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To Cite:
Edem GD, Ekanem AU, Ettetor EU, Ahorituwere OG. Relationship between cyanide poisoning in cassava effluent and epididymis of Albino Wistar rats. *Drug Discovery* 2025; 19: e1dd2013
doi: https://doi.org/10.54905/disssi.v19i43.e1dd2013

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Peer-Review History
Received: 29 August 2024
Reviewed & Revised: 02/September/2024 to 30/November/2024
Accepted: 04 December 2024
Published: 03 January 2025

Peer-Review Model
External peer-review was done through double-blind method.

Drug Discovery
pISSN 2278–540X; eISSN 2278–5396

DISCOVERY SCIENTIFIC SOCIETY

Relationship between cyanide poisoning in cassava effluent and epididymis of Albino Wistar rats

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ABSTRACT

The epididymis is primarily known for temporary storage and development of sperm to maturation. Cassava effluent (Garri and Fufu effluent) contains many anti-nutrients such as cyanide. This research was conducted to investigate the relationship between cyanide poisoning in cassava effluent and the epididymis of Wistar rats. Twenty Wistar rats weighing between 113-203g were randomly divided into five groups (I-V) of four rats each. Each group was administered both effluents as follows for 28 days. Group I was labeled the control group; Group II was administered with 20ml/kg of Garri effluent for 28 days; Group III was administered with 10ml/kg of Garri effluent for 28 days; Group IV was administered with 20ml/kg of Fufu effluent for 28 days and Group V was administered with 10ml/kg of Fufu effluent for 28 days. The hormonal analysis showed an elevated level of prolactin for all experimental groups compared to the control group. Histomorphological assessment of the epididymis was done using hematoxylin and eosin staining techniques. The histomorphological analysis revealed cassava effluent administration led to histopathological changes in the epithelial layer, blood vessels epididymal lumen, smooth muscle layer and the spermatozoa. The result showed that groups treated with 20ml/kg of Garri showed the greatest alteration. The alterations in all sections of the experimental animals were dose- dependent. Conclusively, cassava effluent due to cyanide toxicity causes severe histopathological changes in the epididymis, which can have adverse effects on its reproductive function.

Keywords: Cassava effluent, epididymis, cyanide, prolactin, histomorphology, Wistar rats

1. INTRODUCTION

Cyanide is a chemical compound with the functional group C≡N. Nitriles, which are organic cyanides, have the formula R-CN and are highly toxic due to the CN functional group. Cyanide is reportedly utilized in various fields, including photography, laboratory reactions, industry, pesticides, and rodenticides. Additionally, it is present in certain fruits such as bitter almonds, apple seeds, plum seeds, and peach seeds (Vetter, 2000). Studies demonstrated that cyanide affects spermatogenesis via the hypothalamic-pituitary-gonadal axis in male rainbow trout (Ruby et al., 1993). Kamalu, (1993) reported that cyanide can reduce the spermatogenic cycle and induce testicular germ cell sloughing, degeneration as well as causing occasional abnormal cells in dogs.

Several studies demonstrated that maternal consumption of cyanogenic plants can lead to the fetal malformations in pigs, horses, sheep, cattle and humans. Evaluated NaCN-induced male reproductive toxicity in F344/N rat strain and found that up 4.5 mg/kg BW of cyanide can cause mild (insignificant) alteration in male reproductive system. However, various other studies have shown that cyanide, even at doses lower than 4.5 g/kg body weight, can cause hepatotoxicity, renal toxicity, neurotoxicity, and oxidative stress in different tissues (Kimani et al., 2014; Mathangi and Namasivayam, 2004; Mathangi et al., 2011). Spermatozoa do not achieve full maturation and fertilizing capacity until passage through the epididymis. During this time, they also gain motility, although spermatozoa do not move until after ejaculation (Arrata et al., 1978).

