RESEARCH ARTICLE

Studies of Morphology and Serum Biochemistry of the Kidney of Wistar Rats Treated with Aqueous Leaf Extract of Cassava (*Manihot esculenta*)

Kebe E. Obeten[1*], Gabriel Udo-Affah[1], Obi O. Ndifon[2], Saviour A. Odey[2]
[1]Department of Human Anatomy, University of Calabar, Calabar, Nigeria
[2]Department of Anatomy and Anthropology, Cross River University of Technology, Calabar, Nigeria

[*]*Address for Correspondence: Dr. Kebe E. Obeten, Research Fellow, Department of Human Anatomy, University of Calabar, Calabar, Nigeria*

Received: 15 March 2017/Revised: 12 May 2017/Accepted: 01 July 2017

ABSTRACT- Cassava leaves (*Mannihot esculenta* Crantz) are largely consumed as vegetable in African, but contain a toxic compound, cyanide. The present study explored the assay for liver enzymes on adult Wistar rats. Twelve's adult Wistar rats weighing 110-150g were distributed into three groups of four rats each. Groups 2 and 3 were administered orally with *M. esculenta* leaf aqueous extract at 0.2ml and 0.5ml respectively for 14 days. Group 1 was control and received 0.3ml of normal saline. The effect of aqueous extract of *M. esculenta* on the body weight, liver enzyme was evaluated. After the end of the administration (day 14), the weight were taken before sacrificed the next day. Rat's liver were excised and fixed in 10% formal saline, then processed for rapid routine paraffin embedding. Our results showed significant difference ($p<0.05$) in the body weight gain between control and the treated groups. Serum chemistry revealed significant decrease ($p<0.05$) in alanine aminotransferase (ALT), Alkaline phosphatase (ALP), aspartate aminotransferase (AST) in animals treated with 0.2ml and 0.5ml of the extract relative to the control. From the results of this study, it may be concluded that the administration of aqueous extract of *Mannihot esculenta* leaf is toxic to Wistar rats at the dose administered.

Key-words- Wistar rats, SEM, Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST)

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, 610kJ per 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] & lotaustralin [2-($- Dglucopyronosyloxy) methylbutyronitrile] are hydrolyzed to hydrocyanic acid (HCN) by endogenous linamarase. (EC.3.1.1.21, linamarin, $-D-glucosideglucohydrolase) when cassava tissues are disrupted by cutting, grating, bruising or other mechanical means [6]. 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.

Access this article online	
Quick Response Code	Website: www.ijlssr.com
	crossref DOI: 10.21276/ijlssr.2017.3.4.4

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 [7]. In spite of these qualities, the nutritional potentials of cassava leaf meal and cassava protein concentrates remain currently under-researched. 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 on the variety, be 6 times higher than in the roots. Apart from cyanide, tannin and possibly phytin [8] 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 to increased demands for cassava roots and leaves during which the traditional processing methods may be compromised. Apart from the risk of acute cyanide in toxification and death, chronic exposure to sub-lethal levels increases the incidence of goitre, tropical neuropathy, glucose intolerance [9] and Konzo (spastic paraparesis) [10]. 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 as other 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 anti-nutrients. 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 [11].

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 in 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^{o}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* 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. Blood was collected through cardiac puncture from the left ventricle into labeled specimen bottles. Serum was separated by centrifugation for 5 minutes at 1000 rpm and used for assay to determine liver enzyme.

The liver of these animals were removed, evaluated to ascertain the effect of the extract administered in the liver enzymes which includes alanine anminotransferase (ALT), aspirate aminotransfrase (AST) and alkaline phosphates (ALP). Parts of these tissues were processed through paraffin sections for Heamatoxylin and Eosin (H&E) and Paraffin acid Schiff methods to stain for glycogen.

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 (Fig 1).

