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Behavioural Activity and Renal Functions from Toxicity Level of Bitter Cassava Induced-Konzo and Linamarine

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Cassava (Manihot Esculenta) is the most widely consumed starch-based food in Africa, where it thrives in the continent's tropical and subtropical temperatures.

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Aim: The purpose of this research was to better understand the impact of linamarine using a rat model of konzo.
Methods: Twenty-five (25) adults female Wistar rats were split into five (5) groups, with each group receiving the same amount of linamarin over the course of five weeks: group 1 received animal feed and water as a control, group 2 received bitter cassava and water, group 3 received 4.7 ml of linamarin, group 4 received 2.7 ml of linamarin, and group 5 received 0.7 ml of linamarin. The animals were killed so that samples could be tested in a lab. The time taken to go from the cage's entrance to each of its four corners was the metric tracked. Lack of movement, sluggishness, frequent urine and diarrhea, head sniffing and exploration, ears bent backward, puffiness, and teeth chattering were also seen.
Results: The data revealed that the bitterness of the cassava was significantly reduced in the linamarin group compared to the control group, and that the participants in the linamarin group gained weight. The group fed linamarin had much fewer crossings, shorter rearing times, and lower motivation than the other groups, the group fed bitter cassava remained on the rod for longer at the end of the test.
Conclusion: Research shows that consuming unprocessed bitter cassava results in renal impairment and that consuming pure linamarine, cassava extract induces hepatoxicity.

Keywords: Toxicity level; linamarine; konzo disease; cassava diet; renal function; degeneration.

1. INTRODUCTION

Linamarin, a cyanogenic glucoside found in the leaves and roots of many plants, is toxic to humans. All parts of the cassava plant contain the Val- and Ile-derived cyanogenic glucosides, linamarin and lotaustralin, in a ratio of around 97:3 [1]. It is a cyanohydrin glucoside attached to acetone. Plants high in linamarin need substantial processing and purification before they can be used as food. This is due to the fact that when exposed to enzymes and gut bacteria in the human intestine, linamarin and its methylated cousin lotaustralin may degrade to the toxic chemical hydrogen cyanide. Consumption of cassava products containing trace levels of linamarin is prevalent in low-lying tropical areas. Foods made from cassava plants toasted cassava tubers and cassava flour.

Specifically with konzo, an upper motor neuron illness initially detected in African communities by Trolli and subsequently examined by Hans Rosling's research network, high amounts of linamarin observed in foods prepared from incompletely processed cassava roots have been related to dietary toxicity [2]. However, it is believed that the toxicity is caused by the ingestion of acetone cyanohydrin, a breakdown product of linamarin [3].

Glucose intolerance and diabetes have been linked to dietary exposure to linamarin [4], but studies in experimental animals have shown mixed results, suggesting that the primary effect is in exacerbating preexisting conditions rather than inducing diabetes.

Enzymatic cyanide production from linamarin normally occurs upon exposure to linamarase, an enzyme often expressed in the cell walls of cassava plants. Foods made from cassava often undergo extensive blanching, boiling, or fermenting to remove the volatile cyanide chemicals that are created during these procedures.

Linamarin makes up more than 80% of the cyanogenic glucosides found in cassava with its associated enzymes ethyl methyl ketone cyanohydrin and acetone cyanohydrin ß-glucoside. When the enzyme and substrate are brought together, effective detoxification may take place.

Every kind of cassava contains cyanide. Toxic effects from free cyanide are well known such that the lethal dosage of cyanide is 1 mg/kg of live weight, hence the level of cyanide in cassava roots determines whether they are toxic or not. If the cyanide level of the root is more than this threshold, it is considered poisonous. Hydrocyanic acid concentrations in cassava roots have been reported in the literature to be between 15 and 400 ppm (mg CN/kg of fresh weight). The most common range, however, is between 30 and 150 ppm.

