

Histological Effects of Cassava Starch on the Liver of Adult Wistar Rats

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Abstract

Cassava (*Manihot esculenta Crantz*) and its products have been widely studied and reported to have been of great importance, although sometimes with toxic effects. Most of these effects are due to the presence of some anti-nutrient factors in cassava. This study evaluates the effects of cassava starch on the liver of adult wistar rats. A total of 20 adult wistar rats weighing about 150-200g were used in this study and were randomly divided into 4 groups. Group 1 served as the control and received only distilled water and normal laboratory chow. Group 2 received 250mg/kg of starch. Group 3 received 500mg/kg of starch and Group 4 received 1000mg/kg of starch. The administration lasted for a period of 28 days and the extract was administered via oral route. Twenty four hours after the last administration, the animals were anesthetized under chloroform vapour and dissected. Blood for serum preparation was collected through cardiac puncture for histological studies. The liver was harvested and fixed in 10% formal saline for histological studies. Body weight results revealed that there was a significant decrease ($P < 0.05$) in body weight of groups 3 and 4 while group 2 had an insignificant increase ($P > 0.05$) when compared with control. Result from liver weight had an insignificant increase ($P > 0.05$) in groups 2, 3 and 4 when compared with control. The result of aspartate aminotransferase (AST) showed a significant increase ($P < 0.05$) in groups 2, 3 and 4 when compared with control. The result of alanine aminotransferase (ALT) showed a significant increase ($P < 0.05$) in groups 2, 3 and 4 when compared with control. The result of alkaline phosphatase (ALP) showed a significant increase ($P < 0.05$) in groups 2, 3 and 4 when compared with control. Histopathological findings of the liver of the tested groups revealed that there was moderation, mild portal inflammation in the hepatocytes when compared with control. In conclusion, the aqueous extract of starch caused hepatocellular injury, and biochemical alterations of the liver enzymes.

Keywords: Cassava, Starch, Plants, Wistar rats, Body weight

1. Introduction

Plants are the ultimate sources of food for humans and animals. Being autotrophs, green plants constitute the primary producers of the ecosystems. Almost all tissues in plants such as seeds, fruits, flowers and roots are edible. This is because these tissues are reservoirs of the food nutrients namely carbohydrate, proteins, fats, vitamins and minerals. The tuberous roots of cassava and yam are rich in carbohydrates (Balagopalan *et al.*, 1998). Legumes contain high amounts of proteins thus they serve as alternative source of proteins to man especially in the third world countries where there is scarcity of animal protein due to poverty and poor development of life stock keeping (Ojala, 2001).

Many traditional methods of food preparation such as fermentation, cooking and malting increase the nutritive quality of plant foods through reducing certain antinutrients such as phytic acid, polyphenols and oxalic acid (Balagopalan *et al.*, 1998). An important example of such processing is the fermentation of cassava to produce cassava flour garri: this fermentation reduces the amounts of both toxins and antinutrients in the tuber (Chavan and Kadam 1989). The plant toxicants have been incriminated in the aetiology of several disease conditions such as hemorrhage (Fernandez *et al.*, 1997, Yu *et al.*, 1997), tissue disorders, neurologic disorders (Osuntokun, 1994), teratogenicity, abortion (Fernandez *et al.*, 1997) and various forms of ulcer. The presence of toxicants in plant foods is a cause for great concern to mankind due to their health and economic implantations. This is so because there is great dependence on plant foods in tropical countries, Consequently, screening of commonly consumed tropical plant food materials for the presence of toxicants (both of primary and secondary origins) is an important step in the study of endemic diseases as well as to ensure safety in the dietary use of such plant materials (Yu *et al.*, 1999).

