Chronic cassava meal modulates body weight, histology and weight of reproductive organs in male albino rats

Olisemeke U. Egbune[1,*], Egoamaka O. Egbune[2], Osuvwe C. Orororo[3], Theresa Ezedom[3], Ogheneyoma Onojakpor[4], Ahmed M. Sabo[1], Kemakolam Amadi[1]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, University of Jos, Jos, Nigeria

[2] Department of Biochemistry, Faculty of Science, Delta State University, P.M.B. 1, Abraka, Nigeria

[3] Department of Chemical Sciences, Faculty of Science, Edwin Clark University, Kiagbodo, Delta State, Nigeria

[4] Department of Consumer and Food Sciences, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa

*Correspondence to Egoamaka O. Egbune. Email: egbuneegoamaka@gmail.com

Abstract

Objective: This study evaluated the effect of chronic cassava meals on some reproductive parameters of male albino rats.

Methods: Twenty-four sexually mature albino rats were divided into four groups which received oral treatments from a variety of cassava products containing cyanogenic glycosides in varying quantities: Group A—Control (received 300 g normal rat feeds); Group B (received 50 g red garri at a concentration of 150 ppm cyanogenic glycosides/rat/day); Group C (received 50 g white garri at a concentration of 200 ppm cyanogenic glycosides/rat/day); and Group D (received 50 g raw cassava at a concentration of 400 ppm cyanogenic glycosides/rat/day). After 60 days of treatment, male reproductive organs were harvested from the rats for histological examination. Also noted were the weights of the body and the organs.

Results: The weight of the body and reproductive organs significantly changed in group D after receiving raw cassava at a dose of 400 ppm/rat/day. Compared to the group treated with red garri at 100 ppm/rat/day cyanogenic glycosides, which showed no significant changes in body weight gain or the weight of the reproductive organs, the white garri group showed substantial changes in the testis and prostate weight. The group given 150 ppm/rat/day of red garri did not exhibit any changes in histology, but the groups given 150 ppm/rat/day of white garri and 400 ppm/rat/day of raw cassava displayed testicular atrophy, degeneration, vacuolation, decreased secretion, and desquamation of glandular epithelium in the prostate.

Conclusions: This study has revealed that the concentration of cyanogenic glycosides is higher in raw cassava and white garri than red garri, and that 400 ppm/day of cyanogenic glycosides may have negative impacts on their ability to reproduce.

Keywords: Cyanide; Garri; Cassava; Reproductive organs

Introduction

Cassava (*Manihot esculenta* Crantz) is a member of the Euphorbiaceae family. It is farmed in various regions of the world including Asia, Africa and Latin America. Due to its unique nutritional prowess, it is widely utilized for food and industrial purposes [1, 2]. Mombo et al. [3] and De Moura et al. [4] have estimated the consumption of cassava in America and in Africa to be 105 ± 330 g/day and 940 ± 777 g/day, respectively. Cassava products such as garri, starch and *fufu* are consumed in large quantities daily in the tropics by people of all social-economic status, religion and ethnic groups [5, 6]. There are two types of cassava—sweet and bitter, with the bitter variety used in making garri, being more common [7]. Garri is classified as white or red due to the presence or absence of palm oil [8].

One factor that limits the utilization of cassava as food is the fact that it contains toxic compounds known as cyanogenic glycosides such as linamarin and lotaustralin. When these compounds are broken down within cells, they yield acetone cyanohydrins, cyanide and other products which have been shown to be toxic to cells [8, 10]. Cyanide has both acute and chronic toxic effects arising from its ability to restrain cellular respiration and its negative effects on metalloenzymes [11, 12]. According to Montagnac et al. [13], the cyanide content of cassava leaves is between 53 and 1300 mg, while cassava roots contain between 10 and 500 mg cyanide equivalents/kg of dry matter/kg. To reduce the cyanide content of cassava, different processing/detoxification methods are usually applied, such as fermentation and baking [6, 14].

Cyanogenic glycosides can be detoxified using the right processing methods, which lowers the danger of cyanide poisoning. However, improper processing of the cassava can result in acetone cyanohydrin being in a state where the cyanide molecules are allowed to roam freely as radicals and mix with other substances in the body to form the substance that causes acute cyanogenic intoxication. Numerous health issues have been linked to extend eating of improperly processed bitter cassava cuisine, which results in a sustained high cyanogens intake [15]. Increased blood cyanide levels may cause oxidative stress (OS) as a result of mitochondrial malfunction [16].

It has been suggested that long-term ingestion of cassava in humans may lead to neurological conditions including tropical ataxic neuropathy (TAN) and Konzo, which are characterized by motor dysfunction and cognitive failure [17]. According to Udeme et al. [18], rats' levels of aspartate aminotransaminase, glucose, alanine aminotransaminase, and alkaline phosphatase were significantly raised after consuming cassava products. In addition to these negative consequences, cyanide poisoning has also been linked to histological changes in the thyroid gland [19], reproductive toxicity in male dogs [20], evidence of cell death (necrosis) in an in vitro study [21], negative effects on motor activity, kidney, and liver function in Wistar rats [22 23]. In addition, Paulinus and Obaika [24] found that after cyanide exposure, there was an increase in serum lactate dehydrogenase levels, indicating a switch from aerobic to anaerobic metabolism leading to lactic acidosis.

Infertility is a serious health challenge in the world today affecting about 15% of all couples trying to conceive [25]. Of this figure, male infertility accounts for about 50% of the cases yet no identifiable cause can be found in over 20% of infertile males [26]. It has been shown that the male reproductive system is extremely sensitive to many chemicals and different food substances consumed on a daily basis [27,28,29]. In this light, chronic consumption of cassava meals could be linked to infertility in males either on a long- or short-term basis. Studies evaluating the effect of chronic consumption of cassava meals on the male reproductive system

are limited; hence, this study evaluated the effect of chronic cassava meals on some reproductive parameters of male albino rats.

Results

The result of the phytochemical screening of the garri samples and raw cassava is shown in Table 1. Cardiac glycosides were observed more in the raw cassava than in either white or red garri. Alkaloids were not found the white garri, but were seen in high concentration in the raw cassava.

Table 1. Phytochemical screening of various cassava samples (100 g)

Constituent	White	Red	Raw cassava
	4	5	6
Cardiac Glycosides	+	+	+++
Alkaloids	–	+	+++
Tannins	–	–	
Saponins	–	–	–
Flavonoids	+	–	+
Carbohydrates	++	++	++
Steroids	+	+	++
Anthraquinones	–	–	–
Terpenoids	–	–	–
Cyanogenic glycosides	++	++	+++
Quantity of Cyanogenic glycosides (in ppm)	400	300	800

The effect of various cassava meals on body weight gain of experimental animals is presented in Table 2. Rats in the control group had the highest body weight gain. Body weight gain of rats administered 150 and 200 ppm/day Cyanogenic Glycoside (Groups B and C) was not significantly different, but was lowered that the control group. However, rats administered 400 ppm cyanogenic glycosides/ rat/day (Group D) have a significantly lower ($p < 0.05$) body weight gain compared to control.