Cassava (Manihot esculenta) is an extremely important root crop grown all over the globe. It is possible to keep the starch-rich tubers in the soil

for long periods of time and harvest them asneeded, making cassava a crucial staple crop in Africa [5].

Cyanogenic glucosides and specific glucosidases that can break the glucosidic bond are compartmentalized in the apoplast, which is only one of several tissues or sub-cellular iicompartments in plants [6]. Cellular disturbance (from things like eating insects or food processing) triggers this two-part mechanism. After the -glucosidase has done its job, the resulting cyanohydrin dissociates into a toxic ketone.

The bulk of cyanogenic glucosides [7], are synthesized at the tip of the shoot. The tubers may accumulate up to 1.5 g kg1 dry weight of these substances once they have been introduced there [8,9]. There is a serious nutritional detriment caused by the presence of cyanogenic glucosides in cassava, which is a major problem in places of extreme poverty when it is the only available staple crop. Consumption of incompletely processed cassava-derived products, in addition to an imbalanced diet lacking in sulfur amino acids, may lead to chronic cyanide toxicity [10]. Tropical iiataxic neuropathy [11,12] and konzo are two potentially fatal disorders that might result from this.

Acute cyanide overdose inhibits cellular respiration by inactivating mitochondrial cytochrome aa3 oxidase [13]. Unfortunately, the iiloss iiof iiproteins, vitamins, and minerals that occurs during the careful processing of cassava tubers to remove the cyanide-generating components significantly reducesiithe inutritional value of this important crop [14].

Linamarin is an acetone cyanohydrin glucoside found in cassava, lima bean, and flax leaves and roots. It's thought to have a part in a plant's defense mechanism and in the transfer of nitrogen from the leaves to the roots of young plants. When the cells of cassava roots are broken, an enzyme called linamarase is released, and this enzyme combines with linamarin to produce hydrocyanic acid or prussic acid, both of which are toxic.

Linamarin and lotaustralin are the two cyanogenic glucosides, [15] and their roots and leaves have the highest levels of linamarin [16]. Linamarin is dangerous because it may form hydrogen cyanide, a very toxic toxin.

Cyanide is poisonous to higher organisms because it forms cyanohemoglobin when combined with iron. Higher plants and microorganisms are unable to produce adenosine triphosphate because cyanide prevents electrical transmission [17] by the interaction of cyanide with cytochrome oxidase is the cause of this disruption.

According to research conducted [18], the lethal dosageiis 1 mg/kg iiof body weight. Cassava roots are classified as either ipoisonous ior inon-toxic depending iion iithe iiconcentration iof iicyanideiinside the root. Therefore, the root is harmful if its cyanide content is over the median safe range. Values between 30 and 150 ppm of hydrocyanic acid are often seen in cassava roots, with reports spanning the whole range from 15 iito 400 ppm (mg iiCN/kg iiof iifresh iiweight).

The hydrolytic enzyme linamarase is still active and catalyzes the reaction that yields one molecule iiof glucose, one molecule of acetone, and one molecule of hydrocyanic acid after processing cassava roots, as reported [19]. Microorganisms that produce ß-hydrolytic enzymes may also hydrolyze glucosides and the optimal pH range for linamarase is 5.5 to 6.0.

When cyanide is released slowly, animals usually have a detoxifying mechanism that keeps them from dying. This mechanism is present in both monograstic swine (with a stomach pH of 3.0) and polygrastic bovines (with a stomach pH of 7.0) [20].

Microorganisms may prosper in cyanide-containing substrates because they are able to detoxify the cyanide by splittig the cyanide radical into carbon and nitrogen through anaerobic metabolism, alternative respiratory chain metabolism, and cyanide detoxification.

1.1 Aim and Objectives of the Study

The aim is to research the role and effects of linamarin on rats model of Konzo disease

Objectives include:

To use a rat model of konzo to test the effects of linamarin that can catch and spread konzo

Study the ameliorative effects of a protein-rich diet in a linamarin-induced rat model of konzo disease.

Ascertain the link between linamarin and kozo disorder.