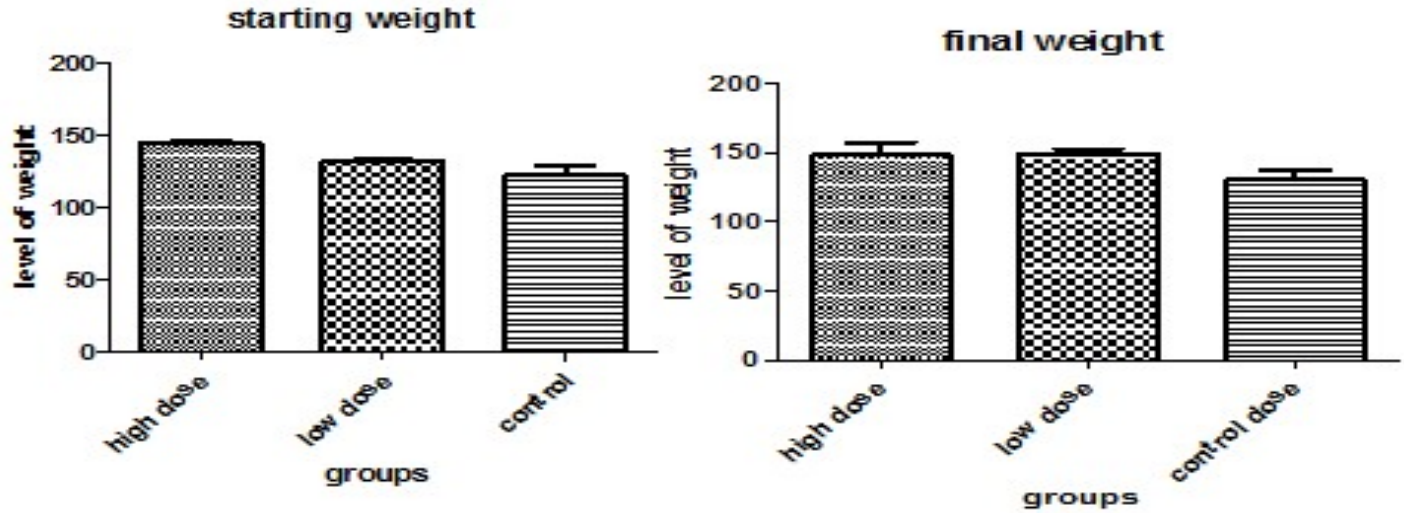

Fig 1: Comparison of initial and final mean body weights in the different experimental groups. Values are mean ± SEM

Table 1: Effects of treatment on body weight of rats

Control		Group B		Group C	
Before	After	Before	After	Before	After
122+ 6.3g	131± 6.6g	132+ 1.0g	150+ 3.1g	145+ 0.8g	148+ 9.4g

Biochemical analysis

Liver serum enzyme alkaline phosphate (ALP)

Results obtained revealed that group A animals had normal level of ALP (153±1.22), while group B and C animals showed significantly reduced values compared to the control group. (91.2±3.24 u/L) and (53.8±2.11u/l) respectively (Fig 2).

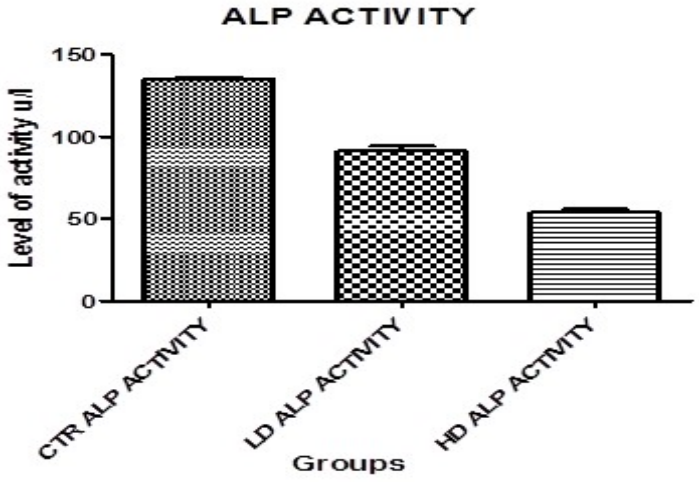

Fig 2: Comparison of serum alkaline phosphatase levels in control and test groups. Values are mean + SEM. *significantly different from control at p>0.05

Alanine amiotransferase (ALT) concentration

Group A animals showed normal level of ATP (5.1±0.21u/l), group B animals showed reduced concentration which is significant (P<0.05) compared to control group (1.4±0.22u/l) while group C animals showed ALT concentration significantly reduced (P<0.05) compared to control group (0.41±0.06u/l) (Fig 3).