Table 2. Effect of various cassava meals on body weight

Group	Treatment	Weight before (g)	Weight after (g)	Weight gain (g)
A	Control	171.33 ± 14.31	233.34 ± 17.45	75.67 ± 23.64^{a}
B	Red garri	190.35 ± 22.16^{a}	249.18 ± 23.75^{a}	50.42 ± 17.11^{b}
C	White garri	182.60 ± 13.43^{a}	240.72 ± 30.90^{a}	49.81 ± 18.84^{b}
D	Raw cassava	157.33 ± 10.23^{b}	190.13 ± 10.59^{b}	32.40 ± 6.38^{c}

Values are expressed as mean ± SEM, ($n=6$) bearing different letters in column differ significantly ($p<0.05$)

The effect of various cassava meals on reproductive organ weight is shown in Table 3. The weight of the testis, epididymis and prostate was significantly decreased ($p < 0.05$) in rats treated with 400 ppm cyanogenic glycosides present in raw cassava compared to the control group, but group B and C did not show any significant difference ($p > 0.05$) when compared to the control group.

Table 3. Effect of various cassava meals on reproductive organ weight

Group	Treatment	Testes (g)	Epididymis (g)	Prostate (g)
A	Control	1.50 ± 0.90	0.38 ± 0.3	0.54 ± 0.2
B	Red garri	1.46 ± 0.06^{a}	0.36 ± 0.2^{a}	0.48 ± 0.3^{a}
C	White garri	1.30 ± 0.05^{a}	0.34 ± 0.2^{a}	0.46 ± 0.3^{a}
D	Raw cassava	1.35 ± 0.02^{b}	0.20 ± 0.04^{b}	0.34 ± 0.01^{b}

Values are expressed as mean ± SEM, (n = 6) bearing different letters in column differ significantly ($p < 0.05$)

Histopathological studies

Testis

In the control group, normal testis histology with regular seminiferous tubules and spermatogenic cell lines with abundance of spermatids in the seminiferous tubules were observed (Fig. 1). Group B treated with red garri at 150 ppm/rat/day showed no changes in the histological architecture of testis compared to the control (Fig. 2).

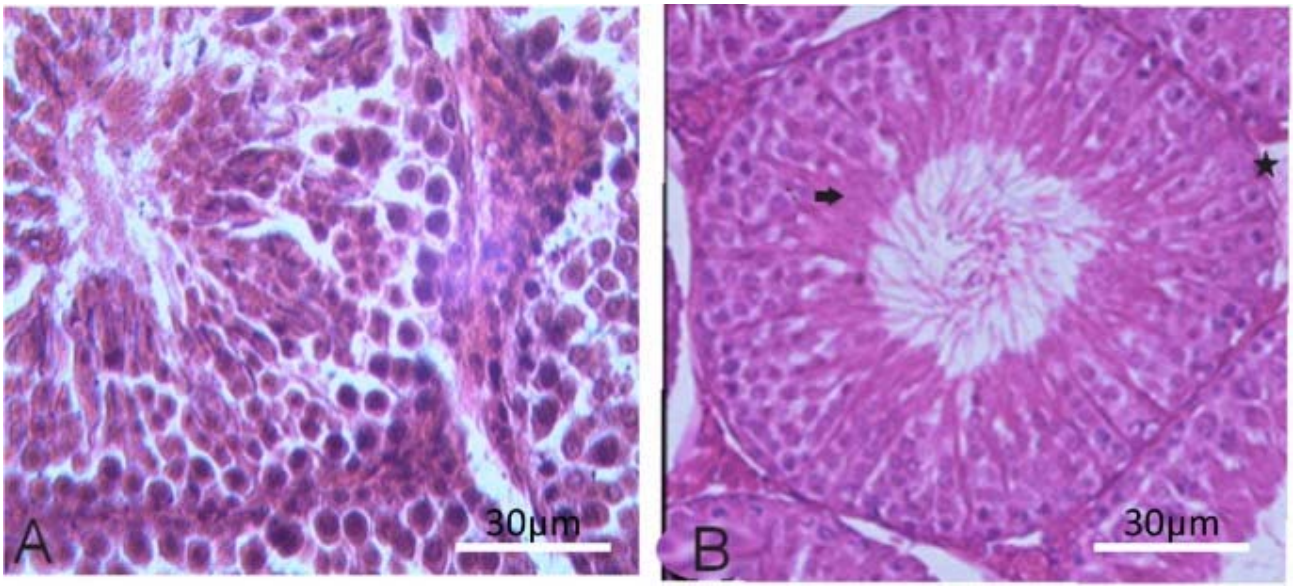

Fig. 1. Photomicrograph of the Testis of the Control Group, showing seminiferous Tubules (→), Interstitial Tissue (★)

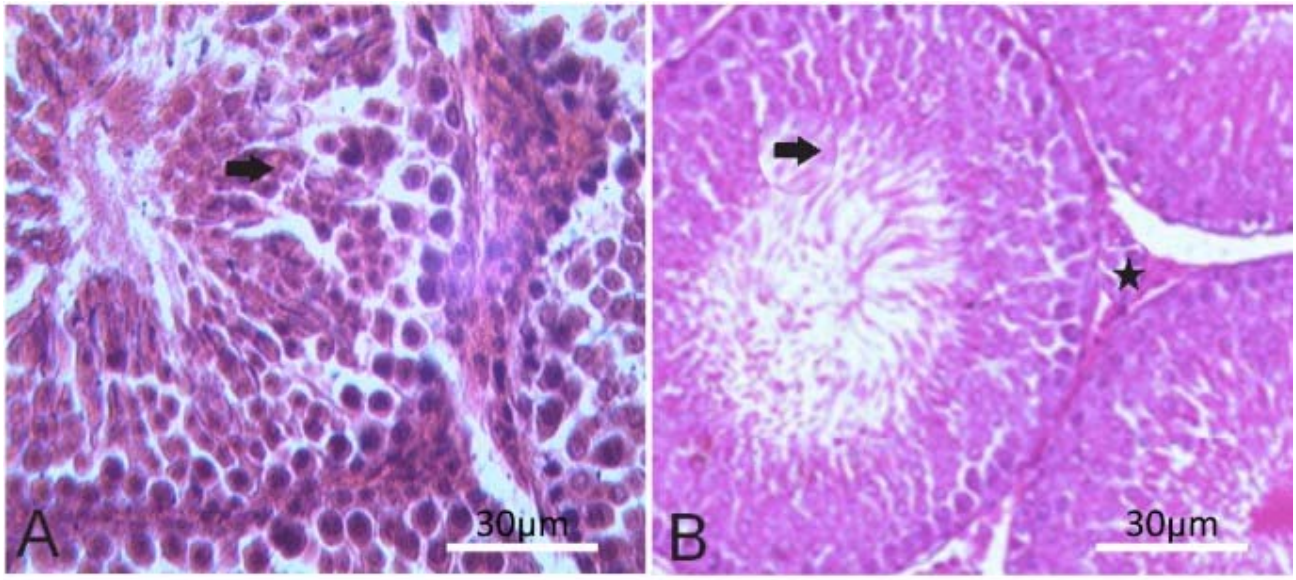

Fig. 2. Photomicrograph of the Testis of Group B, treated with 50 g/day/rat red garri showing seminiferous tubules (➞), Interstitial Tissue (★) as seen on Control Group

Group C and D treated with white garri at 200 ppm/rat/day and raw cassava at 400 ppm/rat/day showed histological alternation including atrophy and degenerated seminiferous tubules; also, there was a than population of spermatogenic cells, spermatocytes spermatids and spermatozoa in the tubules (Fig. 3a, b). This histological alteration was more prominent in group D compared to group C.

