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Genotoxicity and Ecotoxicological Effects of Cassava Effluent in Swiss Albino Male Mice Using the Sperm Morphology and Peripheral Blood Micronucleus Assays

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Abstract

Cassava is a very important staple food in most developing countries but effluents generated during its processing are not usually disposed properly, thus, contributing significantly to environmental pollution. Several reports have demonstrated the acutely toxic and genotoxic effects of cassava effluent in microbial organisms, plants and aquatic animals, the effects of pollutants present in these effluents have not been clarified yet in terrestrial animals. The study aimed to evaluate the haematological, histopathological and genotoxic potential of cassava effluents from Olorun-Sogo cassava mill in Ilorin, Kwara State, Nigeria, on the male germ and

somatic cells of Swiss albino mice using sperm morphology and micronucleus assays. Cassava effluent dilutions of 0.5, 1.0 and 2.0% were administered by intraperitoneal injection to mice, totalizing 0.5mL for 5 consecutive days and a 5-week exposure period was considered. The effluent induced a concentration – dependent statistically significant increase ($p< 0.05$) in the micronucleus and number of sperm with abnormal morphology at all concentrations as well as diverse histopathological changes in the testes. Haematological parameters were not significantly affected ($p>0.05$). The liver shows slightly distorted architecture while the kidney shows slight distortion in the renal corpuscles and the surrounding parenchyma. Alterations in alveolar space were observed in the lung while the spleen revealed a slight distortion in it tissue. The physico-chemical analysis also revealed the presence of significant amounts of cyanide and heavy metals (lead, iron, cadmium and silver). The present findings are relevant in environmental waste management and assessment of the hazardous effects of cassava effluents

Keywords: Cassava effluents; sperm morphology; histopathology; cyanide and genotoxicity

1.0 Introduction

Pollution is occurring on a wide and unprecedented scale around the world due to developmental activities such as construction, transportation, manufacturing and increase in population in most developing and developed countries. These pollutions not only pollute the air, soil and water but also diminish the natural resources. Untreated or improperly discharge of these wastes into aquatic bodies may render it unfavorable to the health of populace and other living systems [1, 2, 3, 4, 5]. Wastewater pollution has been reported to have adverse health effects such as gastrointestinal disorder, cardiovascular diseases, liver dysfunction, chromosomal damage, infertility, premature ageing and cancer in exposed organisms [1, 2, 6, 7]. In the ecosystem, it has been reported that bioaccumulation and biomagnification

effect of these chemical compounds present in the waste may get to a threshold level that may be deleterious to man [8].

Waste generated by cassava processing pose serious environmental pollution especially with increased industrial production of cassava flour and starch. These wastes such as peelings, fibrous by-products and wastewater effluents are indiscriminately disposed into the environment without prior treatment to reduce the volume, toxicity and mobility of the hazardous substances [9].

Over the years, cassava has been transformed into a number of products both for domestic (depending on local customs and preferences) and industrial uses. The three most common fermented cassava products in Nigeria are garri, fufu and lafun [10, 11, 12]. While garri involves fermentation of soaked roots, fufu and lafun on the other hand involves fermentation of soaked roots [13]. Cassava in the fresh form contains cyanide, which is extremely toxic to humans and animals; there is therefore a need to process it to reduce the cyanide content to safe levels [14].

Cassava effluents are liquid wastes from the cassava mill which are usually discharged on land or water in an unplanned manner. Agunbiade *et al*. [8] reported that anomalies in cell division process and chromosome aberration in the sperm of albino mice could be as a result of heavy metals interaction in the effluents from carbonaceous bottling plant. The genotoxic and histopathological effects of the effluents from carbonaceous bottling plant established in the study indicated that the effluents contain toxic substances which may constitute a risk to the environment and human health. Adeyemo, [15] also reported that cassava wastewater effluents contain pollutants that are deleterious to a natural fish population. In developing countries like Nigeria, there is need to give attention to industrial waste management as a result of sharp improvement in the manufacturing performance. The indiscriminate discharge of waste can not only be attributed to lack of proper

education on waste disposal but also to inadequate functioning of environmental monitoring group.

