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The Effects of Cassava Starch on the Pancreas of Adult Wistar Rats

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Abstract
Cassava starch, a staple food from the root of cassava (Manihot esculenta Crantz) has been found to contain some antinutrients (coumarins and cyanogenic glycosides) phytochemicals (tannin and flavonoids) which are toxic to the body. 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 histochemical studies. The pancreas 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 relative pancreas weight showed an insignificant decrease ($P>0.05$) in groups 2, 3 and 4 when compared with control. Result from blood glucose level showed that there was a significant increase ($P<0.05$) in group 3 & 4 when compared with control, while group 2 had an insignificant increase ($P>0.05$) when compared with control. Result from histology of the pancreas of the tested group revealed that there was no damage caused by cassava starch when compared with control. In conclusion, the aqueous extract of Starch was not toxic to the pancreas and also possesses hyperglycemic activity.
Keywords: Cassava, Starch, Wistar rats, Body weight

Introduction

All tissues in plants such as seeds, fruits, flowers and roots are edible and are regarded as the major source of food for humans and animals. This is because these tissues are reservoirs of the food nutri ents namely carbohydrate, proteins, fats, vitamins and minerals. Fruits and vegetables are rich in simple sugars, vitamins and minerals. The tuberous roots of cassava and yam are rich in carbohydrates. 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 (Hahn and Keyser, 1985).

Cassava is a major food crop in Nigeria (FAO, 2001) which is strategically valued for its role in food security, poverty alleviation and as a source of raw materials for agro‐allied industries in Nigeria with huge potential for the export market. The presence of cyanogenic glycosides constitutes a major limitation to the use of cassava in both human and animal foods. Cassava tubers are traditionally processed by a wide range of methods which reduce their toxicity and convert the perishable fresh root into stable products (Casereda *et al*., 1996). This toxic agent present in cassava is a factor that can affect the proper functioning of the body systems depending on the percentage concentration. Cyanide of dietary origin has been implicated in the etiology of various disease conditions (Tylleskar *et al.,* 1992; Rosling, 1987). Some abnormalities that border on pathological changes as well as metabolic integrity of the organism under repeated intakes of cassava with high cyanide content do occur. Known cases of acute cyanide poisoning from the consumption of cassava are rare, probably because of preparation process of cassava for consumption can destroy the linamarase and remove much of the free acid however, the possibility of chronic toxicity has not been eliminated (Osuntokun, 1970).

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. 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 (Nartey (1980). 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).

These antinutrients are characterised by diversity of biological (toxicological) effects including

carcinogenic, hepatotoxic, neurotoxic, immunotoxic effects; In addition they are used for their oestrogenic, dermatoxic, haemorrhagic, mutagenic, immunosuppressive and teratogenic properties (Fernandex *et al*., 1997; Yu *et al*., 1997).All varieties of cassava contain cyanide which exists both in the free form and incombination with glycosides, linamarin 2-(β-D glucopyranosyloxy) isobutyronitrile(>90% total cyanogen) and lotaustralin 2-(β-D-glycopyranosyloxy)-2methylbutyronitrile(<10% total cyanogens) (Ariffin *et al*., 1992; McMahon *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 as20 milligrams of cyanide (CN) per kilogramme of fresh roots, where as bitter ones may contain more than 50 times as much (1 g/kg) (Oke 1968).

Cassava 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) (Harborne, 1973; Tanaka *et al*., 1983). These coumarin compounds possess diverse biological and pharmacological properties such as anti-oedema, anti-inflammatory, antitumor, anticoagulant, immunostimulatory, anti- convulsant and hypotensive activity (Hoult and Paya, 1996; Aoife and Richard, 2004). Biochemically, they possess the ability to scavenge (quench) reactive oxygen species (free radicals), stimulate respiration ionopheretically; inhibit 5- and 12-lipooxygenases 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). It can also cause severe calcific pancreatitis in humans, leading to chronic pancreatitis. 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 (Nwachukwu and Edwards 1987). Studies have 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). 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; Abiye *et al*., 1998).