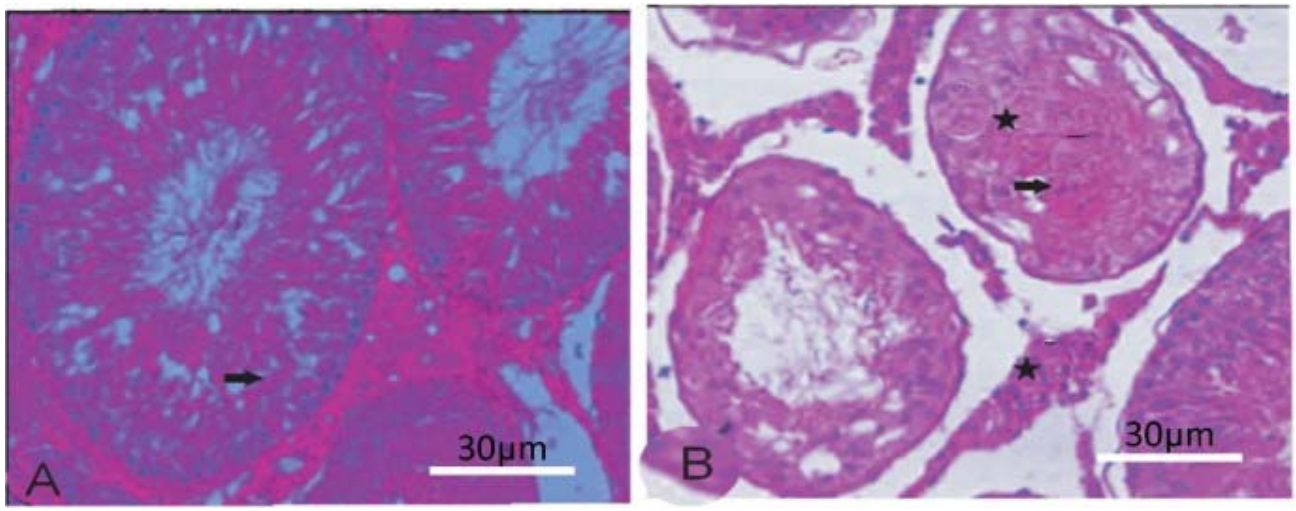

Fig. 3. Photomicrograph of the Testis (A) Groups C, Treated with 50 g/day/rat White garri showing Vacuole (➞) in the germinal epithelial layers; (B) group D treated with 50 g/rat/day raw cassava showing vacuole (➞) atrophy degenerated seminiferous tubules (★)

Epididymis

In group A (control group), epididymal histology with luminal cell lines with abundant number of sperm was observed (Fig. 4), and there was no observable difference in groups B (red garri at 150 ppm/day) when compared to the control. In the groups C and D, there was low sperm density compared to the control groups, and increase in the number of clearing cells with a

vacuolation in luminar cell layer was observed. These alterations were more pronounced in group D than group C (Figs. 5, 6).

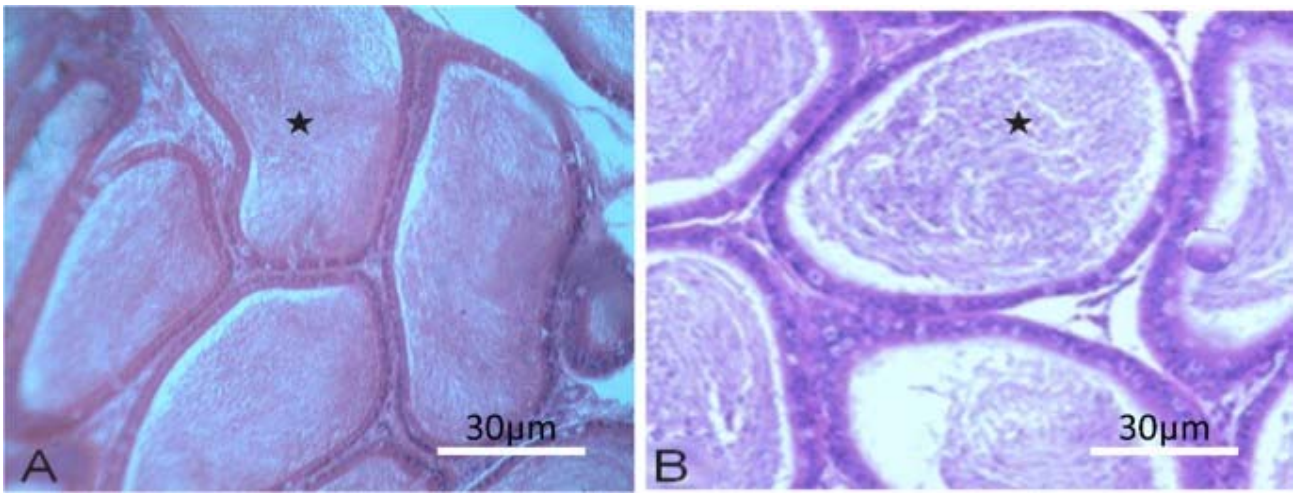

Fig. 4. Photomicrograph of the Epididymis (A) shows normal Epididymis of the Control Group with bulk of sperm (★)

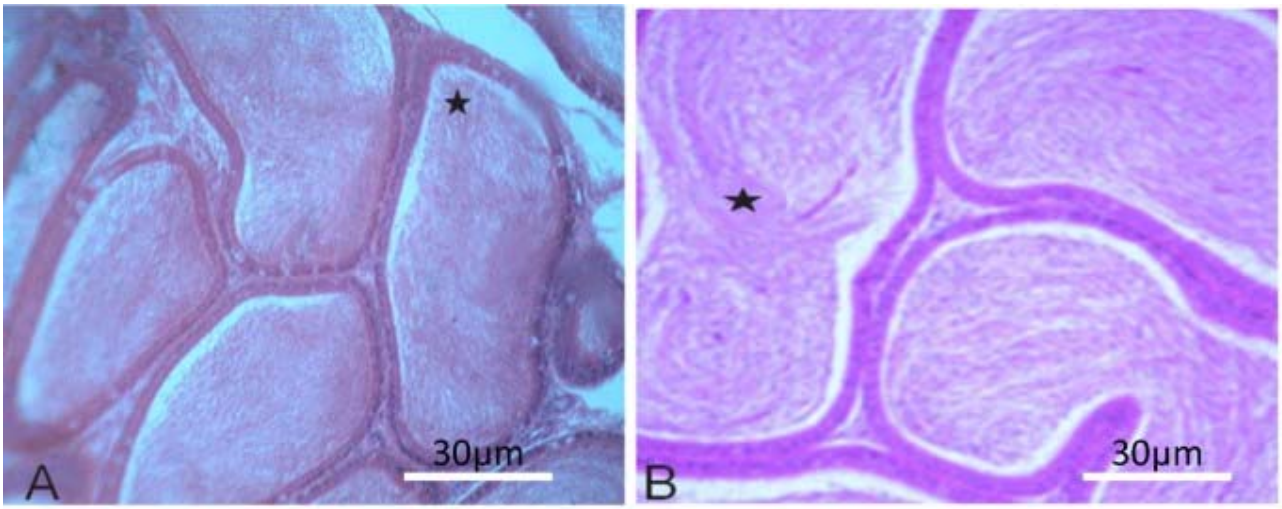

Fig. 5. Photomicrograph of the Epididymis (A) shows normal Epididymis of the Group B treated with 50 g/rat/day with bulk of sperm (★) as seen in Control Group