The organic fraction of human seminal plasma contains phosphate esters, particularly glycerylphosphorylcholine (GPC), phosphorylcholine (PCh), and inorganic phosphate. GPC is found in relatively high concentrations in the semen of many male animals, including men (Arrata et al., 1978). GPC is synthesized by the epithelial cells of the epididymis, apparently under androgenic control. Consequently, it is reported that GPC might be a helpful indicator of epididymal function (Arrata et al., 1978). Cassava tubers vary widely in their cyanogen content, although most varieties contain 15 to 400 mg HCN/kg fresh weight (Coursey, 1973). Occasionally, varieties with high cyanide content (1300 to 2000 mg/kg) are also encountered (Dufour, 1998). The root parenchyma, which is the edible portion, generally contains the lowest cyanide content (De-Bruijn, 1973).

However varying reports are available on the distribution of cyanide within the root tissue of bitter and sweet cultivars. The cyanide content in cassava peel is much higher than the pulp, and different peel to pulp ratios of cyanide have been reported by many scholars (Dufour, 1998; De-Bruijn, 1973). Cassava leaves also contain high concentrations of cyanogenic glycosides, and the values fall mainly in the range of 1000 to 2000 mg/kg on dry a matter basis (Gomez, 1985). The toxicity of cassava arises from the release of hydrocyanic acid (HCN) during hydrolysis of the cyanogenic glycosides by the glycosidases of the intestinal microflora (Fomunyam et al., 1984). Linamarin have been reported to be absorbed through the intestinal mucosa.

HCN can also be released *in vivo* by glycosidases of the liver and other tissues, causing *in situ* cytotoxicity (Padmaja and Panikkar, 1989). Cyanide is a potent toxin inhibiting cellular respiration (Solomonson, 1981). Chronic toxicity of cassava have been implicated in several diseases, such as tropical ataxic neuropathy, endemic goiter Ermans et al., (1980), Akintonwa and Tunwashe, (1992), Essers et al., (1992), Delange et al., (1982) and spastic paraparesis (Ermans et al., 1980). Many of these conditions result from the consumption of poorly processed cassava. Chronic cassava toxicity in chickens was reported to lower egg production and egg quality in layers, thereby reducing the hatchability of eggs and livestock, resulting in poor growth performance (Devendra, 1977). Reducing cyanide intake is therefore of vital importance.

Cassava (*Manihot esculenta*) is a South American plant grown as a staple crop in tropical and subtropical regions of Africa and Asia due to its edible starchy tuberous root, which is a primary carbohydrate source (Ninikanwa, 2018). It is one of the most abundant sources of calories in the tropics, making it a crucial staple food in developing countries. After two years of decline, the global cassava market increased by 0.4 percent to $164.1 billion in 2019. In 2019, Nigeria (61 million tonnes), the Democratic Republic of the Congo (32 million tonnes), and Thailand (32 million tonnes) were the largest consumers of cassava, representing 42 percent of global consumption. Ghana, Brazil, Indonesia, Angola, Vietnam, Cambodia, Mozambique, China, and Malawi followed, contributing 37 percent of total consumption. The highest per capita consumption of cassava in 2019 was in Ghana (646 kg per person), Cambodia (572 kg per person), and Angola (494 kg per person).

Cassava products include tapioca, “lafun”, “fufu”, ‘’starch’’, and “attieke” (Awoyale et al., 2021). Garri is a dry, crispy, creamy-white or yellow, granular flour obtained from cassava roots (Escobar et al., 2018). It is a well-established and convenient staple food product produced from cassava in West Africa. It has been a dependable food source for generations. It remains popular due to its relatively low cost compared to other carbohydrate sources in flour form, as well as its ease of handling, production, lightweight nature, and ease of transportation (Samuel et al., 2020). It is commonly eaten raw with water, sugar, groundnuts, and cashew nuts added or baked into a dough called eba, which is the most popular form. It may also sprinkled over cooked cowpea beans in several African countries, including Nigeria, Togo, and the Republic of Benin (Adinsi et al., 2019).

2. MATERIALS AND METHOD

Materials

Materials used in this experiment included the following; detergent, syringes, dissecting blade, wooden block, rotary microtome, cages, feed, weighing balance, feeding bottles, markers, masking tape, cotton wool, hand gloves, cannula, spatula, sample bottles, water baths, sawdust, needles, refrigerator, tissue roll, paraffin wax, beakers, antiseptics, aluminum foil, measuring cylinder, and oven.