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

MATERIALS AND METHOD

Place of Study

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

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±2^{o}C. The animals were maintained with normal laboratory chow (Growers mesh) and water *ad libitium*; the animals were acclimatized for a period of two weeks and were kept on 12 hours light and dark cycle.

Preparation of Extract

Cassava was procured from Agricultural Farm in Nnobi, Okacha, Anambra State. 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.

AcuteToxicity

The median lethal dose (LD_{50}) of Cassava Starch was carried out in the Department of Physiology, Faculty of Basic Medical Science, Nnamdi Azikiwe University, Nnewi Campus. This was determined using the modified method of Dietrich Lorke (1983). The median lethal dose was found to be above 5000mg/kg.

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; served as control and receives only distilled water
Group 2; received 250mg/kg of Starch
Group 3; received 500mg/kg of Starch
Group 4; received 1000mg/kg of Starch

Collection of Blood Samples and Organ
Twenty four hours after the last administration the animals were anaesthized under diethyl ether. Blood for serum preparation was collected through cardiac puncture for histochemical studies. The pancreas was harvested and fixed in 10% formal saline for histological studies.

Analysis of Blood Glucose Level
Fasting blood glucose was determined using glucometer kit (accu-check) after over-night fasting for 7-11hours. The tail was punctured and blood from the tail was dropped on the strips which have been inserted into the glucometer to obtain blood glucose concentration in mmol/l for each rat in various groups.

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

Stastical Analysis
Data was analyzed using SPSS (Version 16) Software package. All result obtained were expressed as mean and 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 $P<0.05$.

Results
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

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

PANCREAS WEIGHT (g)	MEAN	±SEM	P-VALUE	F-VALUE
GROUP 1 (Control)	0.45	±0.00		
GROUP 2 (250mg of Starch)	0.37	±0.09	0.490	
GROUP 3 (500mg of Starch)	0.46	±0.13	0.368	0.586
GROUP 4 (1000mg of Starch)	0.47	±0.05	0.224	

Table 3: shows the effect of Cassava starch on blood glucose level after 28 days of treatment.

GLUCOSE (mmol/L)	MEAN	±SEM	P-VALUE
GROUP 1 (CONTROL)	1.19	±0.30	
GROUP 2 (250mg of Starch)	1.88	±0.35	0.369
GROUP 3 (500mg of Starch)	3.31	±0.94	0.012
GROUP 4 (1000mg of starch)	2.98	±0.60	0.032