Cassava (*Manihot esculenta Crantz*) is a perennial woody shrub grown for its edible tubers (Chavan and Kadam 1989). It belongs to the family of Euphorbiaceae. Cassava is well adapted to poor soils with marginal nutritional status and pH from 4 to 9 (Cock, 1982; 1985). It is propagated vegetatively with the stem cuttings. It is cultivated extensively in tropical countries where its root tubers constitute a principal source of dietary carbohydrate. Cassava is the most important root crop in the world and ranks second among African staple crops (Nweke *et al.*, 2002; Manu-Adening *et al.*, 2006). As a carbohydrate source, it ranks first, producing 250 x 103

calories per hectare per day compared to 200 x 103 for maize, 176 x103 for rice, 114 x 103 for sorghum and 110 x 103 for wheat (Nartey, 1980). Nigeria is the largest producer of cassava in the world, a third more than in Brazil and almost double the production of Indonesia and Thailand (FAO, 2006).

Starch hydrolysates are also used as basic ingredient in the manufacture of industrial chemicals such as alcohol, gluconic acid and acetic acid (Balagoplan *et al.*, 1998). It is used in the production of adhesives for lamination in plywood, paperboard and footwear and in the packaging industry (Chavan and Kadam 1989). In cable industries, starch is applied in production of paper tubes, cans and cones. It is also used in printing, publishing and library paste. Starch is also used as label adhesives for envelopes postage stamps, gummed tapes, safety matches and many other items. On hydrolysis with acid or enzyme, cassava product starch is used to impact sweetness, texture and cohesiveness to soft drinks, fruit juice, dairy drinks, cake and cookie (Balagoplan *et al.*, 1998).

Cassava contains many antinutrients which include cyanogenic glycosides (linamarin and lotaustralin), coumarins (scopoletin and esculetin and their glucosides, scopolin and esculin), phenolic compounds such as tannins and other phytochemicals (Fernandex *et al.*, 1997).

These antinutrients are characterised by diversity of biological (toxicological) effects including carcinogenic, hepatotoxic, neurotoxic, immunotoxic effects; In addition they are used for their oestrogenic, dermatotoxic, haemorrhagic, mutagenic, immunosuppressive and teratogenic properties (Yu *et al.*, 1997). All varieties of cassava contain cyanide which exists both in the free form and in combination with glycosides, linamarin 2-(β -D glucopyranosyloxy) isobutyronitrile(>90% total cyanogen) and lotaustralin 2-(β -D-glucopyranosyloxy)-2methylbutyronitrile(<10% total cyanogens) (Ariffin *et al.*, 1992; McMahan *et al.*, 1995). They are categorized as either sweet or bitter, signifying the absence or presence of toxic levels of cyanogenic glucosides in their roots. The so-called sweet cultivars may contain as little as 20 milligrams of cyanide (CN) per kilogramme of fresh roots, where as bitter ones may contain more than 50 times as much (1 g/kg). It also contains significant quantities of scopoletin (6-methoxy 7-hydroxycoumarin), esculetin (6, 7 - dihydroxy coumarin), scopolin (6, methoxy, 7 -hydroxylcoumaroyl -7- β -D glucoside) and esculin (6, 7-dihydroxyl coumaroyl-7- β -D glucoside). These coumarin compounds possess diverse biological and pharmacological properties such as anti-oedema, anti-inflammatory, antitumor, anticoagulant, immunostimulatory, anti-convulsant and hypotensive activity (Aoife and Richard, 2004). Biochemically, they possess the ability to scavenge (quench) reactive oxygen species (free radicals), stimulate respiration ionophoretically; inhibit 5- and 12-lipoxygenases and inhibit xanthine oxidase and phenyl alanine hydroxylase. Severe cyanide poisoning, particularly during famines, is associated with outbreaks of a debilitating, irreversible paralytic disorder called konzo and, in some cases, death. The incidence of *konzo* and tropical ataxic neuropathy can be as high as 3 percent in some areas (Wagner, 2010). Studies have shown that prolonged consumption of cassava based products results in the goitrogenic effects of thiocyanate (metabolic product of cyanide) which was responsible for the endemic goiters seen in the Akoko area of South Western Nigeria. It has also shown that a number of illnesses have been attributed to high and continuous consumption of cassava - based diets. The cause of these illnesses is the presence in cassava these toxic materials - namely cyanogenic glycosides and the coumarins (Rickard, 1985). Long - term consumption of cassava starch has been shown to depress the activities of the liver microsomal metabolizing enzymes which transform a wide variety of chemicals including drugs pesticides, carcinogens, food additives as well as endogenous substrates such as fatty acid and steroids (Eze *et al.*, 2009). Prolonged consumption of cassava especially in the presence of protein-calorie malnutrition is associated with chronic poisoning syndromes such as tropical ataxic neuropathy and *konzo* (a sudden-onset of upper motor neurone spastic paraparesis) (Tylleskär, 1991; Mayambu, 1993).