Examine the detoxification process of linamarin toxicity level in animals.

2. METHODOLOGY

2.1 Research Design

25 adult female wistar rats ranging in size from 150 to 250 grams were gotten. Wistar rats underwent a week of acalamatization, during which time they were kept in a cage. There were five different animal groups, all of which were provided with rat diet and water. Five rats were utilized for Group 1 (the control group), and they were kept in a cage with food and water. In the second group, five rats were kept in a cage with water and bitter cassava. Five rats were confined in cage 3 and induced with a mixture of 4.7 cc of linamarin (cassava juice) and water. Five rats were confined in cage 4 and provoked with 2.3 milliliters of linamarin. In group 5, five rats were housed in a cage and given 0.7 milliliters of a stimulant called linamarin.

2.2 Plant Collection and Identification

From the Ministry of Agricultural Farm in Rumuodomaya Rivers State, we gathered roots and leaves from 419 different types of bitter cassava.

2.3 Processing of Bitter Cassava Root

Rumuodomaya Rivers State's Ministry of Agricultural Farm provided the fresh cassava roots that were collected early that morning. After thoroughly peeling off the roots' brownish outer covering, the white inner layer was cut into flakes and dried in the sun for a week. After being bundled up and pounded into a powder using a big motor machine, it was fed to the test animals as bitter cassava feed.

2.4 Rehabilitation Group

Four weeks after beginning cynaide treatment, all animals were given a protein supplement to help in the drug's detorfication.

2.5 Processing of Protein

After purchasing brown beans from the market, they were sent to a massive motorized grinding mill where they were reduced to a powder. Except for the control group, all of the animals in the study were fed this.

2.6 Processing of linamarin

The Ministry of Agriculture's Rumuodomaya farm in Rivers State supplied the University of Port Harcourt's Biomedical Laboratory in the School of Science Laboratory Technology with 419 specially cultivated cassava leaves. About 70 grams of stemless, freshly cut cassava leaves were added to 996.35 millimoles of Hydrochloric acid in a glass mortar. After everything was added together, it was pounded with a lab pistle. The clear supernatant liquid was collected with a pasteur pipette after centrifuging the dark green colored solution. The linamaraze solution was stored in a deep freezer at a temperature of 200 degrees Celsius.Experiments show that a linamarin solution may be frozen and kept for at least five months without deteriorating.

2.7 Statistical Analysis

SPSS version 23.0 was used to analyze the data. Both electronic and manual data processing were used, with the results tabulated for easy viewing. One-way ANOVA was used for mean comparison. The significance level for the 95% confidence interval between the two groups was set at P 0.05. The results are shown as the mean with a standard error bar.

Descriptive statistics were performed using the statistical package for the social sciences (IBM SPSS 23.0) and the business spreadsheet tool Microsoft Excel 2019.One-way analysis of variance (ANOVA) was used to examine the statistically significant differences between groups, and various controls were used for comparison.

2.8 Rotrod Test Procedure

2.8.1 Before testing

Acclimatization: The lab animals were placed in a testing room for 15 minutes before to the activity in order to reduce their anxiety. The wistar rat is introduced to the cage and allowed enough opportunity to investigate its surroundings. The timer shows that five minutes have elapsed since the task was finished.

The mouse is then put on the rotating rod by its tail and kept there while constantly facing in the opposite direction of rotation. It's a race against the clock to see how long the mouse can stay on its hind legs while the rat does a flip and collapses.

2.8.2 Pictures for behavioural test for the wistar rats

The tissue was sliced into tiny pieces using a razor blade.

The tissue was promptly fixed in formolaline to ensure its anatomical integrity was maintained.

To prevent cell damage, tissue was stored in escalating concentrations of alcohol: 30%, 70%, 90%, 95%, and 100%.

Xylene was injected into the tissue to make it see-through.

Tissue is submerged in a paraffin bath heated to 56-60^{0}C and covered with a layer of mottled paraffin wax.