Care of Animals

Twenty (20) male Wistar rats, weighing between 113-203g, were sourced from the Animal House of the Faculty of Basic Medical Sciences, University of Uyo, Nigeria. The animals were weighed, labeled, and kept in wooden cages at the Animal House of the Faculty of Basic Medical Sciences, University of Uyo. They were acclimatized for 2 weeks before the commencement of the experiment. The rats were fed vital feeds and provided with water at will. The cages were kept clean by regularly replacing sawdust every three days. A maximum of 4 animals divided into 5 groups were kept per cage. Group one (1) was designated as control group, while groups two through five (2-5) served as the experimental groups. All animals were handled in compliance with the guidelines of the Faculty of Basic Medical Sciences' Ethics Committee, University of Uyo.

Intervention and Administration of Cassava Effluent

Garri and Fufu effluent were administered orally to the experimental animals. The animals were divided into five (5) groups of four (4) rats per group. These groups were organized as follows: Group 1 rats (control group) received standard diet and tap water; Group 2 rats were given standard diet and 20ml/kg of Garri effluent; Group 3 rats were provided with standard diet and 10ml/kg of garri effluent; Group 4 rats were given standard diet and 20ml/kg Fufu effluent and Group 5 rats were given standard diet and 10ml/kg of Fufu effluent.

Sacrifice and Blood Collection

At the end of the experiment, the animals were anesthetized using chloroform and sacrificed. Blood was obtained from the inferior vena cava (IVF) with the use of a 5ml syringe and needle and transferred into plain serum bottles and allowed to agglutinate for about 45 minutes before being centrifuged for 10 minutes at 4000 rpm to obtain a clear supernatant for serum prolactin analysis. The epididymis of each rat was subsequently harvested and promptly fixed in 10% neutral buffered formalin.

Hormonal Assay

Serum prolactin testing was done with radioimmunoassay (RIA) using a NIADDK rat PRL radioimmunoassay (RIA) kit. Plasma samples were measured in a single RIA run to limit interassay variation, and results were expressed in ng/ml.

Tissue Processing and Microscopy

Processed tissue sections were viewed using a compound light microscope and micrographs from the sections were taken using the microscope's camera attached to a computer.

Statistical Analysis

Data collected from this investigation were analyzed using one-way analysis of variance (ANOVA) using GraphPad Prism statistical software (version 8) and presented as mean ± standard error.

3. RESULTS

Effect of Cassava (Garri and Fufu) Effluent on the Prolactin Level of Male Albino Wistar Rats

Results showed that both effluents significantly and dependently increased the serum prolactin levels in Wistar rats. A significant increase was well observed in the group administered with 20ml/kg of Garri effluent (Figure 1).

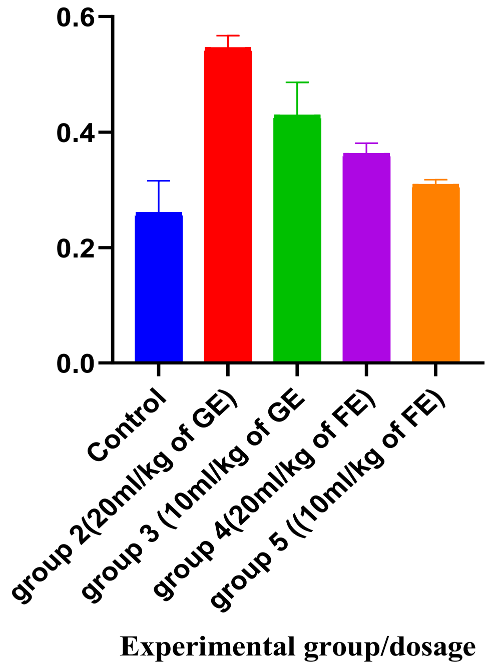

Figure 1 Effect of cassava effluent on prolactin level.

