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Biochemical and Histological Responses on the Liver of Adult Wistar Rats Fed with Varied Level of Cassava

[1]F.B. Oyeyemi, [3]S.A. Babajide, [2]K.T. Elelu, [5]W.A. Oyeyemi and [4, 6]O.A. Iyiola
[1]Cell Biology and Genetics Unit, Department of Zoology, [2]Department of Microbiology,
Faculty of Sciences, University of Ibadan, Ibadan, Oyo State, Nigeria
[3]Department of Anatomy, College of Medicine, [4]Department of Zoology,
Faculty of Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria
[5]Department of Physiology, College of Medicine,
Madonna University, Elele, Rivers State, Nigeria
[6]Department of Molecular Biology and Biotechnology, University of Sheffield,
Firth Court, S10 2TN, Sheffield, United Kingdom

Abstract: The biochemical and histological responses on liver of rats following feeding on unprocessed cassava was examined in this study. About 28 rats were randomly divided into four. Group A which served as the control was fed with 30 g of grower feed per day while groups B-D were given 10 g of unprocessed cassava and 20 g of grower feed per day, 20 g of unprocessed cassava and 10 g of grower feed per day and 30 g of unprocessed cassava per day, respectively. After feeding for 6 weeks, the animal weight, liver histology, Aspartate amino Transferase (AST), Alanine amino Transferase (ALT), Gamma Glutamyl Transpeptidase (GGT) and Alkaline Phosphatase (ALP) were measured. The result shows significant reduction in weight gain among groups B-D animals when compared with the control. Histological studies also revealed diffused hepatic degeneration of the liver in all treatments except the control; this damage is more evident in group D animals. There was a significant increase in ALP, AST, ALT and GGT activity in groups B-D when compared with the control group, the highest was observed in group D. Biochemical evidence of tissue injury in the liver was supported by histological findings of the liver which showed disturbance of normal hepatic cytoarchitecture on rats in groups B-D. It is concluded that feeding on unprocessed cassava is harmful to the liver.

Key words: Unprocessed cassava, histology, cytoarchitecture, hepatic degeneration, liver, AST, GGT

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the most important source of dietary carbohydrates for 750 million people around the world with its starchy root being the main harvested organ (Gleadow *et al*., 2009; Burns *et al*., 2011). Anhwange *et al*. (2011) revealed that tubers of cassava have relatively high content of hydrogen cyanide content compared to *Colocasia esculenta*, *Dioscorea bulbifera* and *Dioscorea domementeroum* in fresh and dried forms.

Cassava serves as a staple food in human diets over 80 countries including Nigeria (Gomez *et al*., 1988). It is a good source of energy with highly digestible carbohydrates. But it has some nutritional drawbacks such as low protein content, low energy density and potential toxic effects due to the presence of linamarin, a cynogenic glycoside that is easily hydrolyzed by the enzyme linamarase (a β-glucosidase) to release Hydrogen Cyanide (HCN). Cyanide liberated from residual linamarin has been shown to be associated with goiter in iodine deficient populations with chronic intake of cassava based food products (Taga *et al*., 2008; Teles, 2002; Abaye *et al*., 1998). Eating food that contain low level of cyanide for a long time develop damage to the Central Nervous System (CNS) and thyroid gland (Jansz and Uluwaduge, 1997).

Recently, Chabwine *et al*. (2011) reported that appearance of konzo in South-Kivu, a wartorn area in the Democratic Republic of Congo was associated with consumption of insufficiently processed cassava root. Thus, the aim of this research is to check the effects of feeding on unprocessed cassava on liver using combination of biochemical and histological tests. Since, liver is involved in detoxifying toxic chemicals.

Corresponding Author: F.B. Oyeyemi, Cell Biology and Genetics Unit, Department of Zoology, Faculty of Sciences, University of Ibadan, Ibadan, Oyo State, Nigeria

MATERIALS AND METHODS

Experimental rats and treatments: A total of 28 adult Wistar rats (*Rattus norvegicus*) bred at the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria and weight between 110-148 g were used for this study. Throughout the experiment, the animals were housed in clean cages placed in well-ventilated house conditions (temperature: 24-28°C; photoperiod: 12 h natural light and 12 h dark, humidity: 45-50%). The cages were cleaned twice daily at 12 h interval. They were acclimatized for 14 days. During which they were fed *ad libitum* with pellet form of grower feed (vital poultry feed) and had free access to drinkable water.