Two tissue changes were made over a period of two hours.

Tissue was embedded in a mottled paraffin block of metal and allowed to cool, solidifying into a paper-thin layer of paraffin that could then be sectioned.

Microtome slices of tissue are very thin.

In order to straighten the tissue segment, it is submerged in a water bath with a humidity of 50%-55%.

After the water has been removed, the slide is placed in an incubator with the temperature set between 37% and 40%.

Fig. 1. Study Experiment 1

Fig. 2. Study experiment 2

Fig. 3. Rotarod test equipment

Fig. 4. Animals were allowed to rest for five minutes

Fig. 5. During testing in rotarod equipment

Fig. 6. During testing in rotarod equipment

Fig. 7. Open field test equipment n as possible after the animal dies to prevent any degradation

Fig. 8. During open field test equipment

2.9 Nissl Stain

Bring sections to water

Stain with cresylecht violet stain for 5 minutes (0.5% cresyl stain)

Rinse in 2 changes of distilled water

Rinse in ii95% alcohol solution for 30isecondds

Rinse in 100% alcohol solution for 30 seconds

Rinse in xylene fori1 minute

Rinse in balsam X xylene mixture fori2 minutes

Rinse in 2 changes of absolute alcohol for 10-30 seconds

Wash in 3-4 changes of xylene

Clean blot and covrship with synthetic resin medium

3. RESULTS

* Value is significant at p≤0.05 when compared to the control; Value significant at p≤0.05 when compared to GP2 (Konzo induced group)

According to the results there was weigh gain in group three (3) and four (4) linamarine group compare to group one (1) which is the control group. In group two (2) there was reduction of weight in animals that feed on bitter cassava.

Finding for the study therefore, indicate that from the extraction of linamarine, the use of active medium inactive endogenous enzymes and allow the direct hydrolysis of the glycosidic bond. Thus, group 3 is significant compare to group 1 the control group.