Histomorphological Effect of Cassava (Garri and Fufu) Effluent on the Epididymis of Male Albino Wistar Rats

Histological findings of group I showed a section of the epididymis of rats with the usual histological structure comprising of a pseudostratified columnar epithelial cell, usual interstitial space with collagen fibers and, epididymal lumen containing densely packed spermatozoa (Figure 2). Section of the epididymis of albino rat in group II administered with 20mg/kg of Garri effluent for 28 days showed a thick muscle layer surrounding the epididymal tubule, hypertrophied epididymal wall and, shrunken tubules with few/scanty/absence of spermatozoa in a majority of the tubules (Figure 3). Section of the epididymis of albino rat in group III administered with 10mg/kg of Garri effluent for 28 days showed a hypertrophied wall of the epididymal tubule and scanty presence of spermatozoa in some of the epididymal tubules (Figure 4).

Similarly, a section of the epididymis of albino rats in group IV administered with 20mg/kg of Fufu effluent for 28 days, showed a hypertrophied wall of the epididymal tubule, congested vessels, and few/scanty spermatozoa in some of the tubules (Figure 5). Histological findings on the epididymis of albino rats in group V, administered with 10mg/kg of Fufu effluent for 28 days, showed a hypertrophied wall of the epididymal tubule, occlusion of the interstitial space with inflammatory cells and thickened collagen fiber within the interstitial space, though with the accumulation of densely packed spermatozoa within the epididymal tubules (Figure 6).

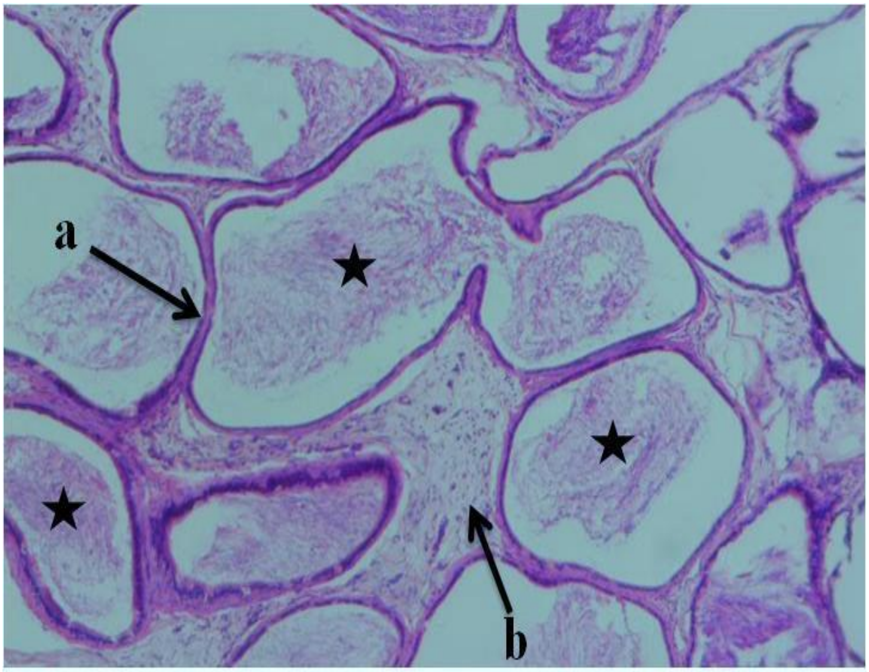

Figure 2 Photomicrograph of the epididymis of control animals given water and feed alone showing a- usual histological structure with pseudostratified columnar epithelium, b-usual interstitial space with collagen fibres and epididymal lumen containing densely packed spermatozoa (star) (H&E). 100x magnification

