ABSTRACT

Mannihot esculenta Crantz (cassava) leaves have been used for therapeutic purposes without considering toxicological effects. This study was designed to evaluate the toxic effects of aqueous extract of leaf of M. esculenta in Wistar rats. Thirty two Wistar rats were distributed into four groups of eight rats each. Groups 2, 3, 4 were administered orally with M. esculenta leaf aqueous extract at 100, 200 and 400 mg kg\(^{-1}\) body weight respectively for 28 days. Group 1 was control and received no treatment. The effect of aqueous extract of M. esculenta on the body weight, liver enzymes (alanine transaminase ALT, alkaline phosphatase ALP, aspartate transaminase AST), total protein, urea and hematological parameters were evaluated. Pathological changes in the liver were also examined. Oral administration of aqueous extract of leaf of M. esculenta resulted in significant increase (P<0.05) in ALT, ALP activities, decrease in body weight and mild to severe histological changes in the liver compared with control. The aqueous extract of leaf of M. esculenta appears to be toxic at the doses tested. The toxic effects observed might be due to the presence of cyanogenic glycosides, a known toxic constituent of M. esculenta..

KEY WORDS: Mannihot esculenta Crantz, toxicity, biochemistry, hematology, histopathology
INTRODUCTION

Medicinal plants are of great importance in the treatment of various ailments in rural areas most especially in Nigeria. The use of herbal preparations made from medicinal plants is widespread in developing countries. In some of these local communities, medical care is not easily accessible due to, in part to lack of healthcare facilities and the high cost of orthodox treatment\(^1,2\). Many plants have been found to be of medicinal values and sources of remedies widely used as alternative tools for the prevention or treatment of many diseases\(^3\). *Mannihot esculenta* Crantz is widespread in the tropics and is usually known as manioc, cassava, tapioca and mandioac\(^4\). It is a dicotyledonous plant, which belongs to the family Euphorbiaceae\(^5\). Its drought tolerance and ability to adapt to various soil and weather conditions make it a choice staple root crop for farmers. *M. esculenta* is a tuber crop whose origin has been traced to South America\(^6\). Cassava leaves have been reported to contain alkaloids, flavonoids, tannins, anthraquinones, phlobatinnins, saponins, reducing sugars and anthrocyanosides, but do not contain cardiac glycosides, and also 3-rutinosides of kaempferol, quercetin; cyanogenic glycosides, lotaustralin and linamarin have been isolated from the fresh leaves of cassava\(^7,8\). The two cyanogenic glucosides, linamarin (2-D-glucopyranosyl-2-ethylpropanenitrile) and lotaustralin (2R)-2-D-lucopyranosylxoy-2-methylbutyronitrile] were derived from valine and isoleucine, respectively\(^9,10\). Studies also showed that cassava leaves contain alpha carotene and vitamin C\(^11\). *M. esculenta* extract has been used as analgesic\(^11\), anti-hemorrhoidal, anti-inflammatory, anti-pyretic, anti-diarharreal and antimicrobial\(^12\), antioxidant\(^13\) and anti-helmintic\(^14\). Cassava leaves have been used for various ailments, therefore the aim of this study is to assess the toxic effects of the leaf aqueous extract using biochemical, haematological and histological parameters.

MATERIALS AND METHODS

**Ethical consideration**

Experimental procedures and protocols used in this study were approved by the Ethics committee of the Ladok Akintola University of Technology, Nigeria and conform to the “Guide to the care and use of animals in research and teaching” (NIH publications number 85-93 revised in 1985).

**Plant collection**

Cassava leaves were collected at a farm in Dada Estate, Egbedore Local Government, Osogbo, Osun State. The leaves were identified by a taxonomist at the herbarium of the Forest Research Institute of Nigeria (FRIN) Ibadan where voucher specimen was deposited.

**Extraction**

*Mannihot esculenta* fresh leaves (1kg) were air-dried at room temperature. The air-dried leaves of the plant were milled into fine powder in a Waring commercial blender. The powdered leaves were macerated in 3 litres of water at room temperature (27 ± 1\(^\circ\)C) and extracted for 48 hours with occasional shaking. The combined ethanol extract was filtered and concentrated to dryness under reduced pressure at 60 ± 1\(^\circ\)C in a rotary evaporator. Freeze-drying and solvent elimination of the resulting aqueous extract was weighed and finally gave 40.78 g. The percentage yield was calculated. A stock solution of 10mg/ml was prepared by dissolving 100mg of leaf extract in 10ml of water.

**Experimental design**

Thirty two healthy rats weighing between 160-220g were obtained from the Animal House of Ladoke Akintola University of Technology Osogbo. The animals were allowed to acclimatize for 2 weeks. They were fed with rat pellets and water throughout the experimental period. They were kept in a well-ventilated environment at room temperature. The animals
were randomly divided into 4 groups, group 1 served as control group and received no treatment; Group 2, 3 and 4 received 100, 200 and 400 mg/kg aqueous extract of *M. esculenta* for 28 days respectively. Animals in each group were weighed every week till the last week of the experiment.