This work is therefore aimed at evaluating the effects of cassava starch on the liver of adult wistar rats.

2. Materials and Methods

2.1. Place of Study

This research work was carried out in the Department of Anatomy, College of Health Sciences, Nnamdi Azikiwe University, Okofia Nnewi.

2.2. Experimental Animals

A total of twenty (20) adult wistar rats weighing between 150 to 200g were used for the study. The rats were purchased from the animal house, Nnamdi Azikiwe University Pharmaceutical Science centre, Agulu and were transferred to the Animal House of the Department of Anatomy, College of Medical Science, Nnamdi Azikiwe University, Nnewi. Animals were kept in standard animal cages in a room temperature of $27\pm 2^{\circ}\text{C}$. The animals were maintained with normal laboratory chow (Growers mesh) and water *ad libitum*; the animals were acclimatized for a period of two weeks and were kept on 12 hours light and dark cycle.

2.3. Preparation of Extract

Cassava was procured from Agricultural Farm in Nnobi, Okacha. The starch was prepared by wet milling of the fresh grounded cassava roots. The cassava root was first harvested after which it was peeled, washed and grounded using local grinder and was soaked in a clean sack cloth for 48 hours and was pressed and the liquid that came out was the starch which was put in a clean plate and then allowed to dry under controlled room temperature.

2.4. Acute Toxicity

The median lethal dose (LD₅₀) of Cassava Starch was carried out in the department of Physiology, Faculty of Basic Medical Science, Nnamdi Azikiwe University, Okofia Campus. This was determined using the modified method of Dietrich Lorke (1983). The median lethal dose was found to be above 5000mg/kg.

2.5. Experimental Design

A total of 20 adult wistar rats weighing between 150 – 200g were used for the study. They were acclimatized for a period of 2 weeks before commencement of administration. The animals were randomly grouped into four (4) groups of 5 animals each. The administration of the extract lasted for a period of 28 days

Group 1; serves as control and receives only distilled water

Group 2; receives 250mg/kg of Starch

Group 3; receives 500mg/kg of Starch

Group 4; receives 1000mg/kg of Starch

2.6. Collection of Blood Samples and Organ

The animals were anaesthetized with diethyl ether in a close container after 24 hours of the last dose of administration. Blood were collected via ocular puncture into a plain container for liver enzyme. The animals were dissected through incision on the abdominal wall vertically and horizontally and the liver were harvested and placed in 10 % formalin in a container.

2.7. Analysis of Liver Enzymes

Determination of ALT and AST was done by monitoring the concentrations of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine. 0.5ml of buffer solution was dispensed into test tubes labeled blank, sample, control blank and control respectively for AST and ALT respectively. 0.1ml of sample and control was dispensed into their respective test tubes. All the tubes were incubated at 37°C for 30minutes. 0.5ml of 2, 4-dinitrophenylhydrazine was dispensed into all test tubes. 0.1ml of sample and control was dispensed into their respective blank test tube. The contents of each test tube was mixed and allowed to stand for 20minutes at 25°C. 5ml of 0.4N sodium hydroxide was added to each tube, mixed and read at 550nm against the respective blank prepared. The activity of the unknown was extrapolated from the calibration curve already prepared (Reitman, and Frankel, 1957).