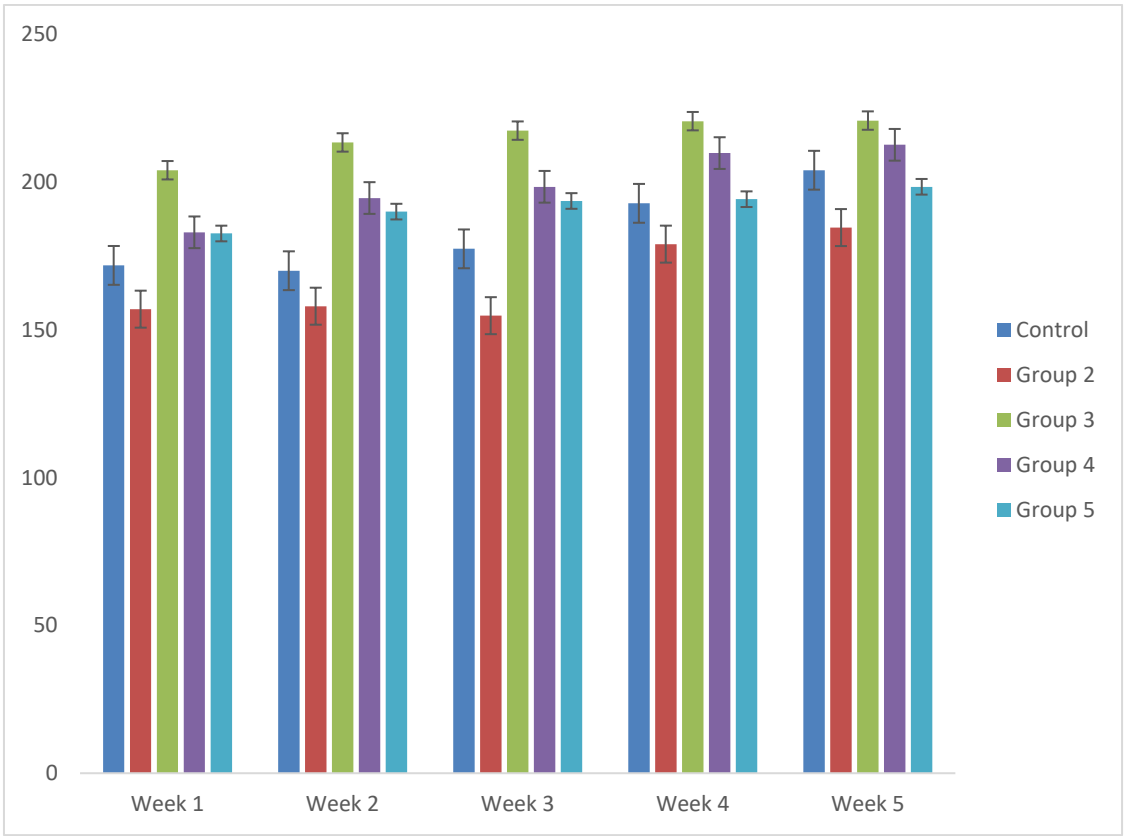

Fig. 9. Bar chart showing the effect of Linamarin on body weight of konzo induced Wistar rats

Legend
Group 1 - Control
Group 2 - Bitter cassava konzo induced
Group 3 - 4.7ml Linamarin (Cassava juice)
Group 4 - 2.3ml Linamarin induced group (cassava juice)
Group 5 - 0.7ml linamarin induced group (cassava juice)