Despite several reports that have demonstrated acutely toxic and genotoxic effects of cassava effluents in microbial organisms [10], plants [16, 17, 18] and aquatic animals [19], the effects of pollutants present in the effluents have not been clarified on terrestrial animals. There is dearth of data on genotoxicity of cassava effluents effect on the male mouse germ and somatic cells. In this study, we investigated the genotoxic effects of cassava effluents in Swiss albino male mice using mouse sperm morphology and peripheral blood micronucleus assays and also toxicological effects of the cassava effluent on the haematology as well as histology of various organs in mice were evaluated.

2.0 Materials and Methods

2.1 Biological materials

Swiss albino male mice (*Mus musculus*) of about 8-10 weeks old were obtained from the Department of Zoology, University of Ilorin, Ilorin, Nigeria. They were acclimatized for 2-3 weeks in pathogen free, well ventilated plastic cages of the animal house of the same Department in order to adapt to their environment. This is to avoid the transiting increases in abnormal sperm occurring at the onset of mouse spermatogenesis in young mice. Throughout the period of exposure, the beddings were regularly changed, discarded and disinfected. The animals were maintained in the animal house of the Department of Zoology, University of Ilorin, Ilorin throughout the period of the experiment at a room temperature of 25°C and in 12 h dark and 12 h light cycles. Food pellets obtained from Aromokeye pharmacy along Challenge, Ilorin and water were provided ad libitum. The experiment was approved by the Ethics committees of the Department of Zoology, University of Ilorin, Ilorin, Nigeria. 25 mice were randomly allocated in five groups (n=5). The groups were as follows;

- Group I (negative control) received a single intraperitoneal injection (IP) of 0.5ml of distilled water for 5 consecutive days.
- Group II (0.5%) received a single IP of 0.5ml of cassava effluent dilution for 5 consecutive days.
- Group III (1.0%) received a single IP of 0.5ml of cassava effluent dilution for 5 consecutive days.
- Group IV (2.0%) received a single IP of 0.5ml of cassava effluent dilution for 5 consecutive days.
- Group V (positive control) received a single IP of 0.5ml of 20 mg/kg bwt of cyclophosphamide for 5 consecutive days.

The IP route was favored since it is one of the fastest and most efficient means of delivering test-chemicals into test-animals in a short-term-assay and gets substances into the circulation faster than oral route. The mice in the treated and control groups were all sacrificed at day 35. Sperm was sampled from the caudal epididymes at 5 weeks from the first injection, this is necessary in order to allow spermatogenesis in male mice to be completed before sacrificing the animals since one mouse spermatogenic cycle from spermatogonia to mature spermatozoa takes 34.5 days [20, 21].

2.2 Sampling site

The cassava effluent sample was sourced from collecting pit which is usually been overflow after a downpour at Olorunsogo cassava mill, Ita Amon (Fig. 1), Iso garri, along Allahuakbar filling station after Pakata roundabout, Ilorin, Kwara State, Nigeria (Latitude 8.60089° N and longitude 4.48825° E). It was established in 1988 and the same collecting pit is still used till date. The sample was collected into 25 L pre-clean plastic container, filtered using 15 cm filter paper (Whatman®, England) to remove suspended particles and poured into a 10 L clean white gallon. These samples were then stored at 4^{o}C throughout the period of study.

2.3 Analysis of the cassava effluent for physicochemical parameters

The effluents were analyzed for a number of standard physicochemical parameters, including pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), Total Dissolved Solids (TDS), alkalinity, salinity, total solids, cyanide, metals (including four heavy metals) namely lead (Pb), cadmium (Cd), iron (Fe) and zinc (Zn) were analyzed in the effluents sample according to standard analytic methods (USEPA and NESREA).

2.4 Sperm morphology assay

Induction of sperm abnormalities was done in accordance with standard procedures [3, 22]. 3 mice from each group were sacrificed by cervical dislocation and their caudal epididymides were removed. Sperm suspensions were prepared from each group by mincing the caudal in of buffered physiological saline, pipetting the resulting suspension. A fraction of each suspension was then mixed (9:1) with normal saline and 1% Eosin Y respectively. For each suspension, 1000 sperm were examined at 1000 folds magnifications; a total of 3000 sperm were scored for sperm abnormalities thus examined for each group at x1000 magnification (oil immersion) using the Olympus microscope.