Alkaline Phosphatase activity was done by Phenolphthalein Monophosphate method. The test tubes were respectively labeled sample, standard and control. 1.0ml of distilled water was pipetted into each tube followed by a drop of the substrate into each test tube. All the test tubes were incubated at 37°C for 5minutes. 0.1ml of sample, standard and control were dispensed into their respective test tubes. The test tubes were incubated at 37°C for 20minutes. 5ml of colour developer was added to each test tube, mixed, and read at 550nm using water as blank. The activity of sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard (Babson *et al.*, 1966).

2.8. Tissue Processing

Tissues are processed through several processes of fixation, dehydration, clearing, impregnation, embedding, sectioning and stained using H & E method.

2.9. Statistical Analysis

Data was analyzed using SPSS (Version 16) Software package. All result obtained were expressed as mean ± SEM in each group. All tested parameters (Pancreatic weight, Liver weight, ALP, ALT and AST) were analyzed using one-way ANOVA, followed by post HOC LSD and Body weight was analyzed using Student dependent T-test. All values were considered significant at P<0.05.

3. Results and Discussion

3.1. Analysis of Body Weight

Table 1: shows the effect of starch on body weight after 28 days of treatment.

	Body weight (g)	MEAN	±SEM	P-VALUE	T-VALUE
GROUP 1 (Control)	INITIAL	152.50	±8.53		
	FINAL	180.00	±9.12	0.002	-11.000
GROUP 2 (250mg of Starch)	INITIAL	170.00	±5.77		
	FINAL	177.50	±6.29	0.215	-1.567
GROUP 3 (500mg of Starch)	INITIAL	182.50	±10.30		
	FINAL	152.50	±2.50	0.046	3.286
GROUP 4 (1000mg of Starch)	INITIAL	177.50	±2.50		
	FINAL	155.00	±2.88	0.018	4.700

3.2. Analysis of Liver Weight

Table 2: shows the effect of starch on the relative liver weight after 28 days of treatment.

LIVER WEIGHT (g)	MEAN	±SEM	P-VALUE	F-VALUE
GROUP 1 (Control)	2.47	±0.27		
GROUP 2 (250mg of Starch)	2.55	±0.19	0.274	
GROUP 3 (500mg of Starch)	3.02	±0.13	0.592	0.487
GROUP 4 (1000mg of Starch)	3.08	±0.18	0.804	

3.3. Activities of Serum Levels of AST, ALT and ALP

Table 3: shows the effect of starch on Liver enzymes after 28 days of treatment.

LIVER ENZYMES		MEAN	±SEM	P-VALUE	F-VALUE
ALT (U/L)	GROUP 1 (Control)	12.50	±0.28		
	GROUP 2 (250mg of Starch)	49.50	±2.10	0.000	
	GROUP 3 (500mg of Starch)	60.50	±1.04	0.000	513.846
	GROUP 4 (1000mg of Starch)	81.50	±0.95	0.000	
AST (U/L)	GROUP 1 (Control)	8.00	±0.40		
	GROUP 2 (250mg of Starch)	95.50	±0.28	0.000	6038.00
	GROUP 3 (500mg of Starch)	119.00	±0.81	0.000	
	GROUP 4 (1000mg of Starch)	128.50	±1.04	0.000	
ALP (U/L)	GROUP 1 (Control)	51.00	±1.22		
	GROUP 2 (250mg of Starch)	45.50	±0.28	0.000	22.846
	GROUP 3 (500mg of Starch)	53.00	±1.63	0.000	
	GROUP 4 (1000mg of Starch)	57.50	±0.28	0.000	