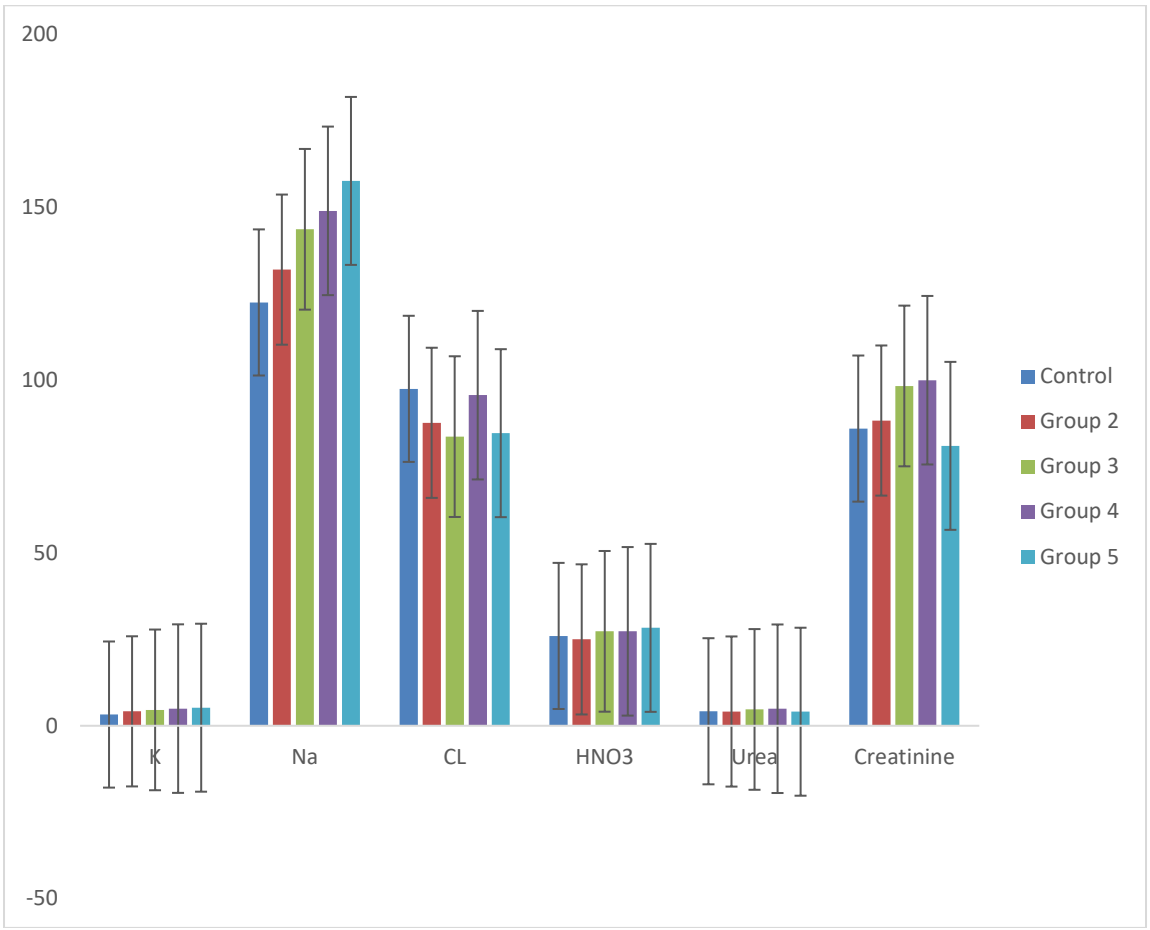

Fig. 10. Bar chart showing the effect of linamarin on electrolyte parameters in konzo induced wistar rats

Legend
Group 1 - Control
Group 2 - Bitter cassava konzo induced
Group 3 - 4.7ml Linamarin (Cassava juice)
Group 4 - 2.3ml Linamarin induced group (cassava juice)
Group 5 - 0.7ml linamarin induced group (cassava juice)

There was an increase in Sodium and chloride in group 2 and 3 compare to one group control. In group 4 there was an increase in sodium, potassium chloride when compared to group 1. In group 5, there was significant in sodium, potassium chloride when compared to group 1. There was an increase in sodium when compared to group 2.

3.1 Behavioural Exercise

3.1.1 Rotarod field test

According to the rotarod testresult, group 2 bitter cassava stayed longer on rod at the last trial, this may be due to toxicity of the hydrogen cyanide to bitter cassava. There was reduction in animal weight and shorter latency to fall from the rotarod in comparism to the other groups [21].

3.1.2 Open field test

According to the results of the behavioural exercise, the wistar rats were tested in the fourth week, the activities was carried out in the afternoon, there was a decrease in group in the number of crossings, time spent rearing, and motivation compare to the other groups. The parameter recorded was latency to move from the entrance of the cage to the four corners of the cage.I observe motionlessness, slow movement, urination and loose stools, sniffing and exploratory of head, backward turned ears, fluffiness and teeth chattering which tends to be successive.

4. DISCUSSION

The control group of Wistar rats in these tests weighed much more than the experimental group, which employed classically induced Konzo rats. This study found that during the course of the experiment's five weeks, the body weight of the animals under study fluctuated significantly on a daily basis as seen in Fig 9.

Results show weekly shifts in the animals' body weights as observed. The individual's weight decreased. Significant weight growth was seen in the experimental group of wistar rats compared to the control group of wistar rats.