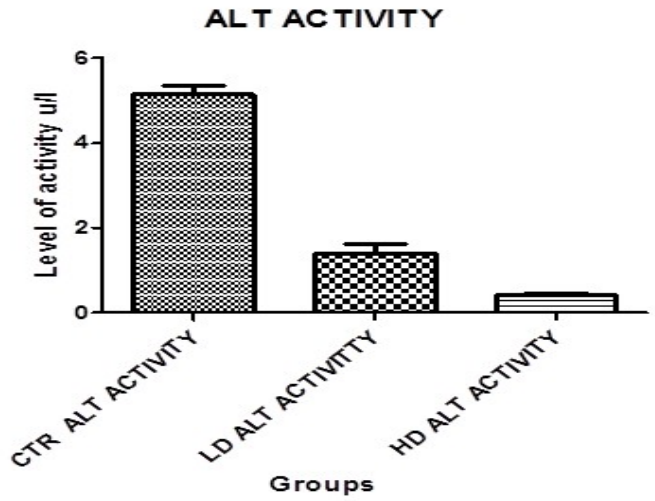

Fig 3: Comparison of serum alamine aminotransferase levels in control and test groups. Values are mean + SEM.*significantly different from control at p<0.05

The effects of extracts on the Aspartate Aminotransferase (AST) concentration

The effects of treatment on the Aspartate aminotransferase (AST) revealed that the groups that received 0.2ml and 0.5ml of leaf extract showed their concentrations as 15+0.70u/l and 11+0.69u/l. while the control is 28±0.87 respectively. This shows that the results from the experimental groups were lower than that of the control group but significant (Fig 4).

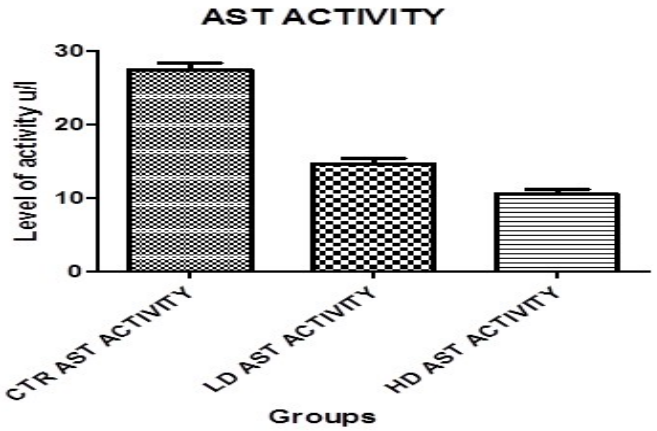

Fig 4: Comparison of serum aspartate aminotransferase levels in control and test groups. Values are mean + SEM.*significantly different from control at p<0.05

Table 2: Effects of extract on biochemical parameters

Control	Group B	Group C
Liver serum enzyme alkaline phosphate (ALP)		
153±1.22u/L	91.2±3.24 u/L	53.8±2.11u/L
Alanine amiotransferase (ALT) concentration		
5.1±0.21u/l	1.4±0.22u/l	0.41±0.06u/l
The effects of extracts on the Aspartate Aminotransferase (AST) concentration		
28±0.87	15+0.70u/l	11+0.69u/l

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.

Mannihot esculenta is one of the most useful traditional medicinal plants known in Nigeria for its therapeutic value. Cassava leaves 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, which is in with line with research by Granner [13].

Serum AST and ALT are sensitive indicators of liver damage or injury. The ratio of AST to ALT can be useful in differentiating between the causes of liver damage and elevated levels of AST are not specific for liver damage [14]. In this research work, it was observed that they was marked and significant reduction in the activities of ALT. ALT is a cytoplasmic enzyme and increase in plasma, is an indication of mild injuries caused by chemicals to the liver [15]. AST is a mitochondria enzyme whose increased activity in plasma reflects severe tissue injuries [15]. ALP comes mainly from the cells lining bile ducts but also in bones. It is an enzyme that transports metabolites across cell membrane [16] in the treated animals. This indicates that the extract may have an adverse effect on the liver enzymes as reported by [17] who stated that below normal values of liver enzymes may suggest liver dysfunction or insufficient protein intake. This work is not in agreement with [15], who reported significant elevation in the activities of serum ALT and ALP and insignificant changes in plasma AST implicating the actions of cyanogenic glucosides, linamarin and lotaustralin the toxic component of *Manihot esculenta* extract.