3.4. Histopathological Findings

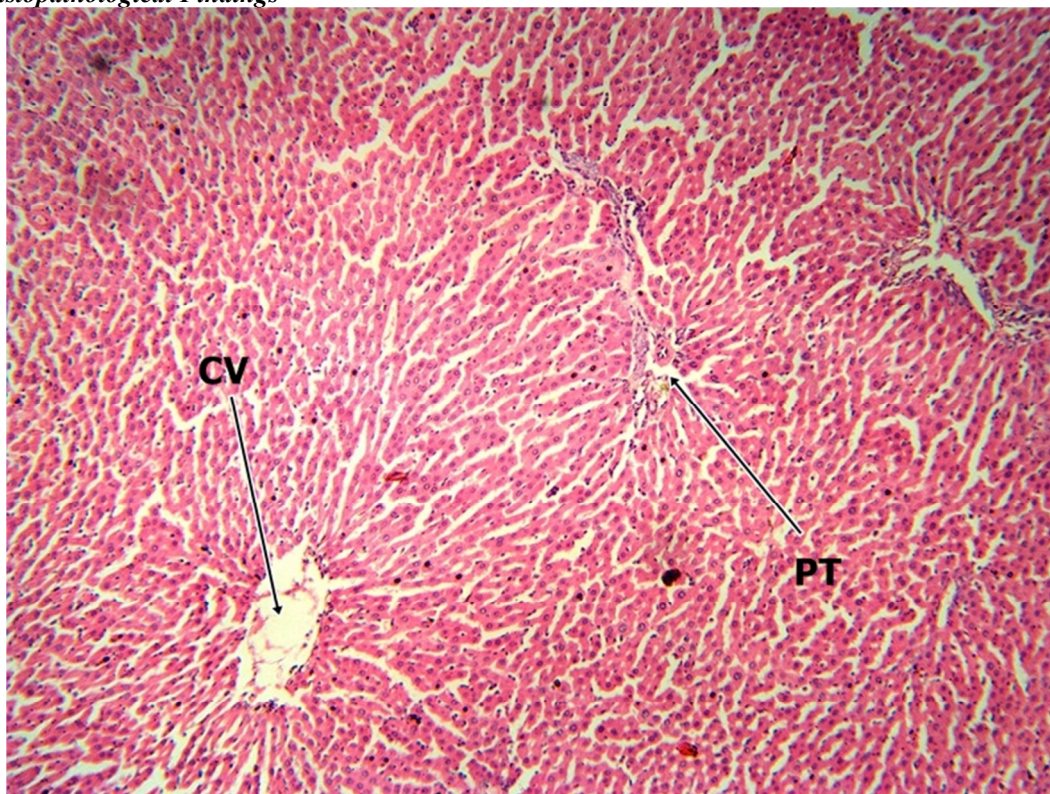


Plate 1 (CONTROL) Photomicrographs show well preserved liver architecture. The portal triads are evenly spaced around a central vein and there is no portal inflammation. There is no steatosis. Stained by H & E (X 100)