Preparation of unprocessed cassava and administration: Cassava root (tuber) was peeled to removed the external coat (brownish part) and the whitish part sliced and diced into small pieces (size of each pellet for grower feed), this is to enable experimental rats to pick and feed on it properly and easily. Fresh unprocessed cassava was used daily throughout the experiment.

Before the rats were fed with unprocessed cassava, they were given known grams of grower feed and the remaining feed was weighed after 24 h to ascertain the actual quantity of feed each group took during the last 24 h. This was done for a week prior to proper feeding and the average was computed. On the average, it was discovered that each group sufficiently ate 210 g of mash feed meaning that each rat can take 30 g of meal day^{-1}.

Experimental procedure: The animals were randomly and equally grouped into four after the acclimatization. The various groups and their experimental feeding were as follows:

- Group A (control); 30 g grower feed/rat/day
- Group B; 10 g of unprocessed cassava and 20 g of grower feed/rat/day
- Group C; 20 g of unprocessed cassava and 10 g of grower feed/rat/day
- Group D; 30 g of unprocessed cassava/rat/day

The animals were fed for 6 weeks and their weights were taken every week. The rats were examined daily throughout the experimental period for changes in activity, skin fur and eyes.

Collection of blood sample and isolation of liver: After 6 weeks of feeding, the rats were anaesthetized and blood was collected from jugular vein and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 5000 rpm at 10°C for 15 min and utilized for the estimation of various biochemical parameters. Then the animals were sacrificed and liver was removed and fixed in Bouin's fluid for histological examination.

Biochemical studies: Aspartate Aminotransferases (AST) and Alanine Aminotransferases (ALT) were determined by monitoring the concentrations of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine as recommended by Reitman and Frankel (1957) and Alkaline Phosphatase (ALP) was done as described by Klein *et al.* (1960) while Gamma Glutamyl Transpeptidase (GGT) was done using Szasz (1969) Method.

Histological examination of tissue: Paraffin tissue section (5-10 μ) of liver was prepared, stained with haematoxylin eosin and mounted in a neutral medium. Histological examination was done on the tissue according to procedure described by Disbrey and Rack (1970).

Statistical analysis: Data were subjected to statistical analysis. Values were reported as Mean+SEM while ANOVA and LSD were used to test for differences between the groups using Statistical Package for Social Sciences (SPSS) version 16. $p<0.05$ was accepted as significant.

RESULTS AND DISCUSSION

Physical examination: The physical activities of the rats during the period of the experiment were closely monitored. It was observed that rats were very active. The rate of feed consumption before and during the feeding experiment did not change in any of the groups. The mean weight gained by rats following feeding on unprocessed cassava is shown in Table 1. The data revealed that there was a significant reduction ($p<0.05$) in the weight gained by the rats as the level of cassava fed increased. The least mean weight gained by rats during and after the experiment was recorded in group D when compared with the control.

Histopathological effects on the liver: There was no observable gross change in the liver of rats fed with

Table 1: Mean weight gain of Wister rats fed with commercial meal (control) and unprocessed cassava (group B-D)

Groups	Mean weight gain
A	16.17±0.54
B	12.65±0.44*
C	10.70±0.50*
D	7.49±0.78*

*Means significant different at $p<0.05$; values are recorded as mean±SEM

unprocessed cassava compared to the ones fed with grower feed only (control). Feeding on unprocessed cassava caused a dose dependent destruction of the histology of the liver in Wistar rats. The histology of the experimental rats showed some degree of vacoulation which increase as quantity of unprocessed cassava fed by rats increases when compared with the micrograph of the control group (Fig. 1-4). Sinusoid of the liver was observed in the control group (Fig. 1), this was observed partially in group B (Fig. 2) but disappear in group C (Fig. 4). Lastly, central vein of the liver in group B and C (Fig. 2 and 3) contained some blood tissues which are absent in the control group (Fig. 1). Histological observation revealed diffused hepatic degeneration; this is more evident in the experimental rats of group D. However, the control (group A) had a normal hepatic cytoarchitecture in which the nuclei of the hepatocytes are well preserved (Fig. 1). Generally, there was dose dependent destruction of the histology of the liver of Wistar rats fed with unprocessed cassava.