Histopathological Findings

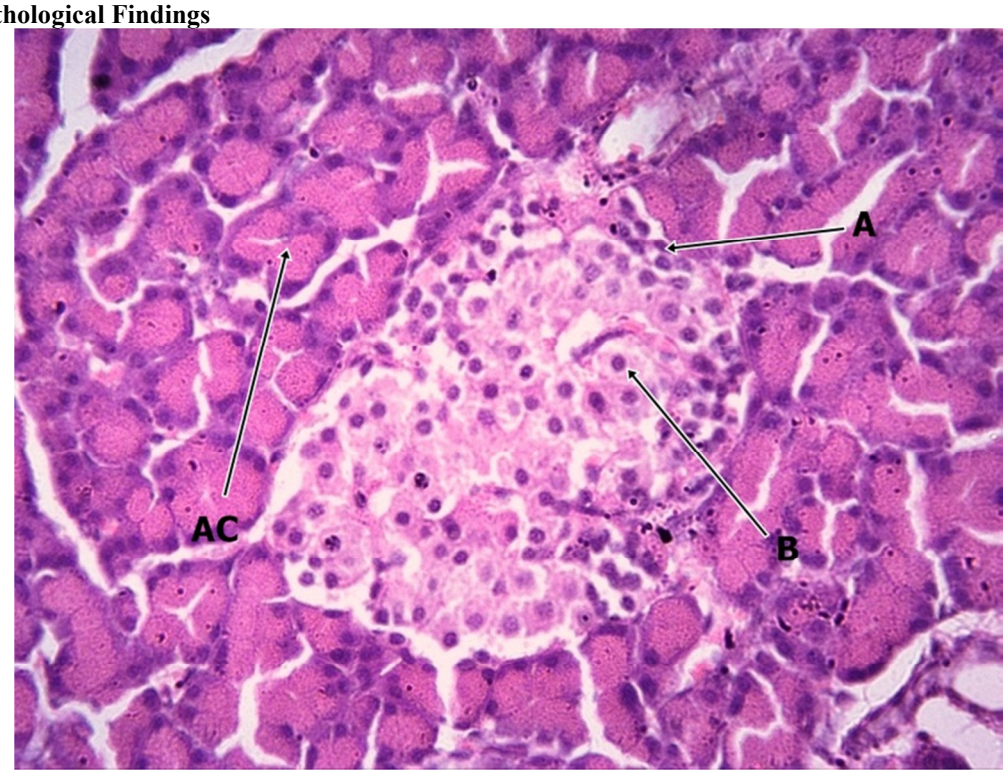

Plate 1 (CONTROL) Photomicrograph shows lobular arrangement of highly cellular glandular tissue composed of acinar cells, ductular structures and Islets of Langerhans. The Islets of Langerhans have peripheral arrangement of A cells and abundant B cells in the central area. The acinar cells contain abundant granular eosinophilic cytoplasm in the apical aspect with basally located nuclei. The interlobular ducts are surrounded by a variably thick rim of dense fibrous tissue. Stained by H & E (X 100)

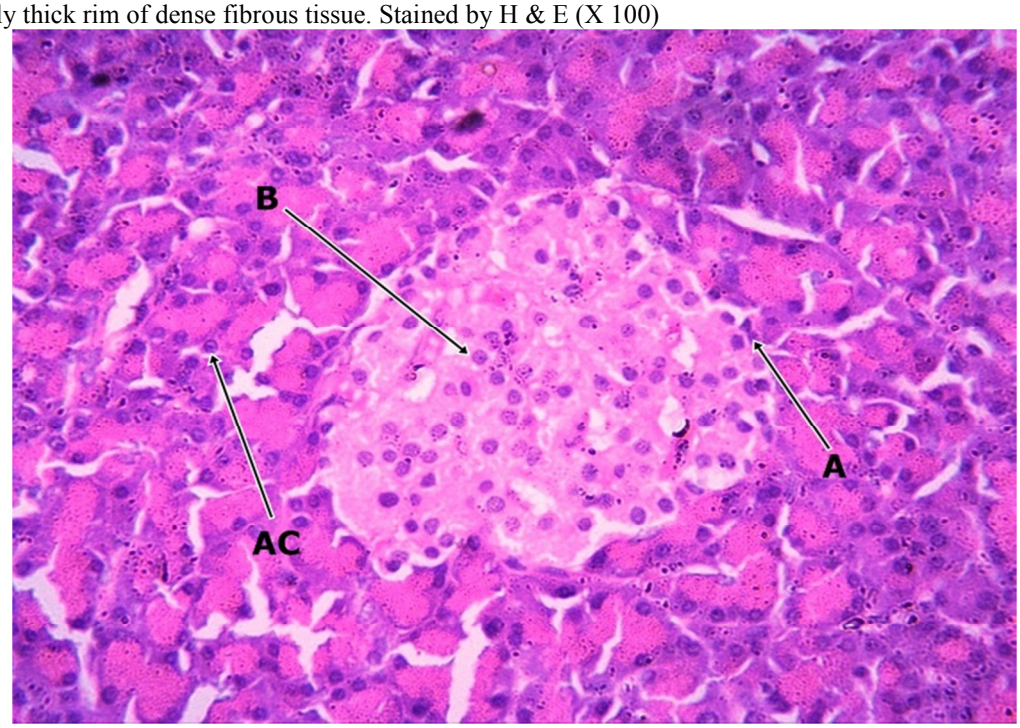

Plate 2 (250mg of Starch). A photomicrograph of pancreas showing lobular arrangement of highly cellular glandular tissue composed of acinar cells, ductular structures and Islets of Langerhans. The Islets of Langerhans have peripheral arrangement of A cells and abundant B cells in the central area. The acinar cells contain abundant granular eosinophilic cytoplasm in the apical aspect with basally located nuclei. The interlobular ducts are surrounded by a variably thick rim of dense fibrous tissue. Stained by H & E (X 100)