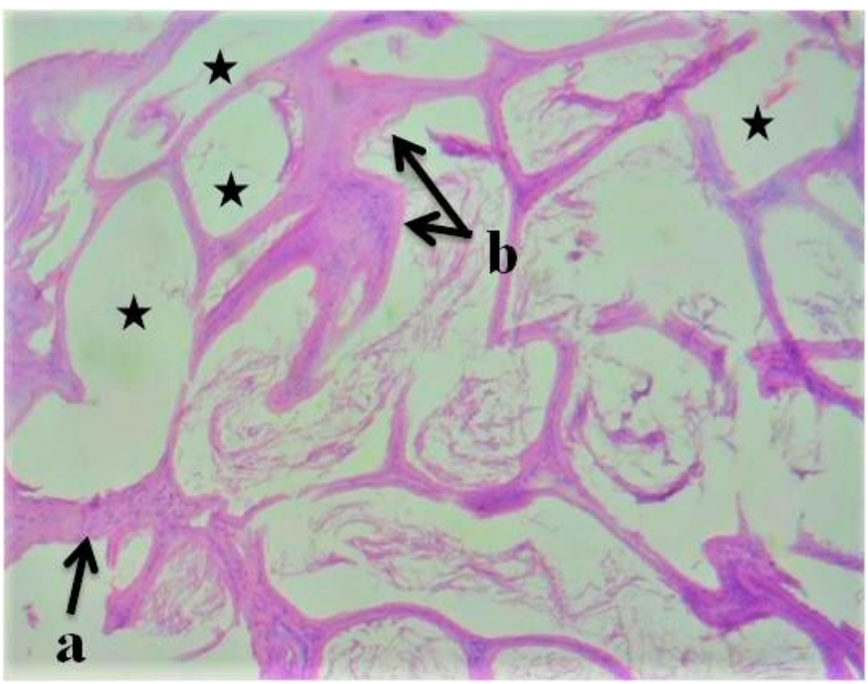

Figure 3 Photomicrograph of the epididymis of group 2 animals given 20ml/kg of Garri effluent showing a-thick muscle layer surrounding the epididymal tubule, b- a hypertrophied wall of epididymal and shrunken tubules with few/scanty/absence of spermatozoa in majority of the tubules (star). (H&E). 100x magnification

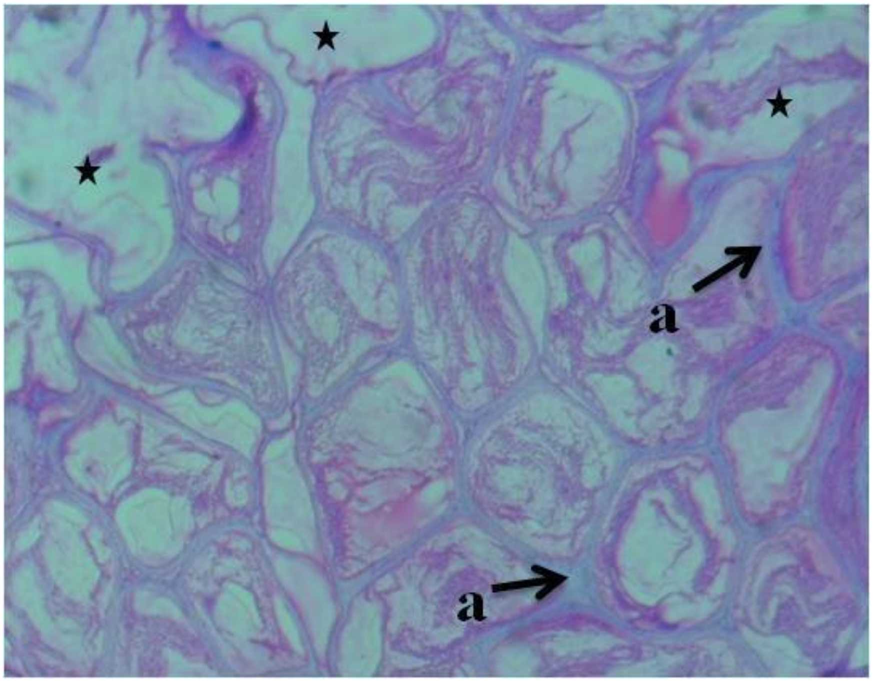

Figure 4 Photomicrograph of the epididymis of group 3 animals given 10ml/kg of Garri effluent showing a-Hypertrophied wall of epididymal tubule and scanty presence of spermatozoa in some of the epididymal tubules (star). (H&E). 100x magnification.