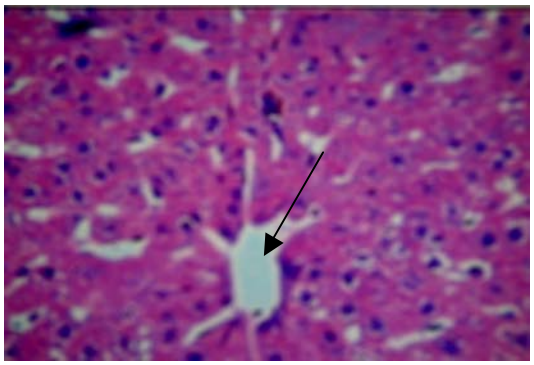

Fig. 1: Liver photomicrograph of the control (H and E) at × 400 showing normal central vein

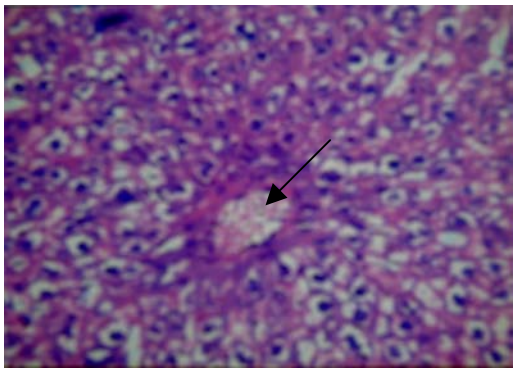

Fig. 2: Liver photomicrograph of rat fed with 20 g of grower feed and 10 g of unprocessed cassava (H and E) at × 400 showing central vein containing minimal blood tissue

Biochemical responses: Table 2 shows the activities of some hepatic enzymes in the serum of both the control and test animals. There was a significant increase in ALP, ALT and GGT activity in group B-D when compared with the control group. While, AST activity was significantly increased in groups C and D when compared with the control. The increased in activities of ALP, ALT, AST and GGT observed in this study were dose dependent. From the result of this study, there was a significant reduction in mean weight gain in animals fed with cassava; this reduction was more prominent in group fed with cassava alone. Rats fed with cassava showed hepatic damage which may be as a result of high cyanide content in the cassava. ATSDR (2006) has earlier reported that the highest tissue concentration of cyanide was found in the liver of rats orally exposed to cyanide contained in cassava. The observed decreased in mean weight gain by the animals in this study might be as a results of liver damage caused by feeding on cassava.

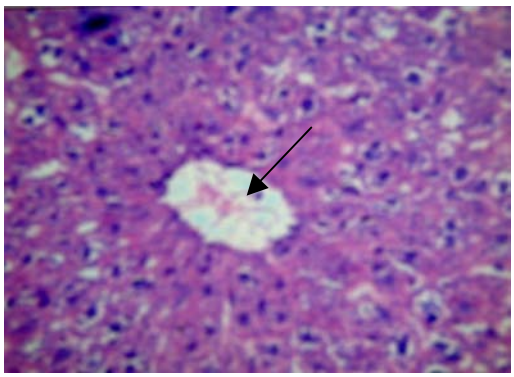

Fig. 3: Liver photomicrograph of rat fed with 10 g of grower feed and 20 g of unprocessed cassava (H and E) at × 400 showing central vein containing blood tissue

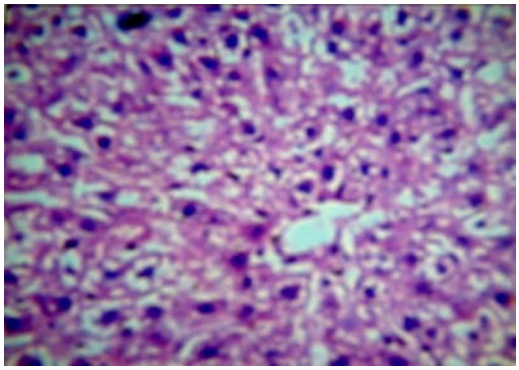

Fig. 4: Liver photomicrograph of rat fed with 30 g of unprocessed cassava (H and E) at × 400 showing highest vacoulation and hepatic degeneration