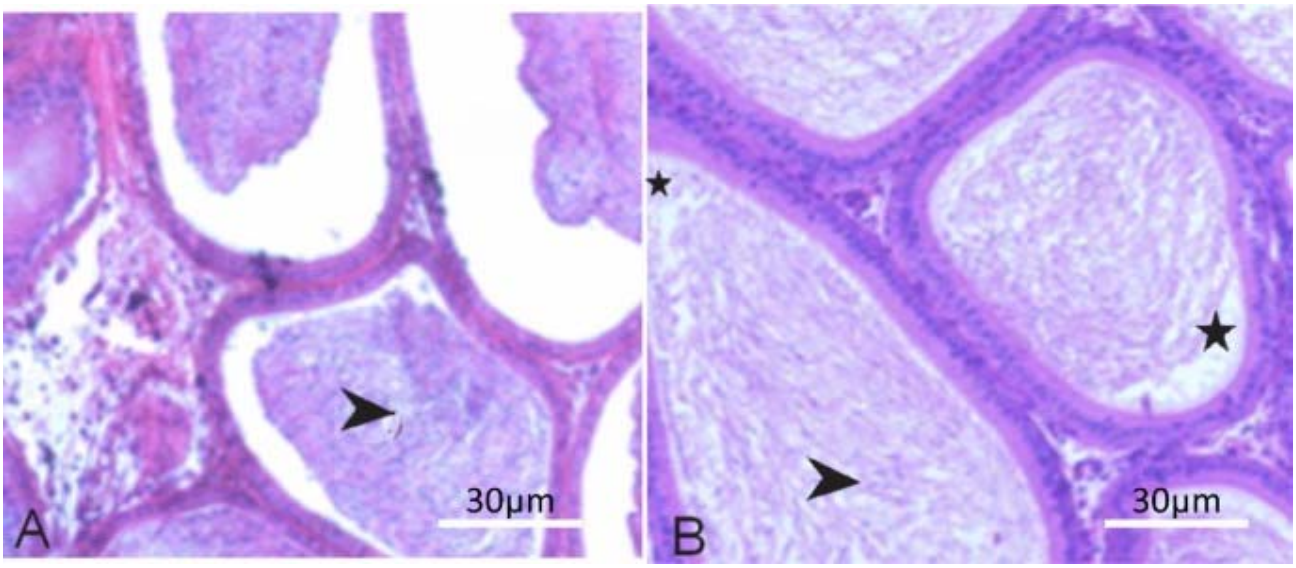

Fig. 6. Photomicrograph of the Epididymis (A) Group C Treated with White garri Showing Low Density of Sperm (★) (B) group D Treated with 50 g/rat/day Raw Cassava showing Low Density of Sperm and vacuoles in the Germinal Cell Lining

Prostate gland

Ample amounts of prostate secretion were seen along with a normal prostate histology and luminal cell lining (Fig. 7). The histological architecture in group B was normal (Fig. 8) White garri was given to groups C and D at doses of 200 ppm/rat/day and 400 ppm/rat/day, respectively. This resulted in less secretion in the lumen (Fig. 9a), and group D's glandular epithelium showed desquamations (Fig. 9b).

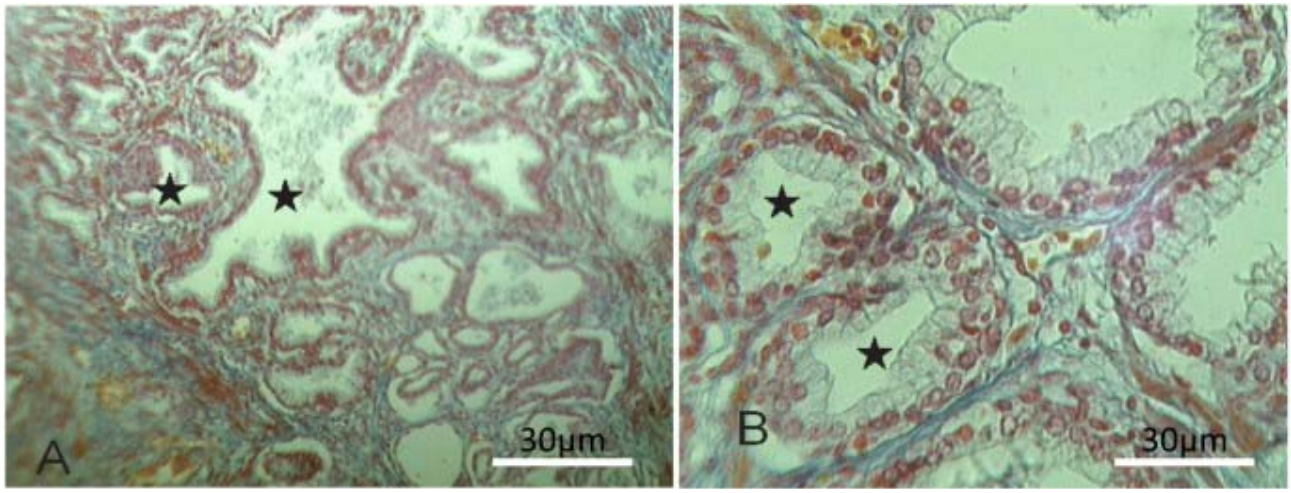

Fig. 7. Photomicrograph of the Prostate Gland; (A) Normal Histology of the Control Group with Prostate Secretion (★)

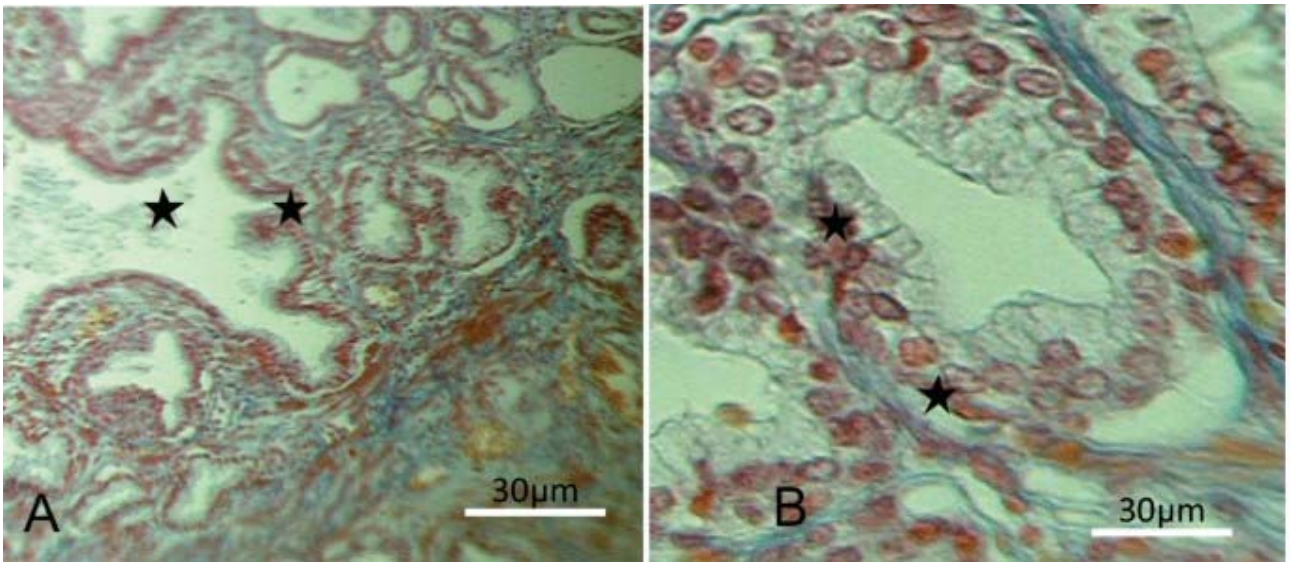

Fig. 8. Photomicrograph of the Prostate Gland; (A) histology of the Prostate Gland of Group B Treated with 50 g/day/rat red garri showing normal prostate Histology with Bulk of Prostate Secretion as seen in the Control Group (★)

