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Original Research Article

EFFECT OF AQUEOUS EXTRACT OF CASSAVA (*MANIHOT ESULENTA*) LEAF ON THE MORPHOLOGY AND MICRO-ANATOMY OF THE LIVER OF WISTER RATS

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ABSTRACT

Background and objective: Cassava leaves (*Mannihot esculenta Crantz*) are largely consumed as vegetable in Africa the present study explored the effect of aqueous leaf extract of *Mannihot esculenta Crantz*, on the microanatomy of the liver of adult Wistar rats.

Materials and methods: Twelve adult Wistar rats weighing 110-150g were distributed into three groups of four rats each. Group 1 served as the control group which received 0.3ml of normal saline, while group 2 (low dose group) received 0.2ml of the extract and group 3 (high dose group) received 0.5ml of the extract. The effect of the extract on the body weight and liver histology was evaluated. After the end of the administration (day 14), the weight were taking before sacrifice. The Liver were excised and fixed in 10% formal saline, and processed for rapid routine paraffin embedding. Tissues were stained with routine Haematoxylin and Eosin, observed under a light microscope and micrograph were taken.

Results: Result shows significant difference ($p<0.05$) in weight gain between control and the treated groups. In the low dose group histology revealed degenerative atrophic hepatocytes while sinusoidal dilatation was observed in the high dose group of the treated groups.

Conclusion: From the results obtained from the study, it could be deduced that; the administration of aqueous leaf extract of *Manihot esculenta* at the doses given induces observable pathological effect on the histology of the liver and may be concluded to have adverse effect on the dosage administered.

KEY WORDS: Cassava leaf, Wistar rat, Liver, Microanatomy.

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INTRODUCTION

Cassava (*Mannihot esculenta Crantz*) is a dicotyledonous plant, belonging to the family Euphorbiaceae [1]. It is a perennial shrub, 2 to 4 m in height and is mainly propagated from stem cuttings. Cassava forms a staple food for an estimated 500 million people in the tropics. It is widely grown in most countries in the tropical regions of Africa, Latin America and Asia.

Cassava is grown over a range of climates and altitudes and on a wide variety of soils. Cassava is tolerant to drought and is productive in poor soil where other staple crops cannot grow [2]. The crop is an important source of carbohydrate for humans and animals, having higher energy than other root crops, 610 kJ/100 g fresh weight. Dried cassava root has energy similar to the cereals [2, 3]. In Africa, the continent with

the largest cassava production, about 93% of the produce is used as food [4].

Although it is the third most important food source in the tropical world after rice and maize, and provides calories for over 160m people in Africa [5] its food value is greatly compromised by the endogenous presence of cyanogenic glucosides. These glucosides, typified by linamarin [2-($ Dglucopyranosyloxy) is obutyronitrile] and lotaustralin [2-($- Dglucopyronosyloxy) methylbutyronitrile] are hydrolyzed to hydrocyanic acid (HCN) by endogenous linamarase. (EC. 3.1.1.21, linamarin, $-D-glucoside glucohydrolase) when cassava tissues are disrupted by cutting, grating, bruising or other mechanical means [6, 2].

Cassava leaves, a byproduct of cassava root harvest is (depending on the varieties) rich in protein (14 - 40% Dry Matter), minerals, Vitamin B1, B2, C and carotenes.

Available literature clearly suggest, that apart from lower methionine, lysine and perhaps isoleucine content, the amino acid profile of cassava leaf protein compares favorably with those of milk, cheese, soyabean, fish and egg. In spite of these qualities, the nutritional potentials of cassava leaf meal and cassava protein concentrates remain currently underresearched. The major drawback to the wide spread use of cassava leaves as food in Nigeria is “cyanide scare” as its content of cyanogenic glucosides could, depending onthe variety, be 6 times higher than in the roots [7, 8]. Apart from cyanide, tannin and possibly phytin [9] may limit the nutritional value of cassava leaves.

While various cassava processing techniques may generally lead to substantial cassava detoxification,conditions, such as famine, drought and failure of otherless - well adapted root crops generally lead toincreased demands for cassava roots and leaves duringwhich the traditional processing methods may be compromised. Apart from the risk of acute cyanide intoxification and death, chronic exposure to sub-lethal levels increases the incidence of goitre, tropical neuropathy, glucose intolerance [10] and Konzo (spastic paraparesis) [11]. It is evident from the foregoing that, for the full nutritional potentials of cassava roots and leaves to be realized, current research efforts must focus more on the development of simple, low - cost but efficient techniques that would rid them of cyanide as well asother anti - quality constituents such as tannin and phytinin the leaves. The present study therefore provides analytical information on the nutrient composition of the leaves of some local and genetically improved cassava varieties as well as the processing effects on some of their inherent antinutriets. We ultimately hope to reconcile the efficacy of such processing techniques with controlled nutritional studies to permit credible local health education programmes with regard to cassava leaf processing and use for human and, or animal feeding.

