Assessment of the effect of aqueous leaf extract of cassava (Mannihot esculenta) on adult Wistar rats

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This study aimed to explore the effect of aqueous leaf extract of Mannihot esculenta on the morphology and histology of the kidney of adult Wistar rats. Three groups of Wistar rats were used. Group A served as the control treated with 0.3 ml of normal saline, while groups B and C served as the experimental groups. Group B and C were treated with aqueous extract of M. esculenta orally at 0.2 and 0.5 ml, respectively for 14 days. At the end, the animals were sacrificed and the kidneys were processed for routine histology. From the results obtained, significant decrease in body weight of the rats treated with 0.5 ml and significant increase in the weight of the rats treated with 0.2 ml of the extract as compared to the control group was observed. The kidney section from the control group revealed normal kidney architecture. Low dose group showed hypertrophy of the tubular cells, occlusion of the Bowman’s space and edematous glomerulus. High dose group animals revealed dilated convoluted tubules, occluded Bowman’s space and glomerular derangement indicating adverse effect of the extract. From the results of this study, it may be concluded that the administration of aqueous extract of M. esculenta leaf is toxic to Wistar rats.

Key words: Cassava, Wistar Rat, kidney, Bowmans space.

INTRODUCTION

Medicinal plants have been identified and used from prehistoric times. Plants make many chemical compounds for biological functions, including defense against insects, fungi and herbivorous mammals. Over 12,000 active compounds are known to science. These chemicals work on the human body in exactly the same way as pharmaceutical drugs, so herbal medicines can be beneficial and have harmful side effects just like conventional drugs. However, since a single plant may contain many substances, the effects of taking a plant as medicine can be complex (Atanasov et al., 2015). The compounds found in plants are of many kinds, but most are in four major biochemical classes: the alkaloids, glycosides, polyphenols and terpenes.

Mannihot esculenta Crantz is a dicotyledonous plant which belongs to the family, Euphorbiaceae (Madukosiri et al., 2010). Cassava is a vital food crop for more than 600 million people worldwide. It thrives on marginal
lands, especially in semi-arid tropical and sub-tropical lands that could be the most severely impacted by climate change. Cassava makes up nearly 50% of the diet in parts of sub-Saharan Africa, where populations are projected to increase by more than 120% in the next 30 years (Amanda et al., 2016). Africa produces more than half of the world’s cassava-about 86 million tons from over 10 million hectares (International Institute of Tropical Agriculture, 2017). Cassava leaves have been reported to contain alkaloids, flavonoids, tannins, anthraquinones, phlobatinnins, saponins, reducing sugars and anthocyanosides. Cyanogenic glycosides, lotaustralin and linamarin have been isolated from the fresh leaves of cassava (Ebuehi et al., 2005). The three cyanogenic glycosides, linamarin (2-D-glucopyranosyloxy-2-ethylpropenitrile), lotaustralin (2R)-2-D-glucopyranosyloxy-2-methylbutyronitrile) and latastralin (2D)-D-lucopyranosyl-2-methylbutyronitrile were derived from valine and isoleucine, respectively (Krieger, 2004; Koch et al., 1992). Studies showed that cassava leaves contain alphacarotene and vitamin C (Miladiyah et al., 2016). *M. esculenta* extract has been used as analgesic (Miladiyah et al., 2016), antimicrobial, anti-inflammatory, anti-pyretic, anti-diarrheaa and antimicrobial (Popoola et al., 2007) antioxidant and antihelmintic (Jayasri et al., 2011).

The kidneys are two reddish brown organs situated high up on the posterior abdominal wall, one on each side of the vertebral column. Functionally, the kidney affects the formation and secretion of urine, production and secretion of erythropoietin, the hormone responsible for controlling the rate of formation of red blood cells, the hormone responsible for controlling renin, an important enzyme in the control of blood pressure (Gannon, 2014). They also function in the removal of waste products of metabolism and excess of water and salts from the blood and maintain its pH (Guyton and Hall, 2011).

Chronic kidney disease (CKD) is a global health burden with a high economic cost to health systems and is an independent risk factor for cardiovascular disease (CVD) (Nathan et al., 2016). Ten percent of the population worldwide is affected by chronic kidney disease (CKD), and millions die each year because they do not have access to affordable treatment, according to the 2010 Global Burden of Disease Study, chronic kidney disease was ranked 27th in the list of causes of total number of deaths worldwide in 1990, but rose to 18th in 2010 (Jha et al., 2013).

This study aimed to explore the effect of aqueous leaf extract of *M. esculenta* on the morphology and histology of the kidney of adult Wistar rats.

**MATERIALS AND METHODS**

**Experimental animals**

Twelve adult Wistar rats weighing about 110 to 150 g were bought from the animal house of the Department of Anatomy, Cross River University of Technology (CRUTECH). The animals were allowed to acclimatize for a period of two weeks before commencement of the treatment. They were housed in cages under standard conditions with 12 h light/12 h dark cycle throughout the duration of the experiment. They were fed with rat chow from Agro Feed Mill Nigeria Ltd and water was provided ad libitum.

**Extract preparation**

Fresh cassava leaves were harvested from Okuku community of Yala Local Government Area of Cross River State, Nigeria. The leaves were verified and authenticated in the Herbarium Unit of Botany Department, University of Calabar. They were plucked and air-dried at a room temperature (27°C for 3 weeks). They were blended to a fine powder using a local mortar and pestle. The blended sample was weighed using digital weighing balance and was 250 g. The aqueous extract was done using water bath extractor. The weight of the extract was 28.7 g. The extract, 28.7 g obtained was stored in the refrigerator for preservation.