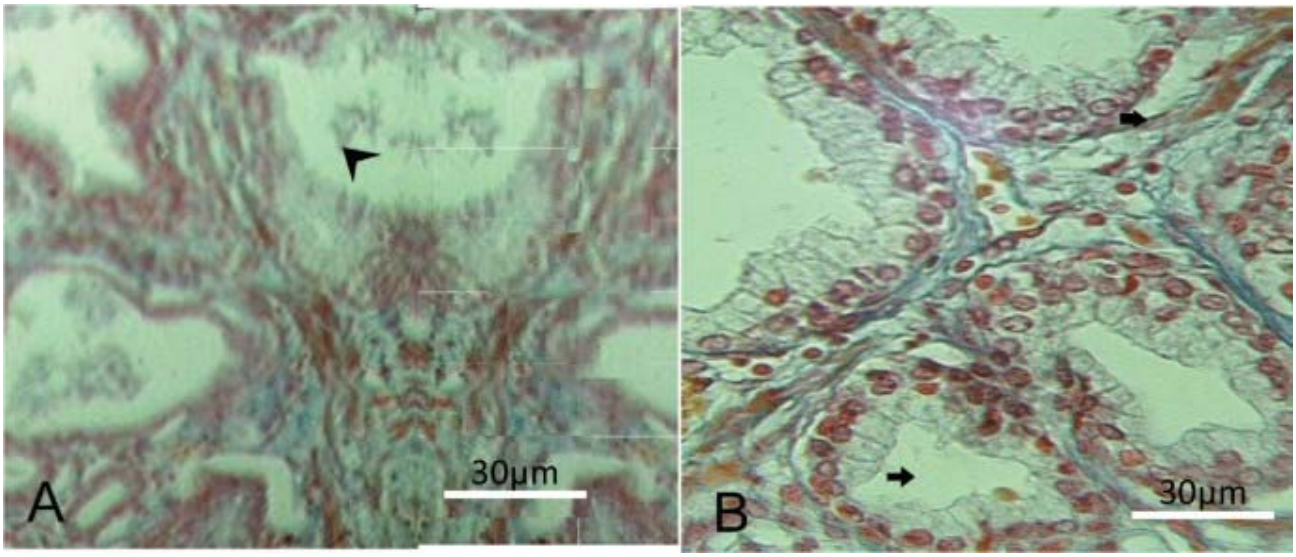

Fig. 9. Photomicrograph of the Prostate gland; (**a**) Histology of the Prostate Gland Group (C) Treated with 50 g/rat/day White garri Showing Low Prostate Secretion (➤). (**b**) the prostate Histology of Group D Treated with 50 g/rat/day raw Cassava Showing Low Prostate secretion (➤), Desquamation of Glandular Epithelium (→)

Discussion

Given the reported high toxic effects of cyanogenic glycosides, this study evaluated the effect of chronic cassava meals on some reproductive parameters of male albino rats. Phytochemical screening of the cassava/garri used in this study revealed the presence of carbohydrates and other phytochemicals which is in consonance with previous reports. In West Africa, cassava starch and garri, a processed product, are crucial food components that make up a significant amount of daily caloric intake [31]. According to Oghobase et al. [32], with a carbohydrate output that is around 40% and 25% greater than rice and maize, respectively, cassava ranks

very high among crops that convert the highest amount of solar energy into soluble carbohydrates per unit of land. Due to its high carbohydrate content, cassava is considered an important edible tuber [33, 34].

Over the course of the treatment, body weight increased in each of the experimental groups. However, compared to the other treatment groups, rats given 400 ppm cyanogenic glycosides/rat/day saw a considerable drop in body weight (Table 2). This may be because of the harmful effects of cyanide. According to Rivadeneyra-Domnguez et al. [22], cyanide, a potent inhibitor of the electron transport chain, encourages oxidative stress. This outcome is consistent with studies by Maliki et al. [23], which found that including cassava in the diet had a favorable impact on rats and caused them to gain more body weight. Incorporating cassava into rat diets can enhance feed intake, which in turn increases the weight of treated animals [35, 36, 66]. Indeed, cassava has a higher nutritional value than sweet potatoes, with 112 cal per 100 g [13]. It is a crucial crop for developing nations because to its capacity to supply high calories. The findings of this study contrast with those of Morgan and Choct [37] and Rosas-Jarqun et al. [38], who reported that chronic ingestion of cassava had no impact on rats' body weight during a 35-day period. While it is currently impossible to justify the discrepancy between our observations of high weight gain and the reduction in weight gain reported by others [15, 39,40,41], it may be caused by the dosage of the cassava administered, the species of animals, the geographic effect on different cassava samples, processing methods and procedures, or other unknown factors [42].

The body's inability to metabolize extremely large levels of cyanide results in acute cyanide poisoning [43, 44]. In humans, cyanide and the ferric ion in mitochondrial cytochrome oxidase interact to block electron transport in the cytochrome system and stop the synthesis of ATP and oxidative phosphorylation. Anaerobic glycolysis is put under more stress due to the suppression of oxidative metabolism, which leads to the generation of lactic acid and the possibility of a serious acid–base imbalance. Enefa et al. [15] claim that continuous ingestion of incompletely processed cassava roots containing cyanogenic glycosides can expose people to cyanide, which can result in neurological diseases, weight loss, growth retardation, and even death (CNS).

The findings of this study suggested that red garri might not be harmful to albino rats' ability to reproduce at 150 ppm/day of cyanogenic glycosides. This could be attributed to the detoxification process that uses the mitochondrial enzyme rhodanese, which catalyzes the reaction between CN^- and thiosulphate to form SCN^- [45, 46]. According to Wrobel et al. [47], sulfur atoms are converted to CN-, which is then expelled over a number of days and is less harmful than CN^-. Red garri's low concentration of cyanogenic glycosides enables the detoxification process because bigger quantities are too high for the liver's rhodanese system to effectively detoxify [48, 49]. Red garri's palm oil content may chelate the CN^- present, reducing its toxicity.

The results of this study also demonstrated that rats given daily doses of 400 ppm raw cassava and 200 ppm white garri induced histological abnormalities in the testes, including atrophy and deteriorated seminiferous tubules (Plate 1c). It has been observed that 14 weeks of exposure to a cyanide-containing cassava diet in dogs causes testicular degeneration and liver lesions [20]. Oxidative stress and anaerobic cellular respiration might also be blamed for these histopathological changes [50, 51]. According to Rivadeneyra-Domnguez et al. [22], cyanide, a potent inhibitor of the electron transport chain, encourages oxidative stress. Cyanide generates oxidative stress in the numerous functional tissues of rats, according to earlier studies [45, 52].