The liver is the largest gland and heaviest organ in the body and it occupies the right quadrant of the abdomen. This organ serves the vital function of maintaining the body’s internal milieu. Three of its basic functions include the production and secretion of bile, which is passed into the internal tract, involvement in many metabolic activities related to carbohydrates, fats and protein metabolism and finally filtration of the blood, removing bacteria and other foreign particles that have gained entrance to the blood from the lumen of the intestine.

MATERIALS AND METHODS

Extract preparation: Cassava leaves from were harvested from a cassava farm located in Okuku community of Yala Local government area of Cross River State, Nigeria. The leaves were verified and authenticated by Mr. Okon of the Herbarium unit of Botany Department, University of Calabar. The leaves were plucked, washed to remove debris and air-dried at a room temperature of about 27°C for three weeks. They were blended to powder, using a local mortar and pestle. The blended sample of Manihot esculenta (leaf) powder was weighed using digital weighing balance and was found to weigh 250g. The aqueous extract of the Manihot esculenta (leaves) was done using Water Bath extractor. The weight of the extract was 28.7g.The extract so obtained was stored in the refrigerator for preservation. Then from the yield of 28.7g of Manihot esculenta leaf extract, the stock solution was prepared by dissolving 2g of the extract in 10mls of distilled water.

Experimental procedure : Twelve adult Wistar rats weighing about 120-150g were used for this research work. They were housed in cages made of wire gauze in the animal house of the Department of Human Anatomy, Faculty of Basic Medical Sciences, Cross River University of Technology (CRUTECH) Okuku Campus. The animals were housed under standard conditions with 12 hours light /12 hours dark cycle throughout the duration of the experiment. The animals were grouped into three groups of four rats each. Group 1 served as the control group which received 0.3ml of normal saline, while group 2 (low dose group) received 0.2ml of the extract and group 3 (high dose group) received 0.5ml of the extract.

Termination of experiment: At the end of the two weeks period, animals in all the groups were sacrificed a day after the end of the administration under chloroform anesthesia and their livers were removed and preserved in labeled bottles containing 10% buffered Formalin for the study.

RESULTS

Effect of treatment on body weight of rats: At the end of the research work, the mean body weight of the animals in the control groups (A) was 131± 6.6g as against its initial weight of 122+ 6.3g, whereas the mean body weight of the treatment groups (B) and (C) were 150+ 3.1g and 148+ 9.4g as against 132+ 1.0g and 145+ 0.8g respectively. The animals in the treated groups (B) and (C) revealed significantly (P>0.05) increased body weight values compared to the control. (Figure 1)

Effect of treatment on the microanatomy of the liver: The microscopic examination of section of the liver from the control group which received 0.3ml of distilled water revealed normal cytoachitecture of the liver with polygonal hepatocytes (h) radiating from the central vein (V). The sinusoids (S) run in between the cords of the liver cells. (Fig 2).

The photomicrograph of a section of the liver tissue from the low dose animals treated with 0.2ml b of (Me) leaf extract showed an ephased architectural pattern with degenerated atrophic hepatocytes (H) (Fig. 3).

Fig. 4: shows the photomicrograph of a section of the liver from the high dose animals treated with 0.5ml of the leaf extract of (Me) showing sinusoidal dilatation. It also shows degenerated hepatocytes (H).

Fig. 1: Comparison of initial and final mean body weights in the different experimental groups. Values are mean ±SEM.

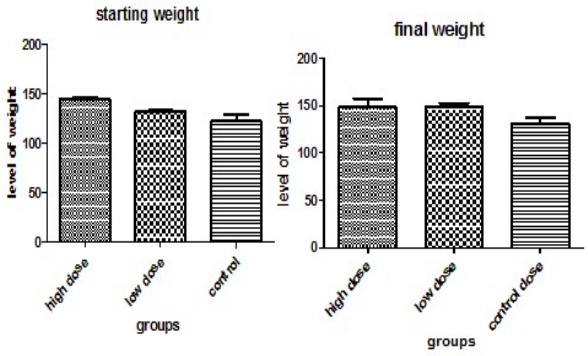


Fig. 2: Photomicrograph of the liver from the control group (A)administered with distilled water for 14 days using H&E×400stain shows well defined hepatic vein (V), Hepatocytes (h) and sinusoids(S). (Control group).