Table 2: Effect of cassava diet on Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP) and Gamma Glutamyl Transpeptidase (GGT) of adult Wister rat

Groups	ALT (UL^{-1})	AST (UL^{-1})	ALP (UL^{-1})	GGT (UL^{-1})
A	31.96±1.87	92.15±6.70	60.09±4.62	322.74±12.15
B	45.60±2.46*	102.67±7.12	75.61±5.08*	408.21±11.89*
C	56.00±2.84*	114.46±3.34*	80.90±3.31*	472.31±19.60*
D	63.44±3.58*	134.39±7.57*	94.66±4.60*	504.70±22.20*

*Means significant different at $p<0.05$; Values are recorded as mean±SEM

This is similar to Iweala *et al.* (2011) report where induced liver damage in rats resulted in their body weight gain reduction. Also, the decreased in mean body weight gain observed in this study might be as a result of utilized sulphur-containing amino acids by the animals to detoxify the cyanide content of the cassava since the liver have been compromised due to hapatotoxicity of the cyanide, thus it might not support the detoxification of cyanide in the cassava.

Physical appearance deteriorations were observed in the rats fed with cassava in this study. Reduction in feeding habit, agility and growth rate/weight were observed in animals fed with cassava, this observation is in agreement with Sunmonu and Oloyede (2010). As the study progressed, the animals fed with cassava looked rough, sick, reddish and bulged eyes and trying to eat up the wire gauge in the cage. The severities of the above mentioned sign were however directly proportional to the quantity of cassava in their diet in that the most severe sigh was found in group D.

The findings revealed increase in the serum AST, ALT, ALP as well as GGT activity in the animals fed with cassava (Table 2). Similar findings have been reported by Okafor *et al.* (2002, 2008) where they observed increased in the activities of these enzymes in human and rats exposed to cyanide in their diet. Increased in serum concentrations of these enzymes might indicate damage to cell membrane of some organs such as liver, kidney and exocrine pancrease (Joan *et al.*, 1988; Bogusz, 1975). AST and ALT play a role in the metabolism of amino acids, an increase in these enzymes level indicate active liver cirrhosis, hepatocellular disease, liver tumour and hepatic disorder (Vaishwanar *et al.*, 1976; Obidike *et al.*, 2005). Thus, increase in ALT and AST observed in this study probably resulted from hepatic dysfunction, especially as it was accompanied by a corresponding increase in GGT and ALP. It could be deduced that increase in serum activity of ALT is directly related to the amount of damage that has occurred to the hepatocytes, this may be due to the fact that pathologies involving hepatic cell might allowed for the escape of large quantities of these enzymes into the blood (Coles, 1986).

The histological studies showed liver damage in rats fed with cassava with highest damage observed in rats fed with cassava only, this similar to Ononogbu and Emole (1978). This result was also in accordance with Ewuola (2009) that observed histopathological damage in the liver of rabbits fed with diet containing suspected toxic substance; Abaye *et al.* (1998) reported liver inflammation and haemorrhage in dogs that consumed cassava containing low amounts of cyanide.

Recently, Chabwine *et al.* (2011) showed that appearance of konzo in South-Kivu, a wartorn area in the Democratic Republic of Congo was associated with consumption of insufficiently processed cassava root this corroborate the finding that eating unprocessed cassava could have a detrimental effects on the body. Also, Cliff *et al.* (2011) showed large epidemics of Konzo during the cassava harvest when the population has been dependent on a diet of insufficiently processed bitter cassava. These two recent observations supported the finding that feeding on unprocessed cassava is harmful to the body particularly the liver. The effects recorded on the liver in this study may be as a result of the presence of cyanogenic glucosides that originates from unprocessed roots of cassava.

CONCLUSION

This study revealed that feeding on unprocessed cassava by Wister rats significantly alter the serum biochemical variables and caused damage and dysfunction to liver which can lead to their poor performance.

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