The epididymal alterations entail the insertion of new proteins and the modification of old proteins by cell-clearing [53, 54]. These epididymal modifications produce an environment that is favorable for spermatozoa to mature [55, 56]. Because sperm motility is crucial for fertilization, anything that affects motility will inevitably impair fertilization [57, 58]. Previous investigations have demonstrated that ATP affects sperm motility [59, 60]. Cyanide may reduce the production of ATP [61, 62]. The ATP energy pool may have undergone changes as indicated by previous studies [63,64,65, 67]. It is hypothesized that the lower prostate output observed in groups C and D (Plate 3C) could be attributed to reduced serum testosterone levels. It is plausible that the desquamation observed was a result of the decrease in serum testosterone levels.

Materials and methods

Collection of cassava and garri samples

Commercial garri samples and raw cassava were randomly collected from six market in Obiaruku, Ukwuani Local Government Area of Delta State (5.8387° N, 6.1580° E) and were kept in tightly sealed envelopes in field cellophane bags before being used for analysis. 100 g of each sample was used for analysis.

Processing of raw cassava root and garri

To reveal the white inner layer, the brownish peel (skin or cortex) was cut away from the raw cassava. The roots were then chopped into chip-sized pieces and left to sundry for three days in a row. The dry cassava pieces were manually ground into a powdery consistency using a grinding device before being fed to the test rats as a combination of regular rat food and cassava chow. The rats received red and white garri in addition to their regular rat food.

Phytochemical screening

Qualitative phytochemical screening for saponins, steroids, tannins, alkaloids, anthraquinone, flavonoids, anthocyanins, cardiac glycoside, and triterpene in the extract were done following standard procedures as described by Harborne [30].

Determination of cyanogenic glycosides

The Association of Official Analytic Chemist (2010) method as described by Maliki et al. [23] was used. In a nutshell, 2 ml of orthophosphoric acid and 40 ml of distilled water were used to soak 4 g of material. To release all bound hydrocyanide acid, the mixture was completely steroid halted and left overnight at room temperature. The final sample was deposited into the distillation flask and broken chips and a drop of paraffin wax (anti-foam 1 mg agent) was added (antibump).

Following filling with other distillation equipment, the distillation flask was used. The receiving flask was then filled with 0.1 g of sodium hydroxide pellets and around 5 ml of distillate. After that, the distillate was poured into a 50-ml volumetric flask and filled to the proper level with distilled water. It was gathered and put in the conical flask, and the filter was then given 1.6 ml of 5% potassium iodide. The resulting concoction was measured against 0.01. From the procedure described above, it was determined that the cyanide level of the cassava

utilized for this study was 300 pm, 400 pm, and 800 pm for red garri, white garri, and raw cassava, respectively, per 100 g.

Mathematical Determination of the concentration of Cyanogenic Glycosides in ppm

100 g of red garri contained 300 pm cyanogenic glycosides.

100 g = 300 ppm.

50 g = *x*.

$X = \frac{50 \times 300}{100}$ = 150 ppm in 50 g Red garri.

100 g of white garri contains 400 pm Cyanogenic Glycosides.

100 g = 400 ppm.

50 g = *x*.\(x = \frac{50 \times 400}{{100}}\) = 200 ppm in 50 g white garri

100 g of raw cassava contain 800 ppm.

100 g = 800 ppm.

50 g = $x = \frac{50 \times 800}{100}$ = 400 ppm

Ethical consideration

The care and use of the animals and the experimental protocol were in accordance with the Ethical Committee and Experimental unit of the Animal House University of Jos. Ethical Clearance was applied for and obtained before the commencement of the study (UJ/2020/05/22).

Animals

For this investigation, male albino rats that were sexually mature and weighed between 109 and 285 g were employed. At the University of Jos' animal house, the animals were kept in well-ventilated iron cages and subjected to a 12-h light/dark cycle at room temperature. Prior to the start of the research, all animals underwent a one-week acclimatization period and were handled in compliance with University of Jos policies for the handling and care of laboratory animals.

Experimental design

The animals were placed into four groups at random and handled as follows:

Group A: Control animals (received 300 g normal rat feeds).

Group B: 50 g red garri at a concentration of 150 ppm cyanogenic glycosides/rat/day.

Group C: 50 g white garri at a concentration of 200 ppm cyanogenic glycosides/rat/day.

Group D: 50 g raw cassava at a concentration of 400 ppm cyanogenic glycosides/rat/day.

Body and reproductive organs weight measurement

The body weights of the animals were measured using the electric weighing balance before and after treatment for 60 days. Euthanasia was done using the chloroform inhalation method. The animal's' testis, epididymis and prostate were dissected and weighed.

Histopathological examination

Histological examination of the testis, epididymis, and prostate gland was performed. The tissue samples were then treated for 24 h with a graded alcohol series, preserved in Bouin's solution, and ultimately embedded in paraffin wax. Using a semi-animated (LeicoaRM 2255) microtome, the paraffin blocks were cut into 5-m-thick slices, which were subsequently stained with hematoxylin and eosin (H and E) for light microscopic examination. For this score, at least ten fields were picked at random for each slide in each scenario. The sections were then examined for histological lesions in the testis, epididymis, and prostate using arbitrary ratings (±). Following that, a cumulative figure for each treatment group was constructed and photographed using a phase contrast microscope (Olympus B51, Tokyo, Japan).

Statistical analysis

The results were expressed as mean ± SEM for six animals per group. The data were analyzed using, one-way ANOVA with Graphpad Prism Statistical Software (version 7). p values less than 0.05 ($p < 0.05$) were considered significant.

Conclusion

This study has shown that the concentration of cyanogenic glycosides is higher in raw cassava and white garri compared to red garri and that cyanogenic glycosides at a concentration between 200–400 ppm/day may bring about reduction in body weight and induce adverse effects on male reproductive functions in albino rats. Therefore, chronic cassava meals may cause infertility in males on the long term, especially if there are challenges in the detoxification process.

Acknowledgements

This research did not receive any specific grant from funding agencies. The research was financed from the authors' own funds.

Conflict of interest

Olisemeke U. Egbune, Egoamaka O. Egbune, Osuvwe C. Orororo, Theresa Ezedom, Ogheneyoma Onojakpor, Ahmed M. Sabo and Kemakolam Amadi declare that we have no conflict of interest.

Ethical approval

The care and use of the animals and the experimental protocol were in accordance with the Ethical Committee and Experimental unit of the Animal House University of Jos. Ethical Clearance was applied for and obtained before the commencement of the study (UJ/2020/05/22).

Availability of data and materials

Data presented in this study are available on request from the corresponding author.