Renal function may be estimated by measuring waste products eliminated by the kidneys, such as creatinine and urea, and the electrolyte concentration in plasma. It can typically determine whether a subject has a kidney problem by identifying them in a plasma sample. Five weeks (thirty-five) of treatment with bitter cassava juice (Linamarin) had a statistically significant impact on renal parameters in wistar rats compared to controls (group 1) at the p 0.05 level. In comparison to the control (group 1), which was given simply animal feed and water (not treated with linamarin), potassium showed a substantial increase across all groups treated with bitter cassava induced konzo and linamarin. Hyperkalaemia is a condition characterized by an unusually high blood potassium level. [22] reports that hyperkalemia raises the risk of heart disease, causes palpitations, and may even increase the chance of a heart attack among its patients. In comparison to the control group (group 1), the sodium levels in the groups given bitter cassava induced-konzo and Linamarin were shown to be significantly higher overall. Renal epithelial cells of hyperbatremia patients ingest more sodium across the apical membrane, leading to greater intracellular sodium concentrations. Consequently, there is an increase in sodium efflux across the basolateral membrane. This also accelerates the pace at which Na+-K+-ATPase is recycled. Na+-K+-ATPase in the basolateral membrane is significantly upregulated in response to chronically elevated sodium levels (hypernatremia), which leads to severe hypertrophy and hyperplasia. These changes to kidney shape and function have no effect on the blood levels of vasopressin and aldosterone. This finding is consistent with the findings of Damtie et al [23], who found that a rise in sodium levels produced a rapid diuretic response in healthy adults. The data also suggested a declining chloride concentration. Hypochloremia, an electrolyte imbalance, develops when the chloride concentration in the blood or other body fluids drops too low and stays there for an extended period of time. Metabolic alkalosis and hypochloremia are closely associated to increased sodium bicarbonate reabsorption in the proximal convoluted tubule under hypovolemic circumstances with high angiotensin II levels [24]. Group 2 was given bitter cassava induced-konzo, which resulted in a drop in bicarbonate, whereas all other linamarin-treated subjects showed an increase. The netabloic alkalosis indicates that the body's fluid has an abnormally high concentration of bicarbonate. Many different things might cause this to occur. The acid-base balance in the blood may be upset by gastrointestinal problems, such as recurrent vomiting. In addition, it may be precipitated by the side effects of liver, cardiac, or renal disorders.

The kidneys eliminate waste products such urea, uric acid, and creatinine in addition to metabolizing and excreting drugs and toxins [25]. Nitrogen waste products such urea nitrogen and creatinine tend to rise in the presence of acute renal impairment. Due to its dependence on protein intake and catabolism, urea excretion is attenuated when urine volume is reduced, leading to passive reabsorption and reduced urea excretion. The present study's hazardous dosage of cassava juice, together with other biochemical characteristics, may have contributed to the failure of the detoxification process in the linamarin-treated rats. The decline in glomerular filtration is further supported by these biochemical indicators. The inability of the test animals to remove potentially harmful compounds due to renal and liver damage caused by the administration of linamarin or cassava juice may account for the konzo illness and other neurological abnormalities found in this research. Urea and creatinine levels were found to be significantly higher in groups 3 and 4, and lower in groups 2 and 5. Ammonia is broken down into urea in the liver, which is then flushed out of the body in the urine through the urea cycle [26]. Urea levels change based on protein consumption and urinary excretion rate [25]. One of the consequences of renal dysfunction is urea retention, which lowers glomerular filtration. The retention of creatinine in the blood is an indicator of renal failure [24], despite the fact that creatinine is a waste product of muscle tissue produced during the metabolism of creatine.

The rotarod test results indicated that the bitter cassava in Group 2 lingered on the rod for a longer period of time than in the previous experiment, which may be attributable to the toxicity of the hydrogen cyanide to the bitter cassava. Animals lost weight and learned to tumble off the rotarod faster than in the other groups. In accordance with the findings of Enefa et al [19]. In the open field test, findings from the behavioral exercise showed that fourth-week Wistar rats showed less desire, spent less time raising their young, and made fewer crossings than rats in the other groups because they were tired from the day's activities. The time taken to go from the cage's entrance to each of its four corners was the metric tracked. There was observation of inactivity, sluggish movement, frequent urine and diarrhea, head sniffing and exploration, ears cocked behind the head, a fluffy appearance, and teeth chattering. Consistent with the findings [26], the events often occur in quick succession. The results of this research, which used the wistar rat as a model, thereby provide more evidence for the effects of bitter cassava induced-konzo and Linamarin on renal functioning.

5. CONCLUSION

This research demonstrates that consuming unprocessed bitter cassava results in renal impairment and that consuming pure linamarine, cassava extract induces hepatoxicity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The ethical clearance letter was signed off on by the University of Port Harcourt's Research Ethics Committee.Animals were housed inside in regular animal cages at the basic medical sciences department.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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