanide extract of *Manihot esculenta* and *Manihot utilissima*. Awe et al. [27] reported that toxicity of *Manihot esculenta* Crantz could be due to depletion of glutathione store or free radical generation or lipid peroxidation revealing abnormality in the liver and kidney architecture. Furthermore, this study is in agreement with a report by Cereda and Mattos [2] that *M. esculenta* extract toxicity may be as a result of the presence of cyanogenic glycosides. The presence of cyanogenic glycosides, linamarin, and lotaustralin pose a potential toxic effect. Linamarin is hydrolyzed by intestinal luminal bacterial beta-glucosidase to release hydrogen cyanide which can cause acute poisoning. It has also been reported that cassava contains some antioxidant compounds namely α-carotene, vitamin C, vitamin A, anthocyanin (flavonoid), saponins and steroids but they do not offer any protective activity to the liver cells [28].

In this study, the effect of *Manihot esculenta* and *Manihot utilissima* cyanide extract on biochemical markers and histological parameters revealed a high amount of cyanide concentration in *Manihot esculenta* (bitter cassava) compared to *Manihot utilissima* (sweet cassava). LD$_{50}$ determined revealed toxicity and caused the death of experimental rats at 10, 20 and 30
mg/kg.bw administered with *Manihot esculenta* and *Manihot utilissima* cyanide extract. The results showed that AST, ALT, ALP, Creatinine, Acid Phosphates and LDH levels of rats administered with *Manihot esculenta* cyanide extract significantly (p< 0.05) increased compared to control rats and rats administered with *Manihot utilissima* cyanide extract. The results also showed a significant (p< 0.05) increase in the activity of AST, ALT, ALP, Urea, and Creatinine on rats administered with *Manihot utilissima* cyanide extract compared to control rats. This is supported by the results on the histological examination of the organs which revealed a more pronounced destruction of the liver of rats administered with *Manihot esculenta* cyanide extract compared to control rats and rats administered with *Manihot utilissima* cyanide extract. The results revealed a dose-dependent effect of the hydrogen cyanide on the liver and kidney tissues of the rats.

5. CONCLUSION

This study, therefore, concluded that *Manihot esculenta* contains a high amount of cyanide compared to *Manihot utilissima*. Cassava tubers (*Manihot esculenta* and *Manihot utilissima*) should not be consumed unprocessed as exposure to cyanide from consumption of cyanogenic glycosides may lead to acute and chronic poisoning, cardiovascular diseases and neurological symptoms resulting from tissue damage exemplified by inflammation, necrosis and death as a result of consumption. Further research should be carried out to improve the rhodanese gene complex in cassava and other ways of reducing the cyanide of cassava should be explored other than fermentation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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