The endpoint of the test is based on the comparison of data for negative, positive and the treated (test) groups. A positive response is deemed to have occurred when at any individual concentration level the increase in abnormal head is significant at $p<0.05$ level and preferable is double the negative control level. For a test to be judged a positive inducer of abnormal sperm, the response should yield statistically significant increases at a minimum of two consecutive concentrations, be reproducible in separate experiments and also show evidence of dose-related increases in abnormalities. A test is judged to be negative if no increase in sperm is seen up to doses or concentration that cause whole animal lethality [22].

2.5 Micronucleus Assay

Peripheral erythrocyte was obtained from the tail of mice (n=3) samples from each treatment and the two control groups according to Alimba *et al.* [23] with little modification. Briefly, blood samples were collected from the tail of each mouse. A drop of blood was placed on a clean, grease free microscope slides to make a thin blood smear. Slides were prepared per mice samples collected from treatments and controls. The smeared slides were allowed to air-dry overnight in a dust free environment. The dried slides were then fixed in absolute methanol for 20 minutes and allowed to dry overnight. The fixed smears were stained in 10% May-Grunwald (rinse and dry over-night) and 5% Giemsa stains (rinse and dry over-night) after which the stained slides were rinsed with distilled water to remove excess stain and allowed to dry. At least 1000 cells/ animal were scored for micronuclei (MN) and other nuclear abnormalities (NA).

2.6 Haematological analysis of the mice

At 35 days of the experiment, three mice were sacrificed by cervical dislocation; blood was thereafter collected from each mouse by cardiac puncture, using syringe and needle and stored in ethylene diamine tetraacetate (EDTA) bottle. The anticoagulant blood was used for haematological parameters, using Coulter HMX Haematology automated analyzer. The haematological parameters determined include white blood cell (WBC), red blood cell (RBC), haemoglobin (Hb), haematocrit/packed cell volume (HCT/PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet (PLT).

2.7 Histopathological studies

At the end of the experiment, testis, liver, kidney, spleen and lung from each sacrificed mice were dissected out and trimmed of excess fat.

2.7.1 Testes

Testes were carefully removed from two mice in the lowest and highest concentration (0.5% and 2.0%) of cassava effluent as well as in the negative and positive control groups at 5 weeks from the first injection. The testes were rinsed in normal saline (0.85g of NaCl + 100ml of distilled water) and trimmed of extraneous tissue. They were fixed in Bouin's solution for 48 hours. They were dehydrated in ascending grades of ethanol (70%, 80%, 95% and 100%), cleared using xylene and embedded in paraffin wax using Leica Histokinete tissue processor for 6 hours. Serial sections of 6µm thickness were obtained on labeled glass slides using a rotatory microtome. The deparaffinized sections were stained routinely with haematoxylin and eosin (H & E) and mounted. The slides were scored randomly at 400X and photomicrographs taken accordingly.

2.7.2 Livers

Liver tissues were carefully dissected from mice in 0.5% and 2.0% concentrations as well as in the two controls. The liver tissues samples dissected were fixed at 10% buffered formaldehyde for 24 h, processed using a graded ethanol, xylene and paraffin series, and embedded in paraffin. The paraffin sections were cut into 4 mm thick slices and stained with hematoxylin and eosin for light microscopic examination. The sections were viewed and photographed using a Nikon light microscope with an attached photographic machine. Two slides were prepared from each liver.

2.7.3 Kidney, Spleen and Lung

The Kidney, spleen and lung in 0.5% and 2.0% concentrations group as well as in the two controls were surgically removed, trimmed of extraneous fat and fixed in 10% buffered formalin and were processed for paraffin sectioning by dehydration in different concentrations of alcohol, cleared with xylol and embedded in paraffin

blocks. Sections of about 5µm thickness were stained with Harris haematoxylin and eosin (H&E) for histological study [24].

2.8 Statistical analysis

The Statistical Package for Social Sciences (SPSS) 16.0 was applied to evaluate the distribution of abnormal spermatozoa. Difference between the negative control and the individual concentration groups were analyzed by means of the One way ANOVA at the $p<0.05$ level. Duncan's New Multiple Range Test (DMRT) and Dunnett were used to compare the difference between the experimental groups and the negative control group. The mean and standard error were calculated and used to present the result for each effluent group.