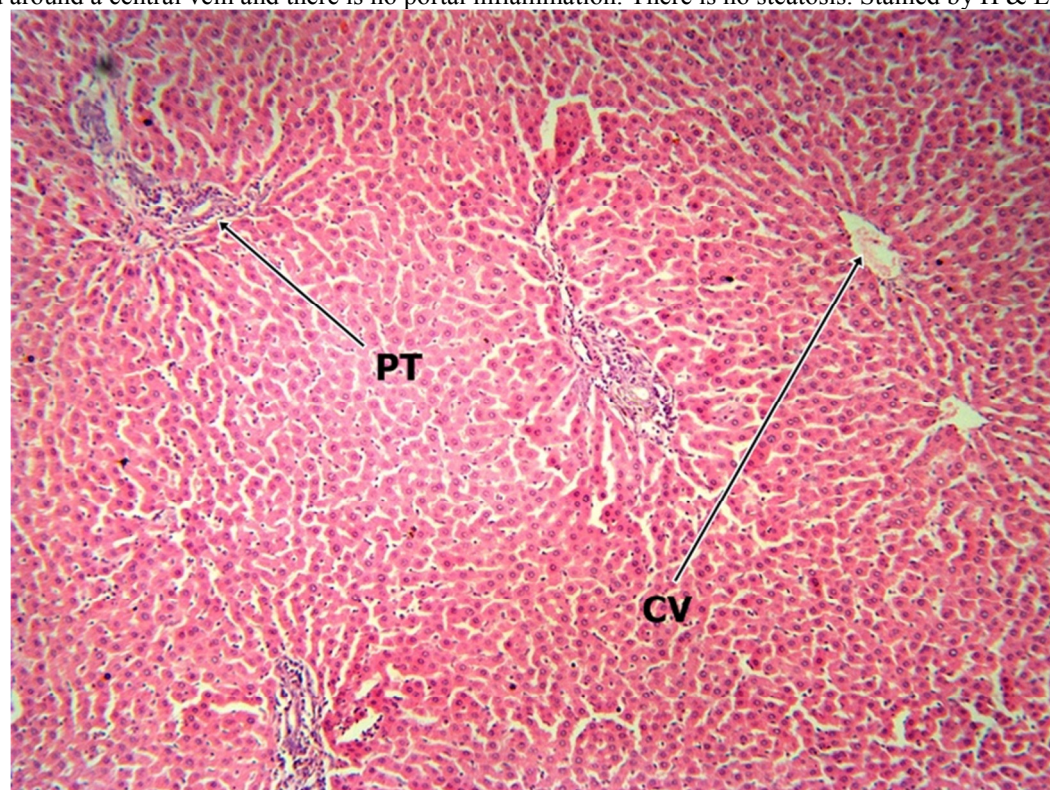


Plate 2. (250mg of Starch). A section of liver showing a well preserved liver architecture. The portal triads are evenly spaced around a central vein and there is moderate portal inflammation with occasional lobular inflammation. There is no steatosis. Stained by H & E (X 100)