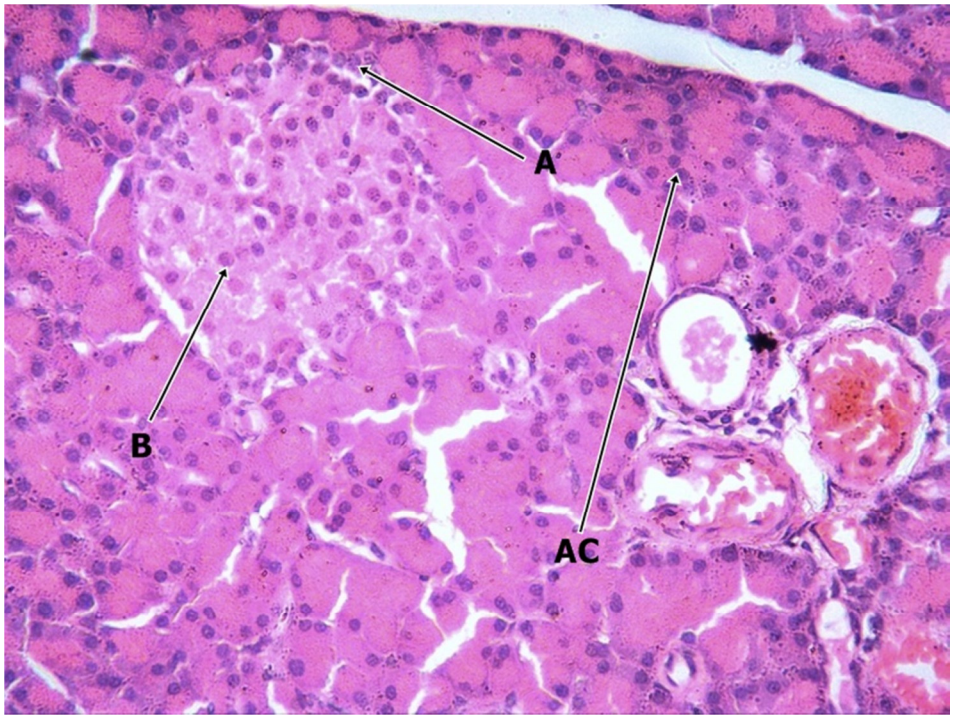

Plate 3 (500mg of Starch). A photomicrograph of pancreas showing a well lobular arrangement of highly cellular glandular tissue composed of acinar cells, ductular structures and Islets of Langerhans. The Islets of Langerhans have peripheral arrangement of A cells and abundant B cells in the central area. The acinar cells contain abundant granular eosinophilic cytoplasm in the apical aspect with basally located nuclei. The interlobular ducts are surrounded by a variably thick rim of dense fibrous tissue. Stained by H & E (X 100)