References

1. Talsma EF, Borgonjen-van den Berg KJ, Melse-Boonstra A, Mayer EV, Verhoef H, Demir AY (2018) The potential contribution of yellow cassava to dietary nutrient adequacy of primary-school children in Eastern Kenya; the use of linear programming. Public Health Nutr 21:365–376
2. Egbune EO, Avwioroko OJ, Anigboro AA, Aganbi E, Amata AI, Tonukari NJ (2022) Characterization of a surfactant-stable α-amylase produced by solid-state fermentation of cassava (*Manihot esculenta* Crantz) tubers using Rhizopusoligosporus: kinetics, thermal inactivation thermodynamics and potential application in laundry industries. Biocatal Agric Biotechnol 39:102290
3. Mombo S, Dumat C, Shahid M, Schreck E (2017) A socio-scientific analysis of the environmental and health benefits as well as potential risks of cassava production and consumption. Environ Sci Pollut Res Int 24:5207–5221
4. De Moura FF, Moursi M, Lubowa A, Ha B, Boy E, Oguntona B (2015) Cassava intake and vitamin A status among women and preschool children in Akwa-Ibom. Nigeria PLoS One 10:e0129436
5. Sanni LO, Babajide JM, Ojerinde MW (2007) Effect of chemical pre-treatments on the physico-chemical and sensory attributes of Sweet potato-gari. ASSET Int J Agric Sci Environ Technol Ser B 6(1):41–49
6. Egbune EO, Aganbi E, Anigboro AA, Ezedom T, Onojakpo O, Amata AI, Tonukari NJ (2023) Biochemical characterization of solid-state fermented cassava roots (*Manihot esculenta* Crantz) and its application in broiler feed formulation. World J Microbio Biotech 39(2):1–12
7. FAO (1990) Food and Agricultural Organization of the United Nations: Production Yearbook 44, Rome
8. Mroso PV (2003) Cassava, an emerging food product: the consequence of its popularity. Int J Food Sci 42:969–979
9. Zidenga T, Siritunga D, Sayre RT (2017) Cyanogen metabolism in cassava roots: impact on protein synthesis and root development. Front Plant Sci 8:1–12
10. Egbune EO, Ezedom T, Anigboro AA, Aganbi E, Amata AI, Tonukari NJ (2022) Antioxidants and antigenotoxic properties of *Rhizopus oligosporus* fermented cassava (*Manihot esculenta* Crantz). Afr J Biochem Res 16(3):39–46
11. Shibamoto TB (2009) Introduction to food toxicology, 2nd edn. Acad Press California, Cambridge, pp 124–154
12. Chaouali N, Gana I, Dorra A,Khelifi F, Nouioui A, Masri W (2013) Potential toxic levels of cyanide in almonds (*Prunus amygdalus*), apricot kernels (*Pruns armeniaca*), and almond syrup. ISRN Toxicol 610648

13. Montagnac JA, Davis CR, Tanumihardjo SA (2009) Nutritional value of cassava for use as a staple food and recent advances for improvement. Compr Rev Food Sci Food Saf 8:181–194
14. Enidiok SE, Attah LE, Otuechere CA (2008) Evaluation of moisture, total cyanide and fiber contents of garri produced from cassava (*Manihotutilissima*) varieties obtained from Awassa in southern Ethiopia. Pakistan J Nutr 7:625–629
15. Enefa S, Paul CW, David LK (2020) Model of Konzo Disease: Reviewing the Effect of Bitter Cassava Neurotoxicity on the Motor Neurons of Cassava-Induced Konzo Disease onWistar Rats. Saudi J Med 5(11):336–348
16. Tshala-Katumbay DD, Ngombe NN, Okitundu D, David L, Westaway SK, Boivin MJ (2016) Cyanide and the human brain: perspectives from a model of food (cassava) poisoning. Ann NY Acad Sci 1378:50–57
17. Bumoko GM, Sombo MT, Okitundu LD, Mumba DN, Kazadi KT, Tamfum-Muyembe JJ (2014) Determinants of cognitive performance in children relying on cyanogenic cassava as staple food. Metab Brain Dis 29:359–366
18. Udeme N, Okafor P, Eleazu C (2015) The metabolic effects of consumption of yellow cassava (*Manihot esculenta* Crantz) on some biochemical parameters in experimental rats. Int J Toxicol 34:559–564
19. Manzano H, de Sousa AB, Soto-Blanco B, Guerra JL, Maiorka PC, Górniak SL (2007) Effects of long-term cyanide ingestion by pigs. Vet Res Commun 31:93–104
20. Kamalu BP (1993) Pathological changes in growing dogs fed on a balanced cassava (*Manihot esculenta* Crantz) diet. Br J Nutr 69:921–934
21. Cunha LA, Mota TC, Cardoso PC, Alcantara DD, Burbano RM, Guimaraes AC (2016) In vitro assessment of the genotoxic and cytotoxic effects of boiled juice (tucupi) from *Manihot esculenta* Crantz roots. Genet Mol Res 15(4):1–8
22. Rivadeneyra DE, Rodriguez-Landa JF (2019) Preclinical and clinical research on the toxc and neurological effects of cassava (*Manihot esculenta* Crantz) consumption. Metab Brain Dis 35(1):65–74
23. Maliki OO, Alagbonsi AI, Ibitoye CM, Olayaki LA (2021) Melatonin and Vitamin C modulate cassava diet-induced alteration in reproductive and thyroid functions. Niger J Exp Clin Biosci 9:133–143
24. Paulinus ON, Obaika US (2013) A comparative study of the toxic effects of prolonged intake of cassava-borne organic cyanide and inorganic cyanide in some rabbit tissues. J Pharm Sci Innov 2:65–69
25. Chandra A, Coper CE, Stephen EH (2013) Infertility and impaired fecundity in the United States, 1982–2010: data from the national survey of family growth. Natl Health Stat Rep 67:1–19
26. Yelsili C, Mungan G, Seckines I, Akduman B (2005) Effects of varicoelectomy on sperm creation kinase. Reprod Toxicol 66:610–615
27. Bonde JP (1996) Environmental factors in comhaire investigation, cause evaluation and treatment. Chapman and Hall, London, pp 267–284
28. Orororo OC, Asagba SO, Tonukari NJ, Okandeji OJ, Mbanugo JJ (2018) *Hibiscus sabdarrifa* L. Anthocyanins-induced changes in reproductive hormones of cadmium-exposed rats. Int J Sci Res 12(4):308–311
29. Orororo OC, Asagba SO, Egbune EO, Efejene OI (2022) Sperm parameters and histological changes in testes of cadmium-exposed rats treated with *Hibiscus sabdarrifa* L. anthocyanins. Sokoto J Med Lab Sci 7(3):114–122
30. Harborne JB (1973) Phytochemical methods: a guide to modern techniques of plant analysis. Chapman & Hall, London