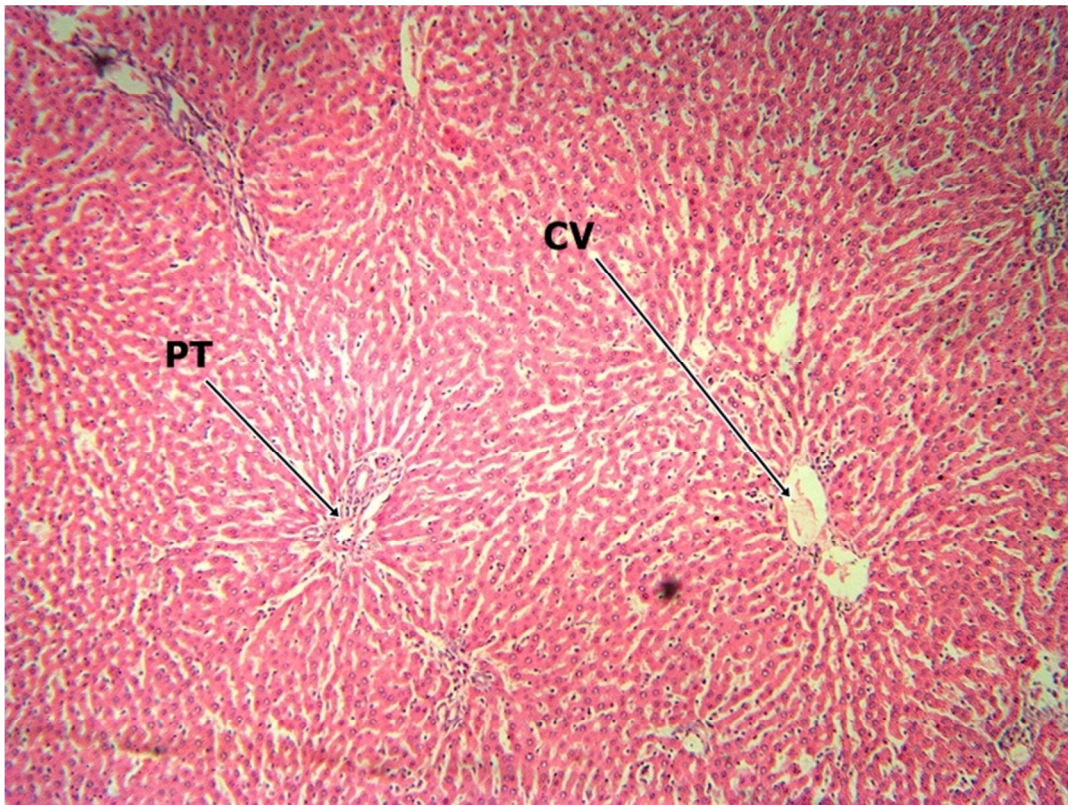


Plate 3 (500mg of Starch) A section of liver showing a well preserved liver architecture. The portal triads are evenly spaced around a central vein and there is mild portal inflammation. There is no steatosis. Stained by H & E (X 100)

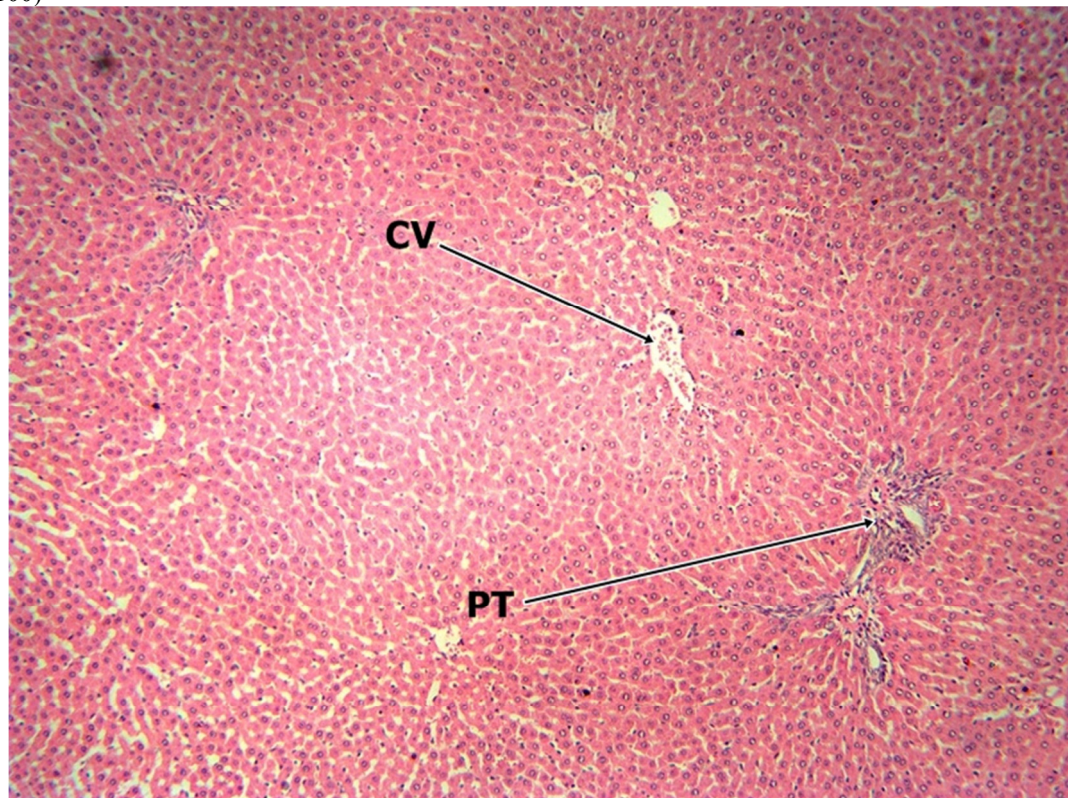


Plate 4 (1000mg of Starch). A section of liver showing a well preserved liver architecture. The portal triads are evenly spaced around a central vein and there is mild portal inflammation. There is no steatosis. Stained by H & E (X 100)