Percentage abnormality for each concentration treatment for both the test groups and control groups were calculated using the formula below:-

$$\% \text{ Abnormality} = \frac{\text{Number of abnormal sperm counted}}{\text{Total number of sperm cells counted}} \times 100$$

3.0 Results

3.1 Physicochemical analysis of the cassava effluent

The physical and chemical analysis of cassava effluent observed in this study is shown in Table 1. The effluent was turbid with offensive odour and acidic with pH value of 6.30. The concentrations of heavy metals were relatively higher than the permissible limits. The concentrations of Fe, Zn, Cd and Pb were 0.342, 0.261, 0.118 and 0.088 respectively. The concentration of cyanide in cassava effluent was 4.48mg/100g (Table 1).

3.2 Sperm head abnormality test (SHAT) after five (5) days of exposure

Table 2 shows the summary of morphological abnormal sperm induced in Swiss albino male mice by different concentration of cassava effluent at 35days. At the lowest concentration level (0.5%), 9.67% of the sperm were abnormal. This was higher than observed in negative control at 5 weeks which was 4.93% abnormalities (p<0.05). At 1.0% and 2.0% concentrations, 8.1% and 9.8% abnormalities were observed respectively. About 12.43% of abnormalities were observed in the positive control. From table (2), it can be observed that it is not concentration dependent. Figure 2 (A-H) is showing the photomicrograph of the various abnormal sperms observed such as folded sperm, tail at wrong angle, sperm without hook, wrong hook attachment, sperm with two hooks, sperm with two tails and others.

3.3 Micronucleus and Nuclear abnormalities induced by cassava effluent

Table 3 shows the frequencies of micronucleus (MN) and other nuclear abnormalities (NA) (such as binucleated, enucleated, nuclear bud, polynucleated and fragmented nucleus) observed after treatment with different concentrations of cassava effluent. At 0.5%, 1.0% and 2.0% concentrations, the cassava effluent dilutions induced 7.83%, 9.90% and 10.37% abnormalities respectively when compared to 0.87% of the negative control at 0.05 p-level. Figure 3 shows the mean aberration of the frequencies of MN and NA induced by cassava effluent which is in a concentration dependent manner compare to the mean aberration recorded in the sperm morphology.

3.4 Haematological assessment of the mice

Table 4 shows the effect of cassava effluent on the haematological parameters of the Swiss albino mice. All haematological parameters were not statistically significant across the groups. There was an initial increase in white blood cell (WBC), red blood cell (RBC), haemoglobin (Hb), *mean* corpuscular *haemoglobin (*MCH) and *mean* corpuscular *hemoglobin* concentration (MCHC) of the mice on exposure to 0.5 % concentration of the effluent which then decreased at 1.0% and increased again at 2.0% concentrations. The effluent decreased the HCT at 0.5 % and 1.0% concentrations but increased it at concentration 2.0 %, while MCV increased at 0.5 % and 1.0% concentrations and decreased on increasing the concentration to 2.0%. PLT on the other hand, increased in value at 0.5 % concentration but thereafter decreased further as the concentration increased to 1.0 % and 2.0% respectively.

3.5 Histopathological analysis

3.5.1 Testes

Histological examination of the testes in negative control, 0.5%, 2.0% concentrations and positive control were carried out. The section in the testis of negative control of the mice showed the normal distribution of sertoli cells, leydig cells, lumen, and basement membrane of seminiferous tubules and around the blood vessels. The histology of the testis of the negative control showed no visible necrosis or lesions. After being treated with cassava effluent, it was seen that the normal cellular architectural of the testicular tissues of the mice was disrupted. There were histopathological lesions such as hypertrophy, atrophy, degradation of seminiferous tubules and congestion of intestinal blood vessels (Figure 4A-D). The lesions were more severe in mice exposed to 2.0% concentration of cassava effluent in which a marked necrosis of spermatogenic cells within the seminiferous tubules (STs) was observed.

3.5.2 Liver

The photomicrograph of the livers dissected at different test concentration shows different lesions such as vacuolation, infiltration and distortion. Mice in negative control show normal liver architecture with normal central vein and portal canal (Fig. 5A). The liver of mice exposed to 0.5% and 2.0% concentrations of cassava effluent show grossly distorted liver architecture with slightly vacuolated portal canal and central vein (Fig. 5B) and grossly distorted liver architecture with undifferentiated liver cells (Fig. 5C) respectively. The liver of the positive control mice exposed to 20mg/kg bwt of cyclophosphamide shows vacuolation of the central vein of the liver cell and distortion of the portal canal (Fig 5D). Also, there was a gross distortion of the hepatocytes of the liver cells.