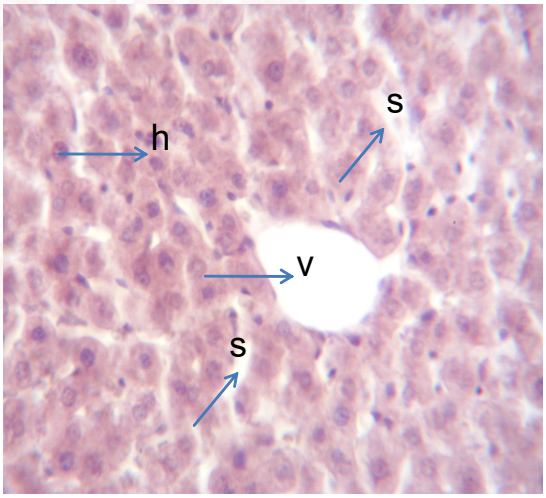


Fig. 3: Photomicrograph section of the liver from the group (B) animals treated with 0.2ml of leaf extract for 14 days shows ephased architectural and degenerating atrophic hepatocytes (H) using H&E stain (X400)

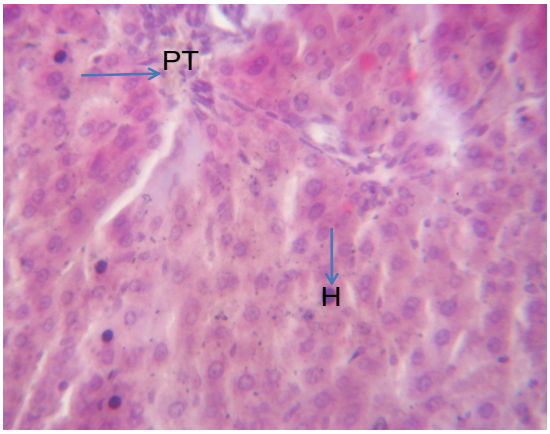
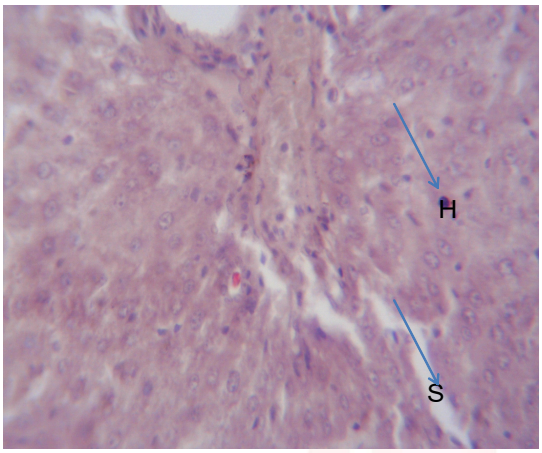


Fig. 4: Photomicrograph section of liver from group (C)animals administered with 0.5ml of the extract for 14days showing atrophic hepatocytes with pynotic-nuelei and ephased architectural pattern. Stain H&E(×400)



DISCUSSION

Natural product of plants origin have been widely reported to exert profound and long lasting effect on human health due to the enormous phytochemical compounds embedded in them [12]. Medicinal plants are known to produce adverse effects under prolonged and continuous usage. Although a drug may be very effective in the treatment of an illness but the effects of these medicinal plants on some vital organs could necessitate its withdrawal from usage.

A variety of medicinal plants or other substances have been reported to affect the liver in one way or the other. It has been reported by Tao *et al*., (2015) that certain drugs and substances damage the liver either by direct biochemical effects, indirect or immunological effects. Substances which cause direct toxicity as reported by them include mercury, gold, antimicrobial, analgesics, carbontetrachloride etc.

Mannihot esculenta is one of the most useful traditional medicinal plants known in Nigeria for its therapeutic value. Cassavaleaves contain an average of 21% crude protein, but values ranging from 16.7 to 39.9% This wide variability is related to differences in cultivars, stage of maturity, sampling procedure, soil fertility and climate. Almost 85% of the crude protein fraction is true protein [7].

The body weight of the rats in the various groups showed variation. The result revealed that the aqueous extract of Manihot esculenta administered at different doses caused increased in the low dose treated animals (0.2ml) and significantly decrease ($P<0.05$) in the body of the high dose (0.05ml) treated animals when compared with the control. The decrease in the body weight of the high dose treated animals might be an indication that the extract causes loss in muscle and adipose tissue which results in excessive mass breakdown of protein. This is in line with the report of [14] The liver section from the control group showed normal cytoarchitecture of the liver with polygonal hepatocytes radiating from a central vein sparse collagenous tissue and sinusoid lining cells with their flattened condensed nuclei.

The liver tissues of animals in low dose group treated with 0.2ml of the extract showed ephased architectural pattered degenerated atrophic hepatocytes. This shows that the extract administered have effect on the liver histology which may be due to the chemical components of the extract which have been reported by [15]. This section of the liver from high dose group revealed sinusoidal dilation and degenerated hepatocytes. This might be an indication that the administration of *Mannihot esculenta* at the dose of 0.5ml over the duration of 14 days has adversely affected the morphology of the liver. The noticeable adverse effect in the integrity of the liver cell perhaps may be due to the chemical component in the extract which can be toxic in prolong use.

CONCLUSION

The results of this experimental work using animal models may not be used to give direct application in man but it gives an insight into the possible toxic effects of the substance. From the results obtained from the study, it could be deduced that; the administration of aqueous leaf extract of *Manihot esculenta* at the doses given induces observable pathological effect on the histology of the liver and may be concluded to have adverse effect on the dosage administered.

Conflicts of Interests: **None**

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