Cassava (*Manihot esculenta* Crantz) and its products have been widely studied and reported to have beneficial, although sometimes with toxic effects (Gomez *et al.*, 1988). Most of these effects are due to the presence of some non-nutrient factors in cassava. Cassava is a staple food in human diets in over 80 countries (Gomez, *et al.*, 1988). Starch a product from cassava tubers is one of the most popular staple foods of the people of the rain forest belt of West Africa and contains mainly starch-20% amylose and 70% amylopectin having lost the soluble carbohydrates (i.e. glucose and sugar) during processing. Cassava products are used as a primary staple food; careful processing to remove these toxic constituents is required to avoid chronic cyanide intoxication (Onabolu *et al.*, 2002).

Result from this study revealed that there was a significant decrease in the body weight at moderate and high dose when compared with control, the possible mechanism of action for this decrease in body weight is not clearly understood, it could be as a result of the presence of some anti-nutritional constituents that is present in the starch thus inhibiting neurotransmitters that are involved in appetite which then cause a weight reduction. This contradicts work done by (Eze *et al.*, 2009), in which there was no significant difference in the mean body weight, when fed with methanolic extracts of garri. This indicates that the oral doses administered had no effect on the growth of rats as significant changes in body weights have been used as an indicator of adverse effects of drugs and chemicals (Hilaly *et al.*, 2004). Nevertheless, the growth of an organism comprises many factors including physiological, biological and cellular processes (Goss, 1990).

Result also revealed that there was an insignificant increase in the weight of the liver while the pancreas had an insignificant decrease, however the mechanism of action is unclear, but could possibly result from the presence of cyanide which is a product of the hydrolysis of linamarin by β -glucosidase (Speijers, 2006). This corresponds with work done by (Eze *et al.*, 2009), in which there was no significant difference in the mean organ weight (heart, liver, spleen, kidneys and testis), when fed with chloroform and methanol extracts of garri.

Findings from the liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)) revealed that there was a significant increase when compared with control, since an increase in these enzymes activity signifies hepatocellular injury and thus acts as biomarkers for liver damage. The increase in AST, ALT and ALP could result from the presence of cyanogenic glycosides, coumarins, phenolic compounds (Tannin) which are toxic substances that is present in most cassava product which causes lipid peroxidation or oxidative stress in most tissue. These anti-nutrients are characterized by their diverse toxic effects (Fernandez *et al.*, 1997; Yu *et al.*, 1997). Study of the effects of hydrogen cyanide exposure in cassava workers, in which there was a significant increase in the AST and ALT level, as a result of the presence of hydrogen cyanide. This study also corresponds with work done by (Eze, *et al.*, 2009), in which there was a significant increase in AST and ALT level, when rats were fed with chloroform and methanol extracts of garri. This study contradicts work done by (Braide *et al.*, 2011), Effect of Cassava based diet on hepatic proteins in albino rats fed with crude oil contaminated diet, in which garri, caused a significant decrease in AST, ALP, and ALT level, through glucose effect

Findings from histopathological study of liver shows that there was mild, moderate portal inflammation seen in the hepatocytes when compared with control, this could be a result of the presence of foreign substances that are present in the starch which causes oxidative damage to the liver cells.

4. Conclusion

Findings from this study therefore indicated that aqueous extract of cassava starch caused hepatocellular injury and also an increase in liver enzymes.

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