**Experimental protocol**

Then, from the yield of 28.7 g of leaf extract, the stock solution was prepared by dissolving 2 g of the extract in 10 ml of distilled water. The different dosages were calculated based on the body weight of the animals. Every 0.5 ml of the extract administered contained 10 mg/ml of the extract for the high dose, while the 0.2 ml of it contained 4 mg/ml for the low dose. The animals were divided into three groups as follows: Group 1 served as the control group and were fed with distilled water. Group 2 served as the low dose group administered with 0.2 ml of the extract, while group 3 served as the high dose group administered with 0.5 ml of the extract. The extract was administered using the oral route. After 14 days of daily administration of the extract, the animals were sacrificed, kidney harvested and processed for histological observation.

**Histological procedure**

The harvested rat kidneys were preserved in 5% formaldehyde for 48 h. Then, dehydrated through ascending grades of alcohol, 2 changes of 70, 90%, and absolute alcohol for 1 h in each change. After dehydration, the tissues were cleared in xylene with 3 changes, 1 h in each. The tissues were placed in 2 changes of molten paraffin wax for 20 min each. They were embedded in molten paraffin wax inside the L-shape Leuchhart mould. The blocks were trimmed and mounted on wooden blocks. Serial sections were cut using a rotary microtome at 5 μ thickness. Sections were floated in a water bath to spread out and later picked on albumuminized slides and dried on at 52°C. To stain, slides were put in staining racks and placed in staining wells containing xylene to dewax, then they were rehydrated in descending grades of alcohol, absolute alcohol (2 changes, 70% alcohol and then water for 5 min after which they were stained with haematoxyline for 5 min. Excess haematoxyline was washed off with water and differentiated with 1% acid alcohol. Sections were counter stained with 1% eosin and washed off with water. They were dehydrated with 70, 90% and absolute alcohol and finally cleared in xylene to remove water. A drop of mountant was placed on the surface of the slides and covered with a 22 by 22 mm cover slip.

**RESULTS**

**Effects of extracts on the body weight**

The mean body weights of the groups of the animals
administered the extract before and at the end of the experimental periods are shown in Figure 1. It was observed that the final mean weights of the groups showed 131±6.6 g for the control group as against its initial mean body weight of 122±6.3 g. While the groups 2 (Low dose) and 3 (high dose) showed 150±3.1 g and 148±9.4 g as their final mean body weight, as against their initial mean body weights of 132±1.0 g and 145±0.8 g, respectively. There was weight gained in the control and low dose animals. However, the animals in the high dose lost weight.

**Histological observation**

**Control group**

Sections showed normal histological features of the kidney. This shows the renal corpuscles (RC) of the cortex composed of the glomerulus (G) within the Bowman's capsule. The Bowman's capsule is lined by thin layer of single squamous cells. The proximal (PCT) and distal convoluted tubules (DCT) are lined by single layer of cuboidal epithelium. The Bowman's space (BS) is also present as shown in Plate 1.

**Group 2 (Low dose)**

Plate 2 represents photomicrograph of section of kidney tissue from the animals treated with 0.2 ml of leaf extract showing the glomerulus with larger urinary space.

**Group 3 (high dose)**

Plate 3 is a photomicrograph of a section of the kidney tissue from the animals treated with 0.5 ml of leaf extract showing the glomerulus with larger urinary space.

**DISCUSSION**

The kidneys play an essential role in regulating the amount of several important inorganic ions in the body, including sodium, potassium, chloride, bicarbonate, hydrogen, calcium and phosphate. They contribute to the maintenance of organic ion balance, eliminate metabolic waste and maintain pH balance (Arroyo, 2008).

From the results obtained, the administration of aqueous leaf extract of *M. esculenta* revealed significant decrease in body weight of the rats treated with 0.5 ml and significant increase in the weight of the rats treated with 0.2 ml of the extract and that of the control group. This result is not in agreement with the work of Awe and Kolawale (2013) which reported significant (P<0.05) body weight loss during four weeks treatment period. The kidney section from the control group revealed normal kidney architecture, darker stained cortex and the pale stained medullar, the renal corpuscles appearing as dense rounded structure and the glomerula being surrounded by narrow Bowmans space.

Low dose group (0.2 ml) administered rats showed hypertrophy of the tubular cells, occlusion of the Bowman’s space and edematous glomerulus. This indicates that the extract administered has affected the renal histology and functions which may consequently...
result in renal failure and can lead to severe reduction in glomerular filtration leading to acute diffuse proliferative glomerulonephritis (Morris, 2012).

High dose group (0.5 ml) treated animals revealed dilated convoluted tubules, occluded Bowman’s space and glomerular derangement indicating adverse effect of
Plate 3. Photomicrograph of kidney (cortex and medulla) of rat administered 0.5ml of leaf extract of *M. esculenta*, showing dilated distal convoluted tubules, occluded bowman space and also showing glomerular derangement; ×400 H&E.

the extract on the cells and tissues of the kidney which may consequently result in diuresis. This result differ from previous studies that have reported the presence of biologically active compounds in *M. esculenta* in particular alkaloids which have been documented to have many pharmacological properties capable of protecting tissues and cells from toxic effects (Prawat et al., 1995; Ebuch et al., 2005).

**Conclusion**

The histological observations suggests that aqueous leaf extracts of *M. esculenta* is toxic to the kidney at the doses given since its administration causes morphological changes and histological damage of the cellular integrity of the kidney. The administration of aqueous leaf extract showed some toxicity to the kidney cells in the Wistar rats. The full potentials of this plant have not been fully exploited. Hence, this review will stimulate further scientific research into the biological activities, with the view to discovering novel or lead pharmaceutical agents.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**REFERENCES**


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