**Biochemical assessment**

Blood samples were collected and plasma separated by centrifugation at 3000rpm for 5 minutes. The plasma parameters were measured using Randox Laboratories UK reagent kits. The activity of aspartate amino transferase (AST) and alanine amino transferase (ALT) were determined colorimetrically at 546 nm using a standard method. Alkaline phosphatase (AP) was measured using colometric method. Total plasma protein (TP) values were measured colorimetrically at 546 nm using the biuret method. Plasma albumin (ALB) concentration was measured colometrically at 630 nm using bromocresol green method. The concentration of blood urea nitrogen (BUN) was determined colorimetrically at 546 nm using the urease cleavage Berthelot's reaction, according to the principle described by Fawcett and Scott.

**Statistical analysis**

Data were expressed as mean ± SEM. Statistical analyses was performed by one-way, ANOVA followed by Dunnett’s test. P values < 0.05 were considered significant

**RESULTS**

The present study showed the toxic effects of the aqueous leaf extract of plant material of *Manihot esculenta*. The results were represented as Mean± Standard Error of Mean (M±SEM). The statistical significance was performed using Dunnett’s test. The treated group of rats exhibited a significant (p<0.05) body weight loss during 4-week treatment period when compared with control (untreated).

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight Loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0 ± 0.0</td>
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<tr>
<td>100</td>
<td>6.3 ± 1.2</td>
</tr>
<tr>
<td>200</td>
<td>12.6 ± 2.4</td>
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<tr>
<td>400</td>
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**Biochemical changes**

There were dose-dependent significant (p < 0.05) changes in ALT and ALP in rats given daily oral doses of *M. esculenta* aqueous leaf extract at 100 (63.60 ± 8.71 and 143.4 ± 7.0), 200 (63.60 ± 8.71 and 168.2 ± 4) and 400 (65.78 ± 8.0 and 181.7 ± 5.0) mg kg\(^{-1}\) respectively for four weeks compared to control (untreated) group (44.86 ± 4.65 and 122.9±4).

**Haematological changes**

There were increase in the values of RBC, PCV and HB but not significant (p >0.05) in the treated groups compared to untreated control group (Table 2). The MCV, MCH and MCHC did not show significant changes at the dose levels studied, but there was significant (p<0.05) decrease in the platelets value at the dose of 400 mg/kg (197.2 ± 66.0) compared to control (464.0 ± 255) untreated group. There was an increase in WBC count at the dose of 400 mg/kg but not significant (Table 2).

**Histopathological examination**

All animals were sacrificed by exsanguinations after light ether inhalation anaesthesia at the end of the experiment. The animals were subjected to post-mortem examination with collection of samples from the liver. The tissues were fixed in 10% neutral buffered formalin for at least 48 h. Sections of the liver (4-5 µm) were processed and stained with haematoxylin and eosin for microscopic examination using standard protocol of Carleton.

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**Histopathological findings**

There were dose-dependent and mild to severe hepatic degeneration, cellular vacuolation with infiltration by mononuclear cells at the dose of 100, 200 and 400 mg/kg respectively, compared to control (untreated) (Figures 1, 2, 3 and 4).

**Table 1**

*Effects of Mannihot esculenta Crantz leaf aqueous extract on body weight and biochemical parameters in rats.*

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Average body weight (g)</th>
<th>Dose mg/kg</th>
<th>TP (dl)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>BUN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>2262 ± 7.3</td>
<td>67.5 ± 3.39</td>
<td>44.86 ± 4.65</td>
<td>177.84 ± 24</td>
<td>122.94 ± 4</td>
<td>9.98 ± 1.31</td>
</tr>
<tr>
<td>Extract 100</td>
<td>206 ± 1.4**</td>
<td>71.24 ± 3.24</td>
<td>63.60 ± 8.71*</td>
<td>168.58 ± 24</td>
<td>143.4 ± 7*</td>
<td>9.56 ± 0.49</td>
<td></td>
</tr>
<tr>
<td>Extract 200</td>
<td>197 ± 1.7**</td>
<td>72.86 ± 3.40</td>
<td>63.60 ± 8.71*</td>
<td>141.04 ± 17</td>
<td>168.2 ± 4*</td>
<td>9.80 ± 0.63</td>
<td></td>
</tr>
<tr>
<td>Extract 400</td>
<td>176 ± 5.3**</td>
<td>73.96 ± 5.22</td>
<td>65.78 ± 8.06*</td>
<td>167.76 ± 22</td>
<td>181.7 ± 5*</td>
<td>10.14 ± 1.11</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 when compared with control, TP= Total protein, ALT= Alanine aminotransferase, AST = Aspartate aminotransferase, AP= Alkaline phosphate, BUN= Blood Urea Nitrogen