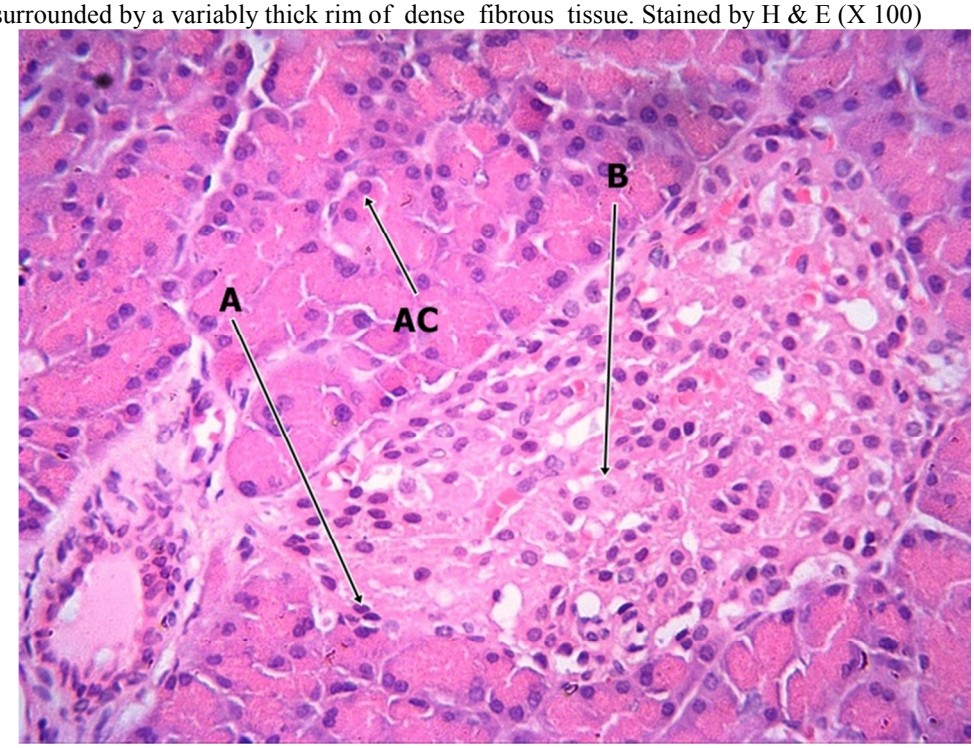

Plate 4 (1000mg of Starch). A photomicrograph of pancreas showing a well lobular arrangement of highly cellular glandular tissue composed of acinar cells, ductular structures and Islets of Langerhans. The Islets of Langerhans have peripheral arrangement of A cells and abundant B cells in the central area. The acinar cells contain abundant granular eosinophilic cytoplasm in the apical aspect with basally located nuclei. The interlobular ducts are surrounded by a variably thick rim of dense fibrous tissue. Stained by H & E (X 100)

Discussion

Cassava is a major food crop in Nigeria (FAO, 2001) which is strategically valued for its role in food security,

poverty alleviation and as a source of raw materials for agro allied industries in Nigeria with huge potential for the export market. The presence of cyanogenic glycosides constitutes a major limitation to the use of cassava in both human and animal foods. Cassava tubers are traditionally processed by a wide range of methods which reduce their toxicity, improve pal ability and convert the perishable fresh root into stable products. This toxic agent present in cassava is a factor that can affect the proper functioning of the body systems depending on the percentage concentration. Cyanide of dietary origin has been implicated in the etiologym of various disease conditions (Tylleskar *et al.,* 1992; Rosling, 1987).

Result from this study revealed that there was a significant decrease in the body weight at moderate and high dose when compared with the 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 chloroform and methanol 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 from this study revealed that there was a significant increase in blood glucose level in group 3 and 4 when compared with control; however the mechanism of action could result from the metabolism of starch in the liver through glycolysis and as a result lead to an increase in glucose level. This study agrees with work done by Yuji *et al.,* 2005 'Effect of Bread containing resistant starch on postprandial blood glucose levels in humans' who reported that there was an increase in the postprandial glucose level when compared with the border line group. This disagrees with work done by (Itam *et al.,* 2012) who reported there was a significant decrease in the glycemic index of Cassava flour starch when comparing between the diabetic and non-diabetics.

However result from histology of the pancreas, revealed that there was no damage cause by cassava starch when compared with control.

Conclusion

Findings from this study therefore indicate that aqueous extract of Starch were not toxic to the pancreas and also posses hyperglycemic activity.

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