CONCLUSIONS

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, it could be deduced that the administration of aqueous leaf extract of *Manihot esculenta* at the doses given induces observable effects on the liver enzymes and may be concluded to have adverse effect on the dosage administered.

REFERENCES

[1] Alves, A.A.C. and Setter, T.L. Response of cassava to water deficit: leaf area growth andabscisic acid. Crop Science 2002; 40, 131-137.

[2] Bradbury, J. H. and Holloway, W. D. Chemistry of tropical root crops: significance for nutrition and agriculture in pacific. ACIAR Monograph No 6. FAO, 1990. Roots, tubers, plantains and bananas in human nutrition, 1988.

[3] FAO, Rome, Italy.FAO, http://www.fao.org. Agricultural Statistics. Food and Agricultural Organization of the United Nations. Rome 2002.

[4] Nweke, F. I., Spencer, D. S. C. and Lynam, J. K. The cassava transformation: Africa's best-kept secret. Michigan University Press, USA. 2002.

[5] Polsen, RA and Spencer D.S. The technology adoption process in subsistence agriculture: The case of cassava in Southwestern Nig. Agri. Sys., 1991; 36:65-78.

[6] Bradbury, J.H., Egan, S.V., Lynch, M.J. Analysis of cyanide in cassava using acid hydrolysis of cyanogenic glucosides. Journal of the Science of Food and Agriculture 1991; 55, 277–290.

[7] Eggum, B.O. The protein quality of cassava leaves. *Brtish. Journal. Nutrition*., 1970; 24: 761-768.

[8] Reeds, W.R., S.K. Sathe and D.K. Salunkhe, Phytater in legumes and cereals. Adv. Food Res., 1982; 28: 1-9.
[9] Akanji A. O. and Famuyiwa O. O. The effects of chronic cassava consumption, cyanide intoxication and protein malnutrition on glucose tolerance in growing rats. *Br J. Nutri* 1993; 69(1): 269-76.
[10] Howlett RA, Parolin ML, Dyck DJ, Hultman E, Jones NL, Heigenhauser G J F, Spriet LL. Regulation of skeletal muscle glycogen phosphorylase and PDH at varying exercise power outputs. American Journal of Physiology. 1998;275:R418–425.
[11] Snell, R.S. Human Anatomy, (3rd ed), 2000, pp:771-773.
[12] Atangwho, I.J., Ebong, P.E, Eyong, E.U., williams, I. O., Eten M U., & Egbung G. E. Comparative chemical composition of leaves of some antidiabetic medicinal plants: 2009.
[13] Granner, O; Murray, R & Rodwell, W. Harper's illustrated Biochemistry Textbook27th ed, New York mcGrall Hill, 172-174; 2006.
[14] Giboney PT. Mildly elevated liver transaminase levels in the asymptomatic patient. *America Family Physician* 2005; 71(6) 1105-10.
[15] Awe E. and Kolawole O. Biochemical, haematological and histopatholgical assessment of toxic effects of *Manhito esculenta* Crantz leaf Aqueous extract in Rats. *International Journal of Pharma and Bio Sciences* 2013; 4(3):228-235.
[16] Giannini E G. Roberto T. Vincenzo S. Liver enzyme alteration: a guide for clinicians. CMAJ, 2005; 173(3): 367-379.
[17] Palmer M. A practical guide to understanding, treating and living with hepatitis & liver disease. Penguin Group USA. 10-12; 2004.

How to cite this article:
Obeten KE, Udo-Affah G, Ndifon OO, Odey SA: Studies of Morphology and Serum Biochemistry of the Kidney of Wistar Rats Treated with Aqueous Leaf Extract of Cassava (*Manihot esculenta*). *Int. J. Life. Sci. Scienti. Res.,* 2017; 3(4): 1127-1131. DOI:10.21276/ijlssr.2017.3.4.4
Source of Financial Support: Nil, **Conflict of interest:** Nil