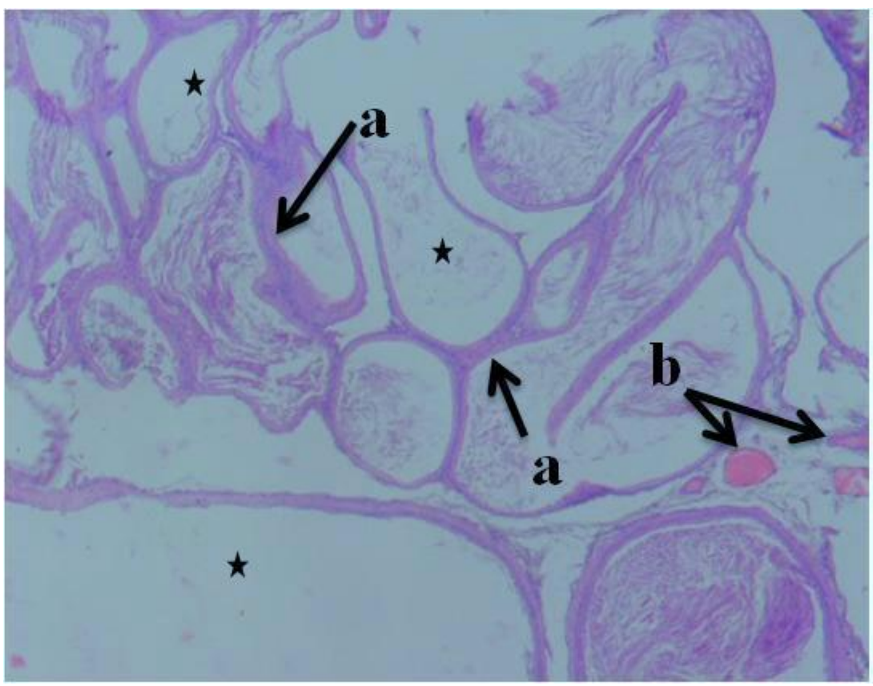

Figure 5 Photomicrograph of the epididymis of group 4 animals given 20ml/kg of Fufu effluent showing, a- Hypertrophied wall of epididymal tubule, b-congested vessels and few/scanty spermatozoa in some of the tubules (star). (H&E). 100x magnification.

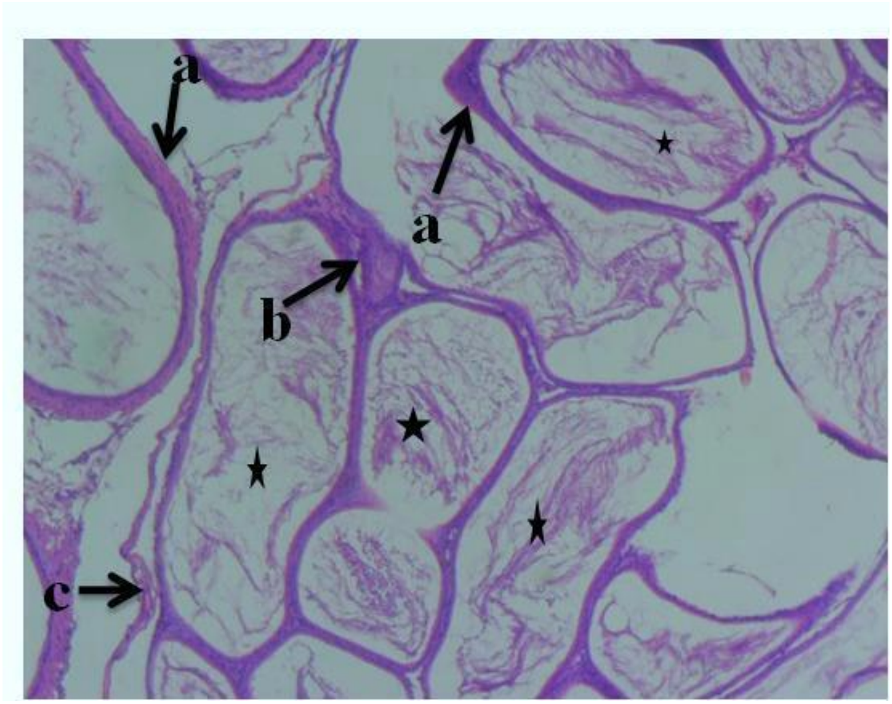

Figure 6 Photomicrograph of the epididymis of group 5 animals given 10ml/kg of Fufu effluent showing a- Hypertrophied wall of the epididymal tubule, b-occlusion of the interstitial space with inflammatory cells, c- thickened collagen fiber within the interstitial space, though with the accumulation of densely packed spermatozoa within the epididymal tubules (star). (H&E). 100x magnification.