**Table 2**

*Effects of Mannihot esculenta Crantz leaf aqueous extract on haematological parameters in rats.*

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Average body weight (g)</th>
<th>Dose mg/kg</th>
<th>PCV (l/l)</th>
<th>Hb (g/dl)</th>
<th>RBC x 10^6 /cm^3</th>
<th>PLT x 10^9 /cm^3</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
<th>WBC x 10^3 /cm^3</th>
<th>LYM x 10^9 /cm^3</th>
<th>NEUT x 10^9 /cm^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>46.8 ± 4.1</td>
<td>12.42 ± 0.6</td>
<td>7.2 ± 0.40</td>
<td>464.0 ± 255</td>
<td>64.4 ± 2.2</td>
<td>17.1 ± 0.3</td>
<td>26.6 ± 1.1</td>
<td>13.6 ± 4.9</td>
<td>10.4 ± 4.0</td>
<td>3.2 ± 1.8</td>
</tr>
<tr>
<td>Extract 100</td>
<td>100</td>
<td>206 ± 1.7**</td>
<td>50.0 ± 4.4</td>
<td>12.98 ± 0.6</td>
<td>7.59 ± 0.60</td>
<td>432.6 ± 286</td>
<td>66.1 ± 2.2</td>
<td>17.1 ± 0.8</td>
<td>26.1 ± 1.5</td>
<td>11.8 ± 3.5</td>
<td>9.4 ± 2.8</td>
<td>2.4 ± 0.9</td>
</tr>
<tr>
<td>Extract 200</td>
<td>200</td>
<td>197 ± 1.7**</td>
<td>52.6 ± 2.7</td>
<td>12.98 ± 0.6</td>
<td>7.93 ± 0.42</td>
<td>319.4 ± 130*</td>
<td>56.3 ± 2.1</td>
<td>17.0 ± 0.4</td>
<td>25.7 ± 1.5</td>
<td>11.8 ± 3.5</td>
<td>10.3 ± 2.1</td>
<td>4.1 ± 1.6</td>
</tr>
<tr>
<td>Extract 400</td>
<td>400</td>
<td>176 ± 5.3**</td>
<td>50.8 ± 2.7</td>
<td>13.10 ± 0.6</td>
<td>7.73 ± 0.35</td>
<td>197.2 ± 6*</td>
<td>65.8 ± 25.0</td>
<td>17.8 ± 0.6</td>
<td>25.7 ± 0.5</td>
<td>14.7 ± 2.5</td>
<td>11.1 ± 1.7</td>
<td>3.6 ± 1.1</td>
</tr>
</tbody>
</table>

*P<0.05, compared to control, PCV= Packed cell Volume, Hb= Haemoglobin, RBC= Red blood cell, MCV= Mean corpuscular volume, MCH= Mean corpuscular hemoglobin MCHC= Mean corpuscular hemoglobin concentration, WBC= White blood cell, N= Neutrophils, LYM= Lymphocyte, N= Neutrophils

**Pathological changes**

*Figure 4.1

Photomicrograph of control normal liver section showing no lesion (×400)*
Figure 2
Photomicrograph of group 1 liver showing mild hepatic degeneration. (×400)

Figure 3
Photomicrograph of group 2 liver showing diffuse, vacuolar Degeneration with infiltration of mononuclear cells (×400).
DISCUSSION

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. The values of ALT and ALP dose-dependently and significantly increase over the control at doses of 100, 200 and 400 mg/kg respectively. The significant changes in plasma ALT and ALP activities with insignificant change in plasma AST at the dose levels studied indicate that the extract caused mild changes in the liver. ALT is a cytoplasmic enzyme and 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. Clinical observations and experimental studies 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 ALP 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.

The result of the previous investigation indicated that M. esculenta methanol and aqueous root extracts in rats show significant changes in body weight, some haematological parameters, serobiochemical parameters (ALT, AST, and ALP). The liver, kidney and lungs are the primary organs affected by metabolic reaction caused by toxicants. The liver is the most affected organ because it is the site of detoxification in the body which showed fatty vacuolation of the hepatocytes. The results obtained from the present study are in agreement with the previous investigation.

Although the compound responsible for the toxic effects of the extract could not be established in this study, a number of investigators have implicated cyanogenic glucosides, linamarin and lotaustralin as the toxic components of Mannihot esculenta Crantz leaf extract. It is therefore not unreasonable to speculate that these chemical compounds most especially cyanogenic glycoside could have contributed to the observed toxic effects of the plant’s leaf aqueous extract.
CONCLUSION

In conclusion, it was observed that prolonged administration of *Manihot esculenta* leaf aqueous extract at the doses tested shows alterations in liver enzymes (ALP and ALT) and average body weight in rats and mild to severe histological changes in the liver. Therefore caution should be taken in its continued administration.

ACKNOWLEDGEMENT

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REFERENCES