31. Sriroth K, Chollakup R, Chotineeranat S, Piyachomkwan K, Oates CG (2000) Processing of cassava waste for improved biomass utilization. Bioresour Technol 71:63–69
32. Oghobase GE, Aladesanmi OT, Akomolafe RO, Olukiran OS, Akano PO, Eimunjeze MH (2020) Assessment of the toxicity and biochemical effects of detergent processed cassava on renal function of Wistar rats. Toxicol Rep 7:1103–1111
33. Li S, Ma Y, Ji T, Sameen DE, Ahmed S, Qin W, Liu Y (2020) Cassava starch/carboxymethylcellulose edible films embedded with lactic acid bacteria to extend the shelf life of banana. Carbohydr Polym 248:116805
34. Nizzy AM, Kannan S (2022) A review on the conversion of cassava wastes into value-added products towards a sustainable environment. Environ. Sci Pollut Res 29(46):69223–69240
35. Adegbeye MJ, Salem AZM, Reddy PRK, Elghandour MMM, Oyebamiji KJ (2020) Waste recycling for the eco-friendly input use efficiency in agriculture and livestock feeding. In: Resources use efficiency in agriculture. Springer, Singapore, pp 1–45
36. Souza CMM, Bastos TS, Kaelle GCB, Bortolo M, Vasconcellos RS, De Oliveira SG, Félix AP (2021) Comparison of cassava fiber with conventional fiber sources on diet digestibility, fecal characteristics, intestinal fermentation products, and fecal microbiota of dogs. Anim Feed Sci Technol 281:115092
37. Morgan NK, Choct M (2016) Cassava: nutrient composition and nutritive value in poultry diets. Anim Nutr 2:253–261
38. Rosas-JarquínChJ R-D, León-Chávez BA, Nadella R, Sánchez-García AC, Rembao-Bojórquez D, Rodríguez-Landa JF, Hernandez-BaltazarD, (2020) Chronic consumption of cassava juice induces cellular stress in rat substantianigra. Iran J Basic Med Sci 23:93–101. https://doi.org/10.22038/IJBMS.2019.38460.9131
39. Akapo AO, Oso AO, Bamgbose AM, Sanwo KA, Jegede AV, Sobayo RA, Idowu OM, Fan J, Li L, Olorunsola RA (2014) Effect of feeding cassava (*Manihot esculenta* Crantz) root meal on growth performance, hydrocyanide intake and haematological parameters of broiler chicks. Trop Anim Health Prod 46(7):1167–1172. https://doi.org/10.1007/s11250-014-0622-5
40. Rivadeneyra DE, Rodriguez-Landa JF (2016) Motor impairments induced by injection of linamarin in the dorsal hippocampus of Wistar rats. J Neurol 31(8):516–522
41. Ebeye OA (2018) The effect of processed cassava products ("Tapioca and Gari") on weight and haematological indices of Wistar rats. Int J Basic Appl Innov Res 7(1):35–40
42. Airaodion AI, Ene AC, Ogbuagu EO, Okoroukwu VN, Ekenjoku JA, Ogbuagu U (2019) Biochemical changes associated with consumption (by rats) of "garri" processed by traditional and instant mechanical methods. Asian J Biochem Genet 2(4):1–11
43. Cope RB (2020) Acute cyanide toxicity and its treatment: the body is dead and may be red but does not stay red for long. In: Handbook of toxicology of chemical warfare agents. Academic Press, Cambridge, pp 373–388
44. Nielson JR, Nath AK, Doane KP, Shi X, Lee J, Tippetts EG, Peterson RT (2022) Glyoxylate protects against cyanide toxicity through metabolic modulation. Sci Rep 12(1):1–16
45. Zuhra K, Szabo C (2022) The two faces of cyanide: an environmental toxin and a potential novel mammalian gasotransmitter. FEBS J 289(9):2481–2515
46. Itakorode BO, Okonji RE, Torimiro N (2022) Cyanide bioremediation potential of Klebsiellaoxytoca JCM 1665 rhodanese immobilized on alginate-glutaraldehyde beads. Biocatal Biotransform 1–10

47. Wrobel M, Jurkowska H, Sliwa L, Srebro Z (2004) Increased in antioxidant activity in cyanide treated rats. Toxicol Mech Methods 14:331–337
48. Satpute RM, Bhutia YD, Lomash V, Bhattacharya R (2019) Efficacy assessment of co-treated alpha-ketoglutarate and N-acetyl cysteine against the subchronic toxicity of cyanide in rats. Toxicol Ind Health 35(6):410–423
49. Atobrah EE (2020) Orange-fleshed sweet potato (ofsp)–cassava composite gari: effects of processing variables and storage on beta-carotene and sensory qualities. Doctoral dissertation, University of Cape Coast.
50. Agarwal A, Leisegang K, Sengupta P (2020) Oxidative stress in pathologies of male reproductive disorders. In: Pathology. Academic Press: Cambridge, pp 15–27
51. Michelucci A, Liang C, Protasi F, Dirksen RT (2021) Altered Ca2+ handling and oxidative stress underlie mitochondrial damage and skeletal muscle dysfunction in aging and disease. Metabolites 11(7):424
52. Cázares-Camacho R, Domínguez-Avila JA, Astiazarán-García H, Montiel-Herrera M, González-Aguilar GA (2021) Neuroprotective effects of mango cv. 'Ataulfo'peel and pulp against oxidative stress in streptozotocin-induced diabetic rats. J Sci Food Agric 101(2):497–504
53. Cooper TG, Yeung CH (2010) Physiology of sperm maturation and fertilization E. Nieschlag. Eur J Endocrinol 25(4):200–210
54. Zhang X (2021) Isolation, molecular composition, and immune regulatory functions of extracellular vesicles from seminal plasma. Doctoral dissertation, Utrecht University
55. Elbashir S, Magdi Y, Rashed A, Henkel R, Agarwal A (2021) Epididymal contribution to male infertility: an overlooked problem. Andrologia 53(1):e13721
56. Ozkocer SE, Konac E (2021) The current perspective on genetic and epigenetic factors in sperm maturation in the epididymis. Andrologia 53(3):e13989
57. Homa S (2020) Handling unhealthy or poor-quality sperm samples in a medically assisted reproduction laboratory. In: Textbook of assisted reproduction. Springer, Singapore, pp 767–777
58. Heidari-Vala H, Sabouhi-Zarafshan S, Prud'homme B, Alnoman A, Manjunath P, (2020) Role of Binder of SPerm homolog 1 (BSPH1) protein in mouse sperm-egg interaction and fertilization. Biochem Biophys Res Commun 527(2):358–364
59. Bae JW, Kwon WS (2020) Investigating the effects of fipronil on male fertility: Insight into the mechanism of capacitation. Reprod Toxicol 94:1–7
60. Marín-Briggiler CI, Luque GM, Gervasi MG, Oscoz-Susino N, Sierra JM, Mondillo C, Buffone MG (2021) Human sperm remain motile after a temporary energy restriction but do not undergo capacitation-related events. Front Cell Dev Biol 9:777086
61. Pacher P (2021) Cyanide emerges as an endogenous mammalian gasotransmitter. Proc Natl Acad Sci 118(25):e2108040118
62. Ilesanmi OB, Ikpesu T (2021) Neuromodulatory activity of trèvo on cyanide-induced neurotoxicity viz neurochemical, antioxidants, cytochrome C oxidase and p53. Adv Trad Med 21(2):297–304
63. Vickram AS, Samad HA, Latheef SK, Chakraborty S, Dhama K, Sridharan TB, Gulothungan G (2020) Human prostasomes an extracellular vesicle–Biomarkers for male infertility and prostrate cancer: the journey from identification to current knowledge. Int J Biol Macromol 146:946–958
64. Vickram AS, Rohini K, Anbarasu K, Dey N, Jeyanthi P, Thanigaivel S, Arockiaraj J (2022) Semenogelin, a coagulum macromolecule monitoring factor involved in the first step of fertilization: a prospective review. Int J Biol Macromol 209:951–962
65. Tonukari NJ, Oliseneku EE, Avwioroko OJ, Aganbi E, Orororo OC, Anigboro AA (2016) A novel pig feed formulation containing Aspergillus niger CSA35 pretreated-

cassava peels and its effect on growth and selected biochemical parameters of pigs. Afr J Biotech 15(19):776–785
66. Ojo I, Apiamu A, Egbune EO, Tonukari NJ (2022) Biochemical characterization of solid-state fermented cassava stem (Manihot esculenta Crantz-MEC) and its application in poultry feed formulation. Appl Biochem Biotech 194(6):2620–2631
67. Tonukari NJ, Anigboro AA, Avwioroko OJ, Egbune EO, Ezedom T, Ajoh AI, Aganbi E (2023) Biochemical properties and biotechnological applications of cassava peels. Biotechn Mol Biol Rev 14(1):1–8