4. DISCUSSION

Cyanide glycoside in cassava effluent can induce oxidative stress in the brain, particularly in the hypothalamus, where prolactin release is regulated. Oxidative stress can damage neurons and alter the normal functioning of neurotransmitter systems, further disrupting the inhibitory control of prolactin secretion (Aitken and Baker, 2006). Chronic exposure to cassava effluent may also trigger inflammation in the pituitary gland. Inflammatory cytokines, such as TNF-α and IL-6, can affect the hypothalamic-pituitary axis, potentially leading to increased prolactin secretion (Hotamisligil, 2006). Inflammation can interfere with the standard regulatory mechanisms, contributing to hyper-prolactinemia. From our result in Figure 1, the experimental groups all had increased prolactin levels, especially group II animals given 20mg/kg of Garri effluent.

Our study supports the claim of Aitken and Baker, (2006) who reported that cyanide glycoside causes hyper-prolactenemia. Group II animals also had the highest level of prolactin elevation and this could be, because group II animals were fed Garri effluent with higher quantity of CN- in comparison to group IV and group V which were administered Fufu effluent. Similarly, group II animals had a higher prolactin level compared to group III which were also administered 10ml/kg of Garri effluent. This investigation suggests that, the level of prolactin is directly proportional to the amount of CN- and dosage of administration. Elevated prolactin levels have also been linked with increased oxidative stress, which can damage sperm DNA, lipids, and proteins thereby leading to poor sperm quality (Aitken and Baker, 2006). Oxidative stress-induced sperm damage is a significant factor in male infertility. This suggests that cassava effluent over the long term can result in male infertility.

Findings from our research showed the following; thick muscle layer surrounding the epididymal tubule, a hypertrophied wall and shrunken epididymal tubules with few/scanty/absence of spermatozoa in the majority of the tubules. These histopathologies were significantly prominent in the group administered with 20ml/kg of Garri effluent. The histomorphological changes in Figure 3, such as the thick muscle layer and hypertrophied wall of the epididymis, consistent with (Aghaei et al., 2014). In their study, it was reported that, oxidative stress caused by cyclophosphamide (CP) toxicity caused disorganization and hypertrophy of the epithelium of epididymis in rats. Furthermore, their findings showed increased in epithelium thickness of epididymis in CP-treated rats. Also, the scanty spermatozoa in Figure 3 would agree with (Abarikwu et al., 2009).

Their study on atrazine toxicity showed that the atrazine-treated animals showed reduced epididymal and testicular sperm number and sperm motility. These animals from their study were documented to suffer impaired spermatocytogenesis with a concurrent drop in the rate and efficiency of spermatozoal production and the viability of spermatozoa. This reduction of sperm was due to the impairment of the antioxidant defense system in the epididymis (Abarikwu et al., 2009). This would suggest the reason behind the scanty spermatozoa present in (Figure 3). Our findings as observed in Figure 4 showed a hypertrophied wall of the epididymal tubule, and scanty presence of spermatozoa in some of the epididymal tubules. These observations agree with Huang et al., (2005) report, which documented degenerated, necrotic, and apoptotic epithelial cells in the epididymal ducts due to oxidative stress from cadmium-induced toxicity.

The lethal dose of cyanide for humans is considered to be between 0.5 to 3.5 mg per kilogram of body weight. Our findings also revealed several histomorphological changes including, a hypertrophied wall of epididymal tubule, congested vessels and few/scanty spermatozoa in some of the tubules. In a study by Adamkovicova et al., (2014), blood vessels appeared dilated and more congested following induced cadmium toxicity in rats. Our findings corroborated a study by Thompson and Bannigan, (2008) who demonstrated cadmium-induced testicular necrosis after ischemia, preceded by the perturbing of blood-testis barrier integrity and disruption of cell-to-cell endothelial and epithelial junctions. This mechanism may be similar to cyanide-induced ischemia (Borron and Bebarta, 2015). These studies are consistent with the presence of congested blood vessels in (Figure 5).

Moreso, animals administered with 10ml/kg of Fufu effluent for 28 days showed hypertrophied wall of the epididymal tubule, occlusion of the interstitial space with inflammatory cells and thickened collagen fiber within the interstitial space, though with the accumulation of densely packed spermatozoa within the epididymal tubules. Sperm compaction (stasis) may occur in the efferent ducts due to increased fluid reabsorption or due to impaction of sperm in blind ending ducts. Sperm stasis generally incites an inflammatory response with time (Radovsky et al., 1999). Movement of sperm through the efferent ducts and epididymis is dependent on the correct fluid dynamics (fluid production by the Sertoli cell and fluid reabsorption by the efferent ducts) and also on smooth muscle contraction within the epididymis and vas deferens.

Disturbances in either of these processes or obstruction of any part of the duct system can lead to sperm stasis, which in turn often leads to inflammation and, or sperm granulomas (De-Grava and Klinefelter, 2014). The administration of cyanogenic glycosides from cassava can lead to the presence of inflammatory cells in the epididymal tissue, occluding its interstitial spaces, through mechanisms involving cyanide-induced hypoxia, oxidative stress, necrosis, and the subsequent inflammatory response. Since epididymis is an organ rich in polyunsaturated fatty acids and has low antioxidant defense, this organ is more susceptible to tissue damage by toxic chemicals interacting with cellular membranes, inducing structural alterations and the recruitment of neutrophils, macrophages and monocytes containing Myeloperoxidase (MPO) (Rudolph et al., 2008).

These processes lead to the recruitment and accumulation of inflammatory cells, contributing to histological evidence of inflammation, and tissue damage (Dinarello, 2000; Rock and Kono, 2008; Way, 1984). This study revealed presence of inflammatory cells within the interstitial spaces as seen in Figure 6 due to CN- present in Fufu effluent. This differs in comparison to Figure 3-5, where inflammatory cells were observed to be absent in higher dosages of Fufu effluent and different dosages of Garri effluent. According to Sies, (1997), lower doses of cyanogenic glycosides might induce a manageable level of oxidative stress that triggers an inflammatory response, leading to the presence of inflammatory cells.

However, at higher doses, the oxidative stress might exceed the tissue's antioxidant capacity, leading to widespread cell death without the intermediate step of inflammation. The overwhelmed antioxidant system might fail to protect cells, resulting in necrosis and the absence of an inflammatory response. This would support the finding of inflammatory cells in group 5 compared to the other experimental groups. Regarding CN- quantity, Fufu effluent is less toxic than Garri effluent but at a lower dosage it can still trigger immune response. From the histopathological changes seen in our findings, the alteration caused by cassava effluent is dose-dependent and Garri effluent having more cyanide glycoside content is revealed to cause more histologic alteration than Fufu effluent.

5. CONCLUSION

In conclusion, this study shows that cassava effluent causes cyanide-induced toxicity with serious histopathological effects on the epididymis and its functions. These changes may have severe implications on reproductive functions.

Acknowledgement
We appreciate the laboratory staff who provided the necessary laboratory support during this research.

Authors Contribution
This research was carried out in collaboration with all authors. Author Gabriel DE designed the work, author Akpan UE carried out statistical analysis, author Eno UE did the proof-reading of the manuscript while author Onome GA managed the literature searches.

Ethical Approval
All animals were handled in compliance with the guidelines of the Animal House of Faculty of Basic Medical Sciences' Ethics Committee, University of Uyo, Nigeria. The Animal ethical guidelines are followed in the study for experimentation.

Informed Consent
Not applicable.

Conflicts of interests
The authors declare that there are no conflicts of interests.

Funding
The study has not received any external funding.

Data and materials availability
All data associated with this study are present in the paper.

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