3.5.3 Kidney

The histological examination of the kidney at the negative control group showed grossly normal renal architecture of the kidney. In the exposed group (0.5% and 2.0% concentrations), histopathological lesions such as infiltrated renal cortex, irregular tubules with obvious glomerular atrophy, distorted renal capsules, infiltrated tubules and abnormal glomerulus and parenchyma surrounding were observed (Figure 6).

3.5.4 Spleen and Lung

The histological examination of the spleen (Figure 7) at the negative control group shows fairly normal architecture of splenic tissue. In the treatment groups, distorted splenic tissue was observed at the lowest and highest concentrations (Fig. 7B and C). The histopathology of the lungs (Figure 8) at the negative control group shows apparently normal lung architecture (Fig. 8A). The groups treated with cassava effluent at different concentrations show slightly distorted lung tissue with increased alveolar

spaces at 0.5% concentration (Fig. 8B) and an infiltrated lung tissue with reduced alveolar spaces at 2.0% concentration (Fig. 8C).

4.0 Discussion

Cyanide is a common cytotoxic agent that kills cells by inhibiting cytochrome oxidase of the electron transport chain. The health significance of cassava effluent that contain this cyanide caused researchers to carry out more work on it and this has led to the development of various research methods used in investigating the cytotoxicity, genotoxicity, mutagenicity and carcinogenicity of this effluent. In this study, Swiss albino male mice were exposed to different concentrations of the effluent and the sperm morphology, peripheral blood erythrocytes, haematological profile, and histopathology of testes, livers, kidneys, spleens and lungs were investigated. The result from the physicochemical analysis of this study confirms that cassava effluent sample contain potentially genotoxic substances such as heavy metals (Pb, Cd, Zn and Fe) and cyanide. Thus, the result revealed that cassava effluent has the ability to induce sperm abnormalities since the formation of normal sperm head involves series of steps and the nuclei that result from these processes are normally very homogenous and have a marked strain specific structural definition [25]. Genotoxic chemicals might increase the frequency of these mistakes [3]. For instance, a spermatid which has a translocation may not form a normal-looking head, hence sperm with abnormal shapes would contain abnormal genetic material [26]. Ubalua, [27] stated that cassava effluent caused problems in some crops. Ivanova *et al*. [28] and Staykova *et al*. [29] have established the genotoxic and mutagenic effects of open water contaminated with heavy metals and cyanide, further confirming the results of the inhibitory effects of these effluents in previous studies [16, 17, 18]. Presence of different types of abnormal sperm observed in this study may lead to reduction of fertility in the male germ cells of experimental animals and human [30].

The sperm abnormalities observed in this study does not correlate with the research carried out by Okunola *et al.* [31]. This could be due to the presence of potentially genotoxic substances present in the cassava effluent. The abnormalities observed in sperm head occur presumably during spermatogenesis [32, 33].

The assumption or criteria for a positive response were also satisfied in this study because the abnormalities almost double the negative control group and was statistically significant at $p<0.05$ in all concentrations when compared to the negative control [22].

The result of the MN assay observed in this study in the exposed group shows an increase in the frequency of micronucleated erythrocytes in a dose dependent manner. An increase in the frequency of MN in treated animals is an indication of induced chromosome damage or aneugenic [33]. This result also indicates that the effluent contain chemicals that are capable of blocking cytokinesis of a normal cell during M phase of the cell cycle leading to formation of a cell with NAs such as binucleated [34]. Binuclei formation in cells may lead to genetic imbalance and carcinogenesis [35].

Our results showed no significant effect of cassava effluent on haematological profile of the experimental mice. The effect of the effluent on haematological profile was generally irregular. This implies that, the effluent may not have serious adverse effect on the total production of RBC by the bone marrow and may not decrease the oxygen-carrying capacity of the whole blood cell, as indicated by the insignificant values of RBC, HCT and Hb across the groups [36]. Also, unaffected MCV, MCH and MCHC indicate that, the cassava effluent may neither interfere with the oxygen-carrying capacity of an individual RBC nor the morphology and osmotic fragility of the RBC [37]. More so, our results indicate that WBC and PLT were not significantly affected by the effluent, suggesting that, cassava effluent may not induce leucopenia and thrombocytopenia respectively.

The histopathology of the testes we observed in this study illustrates some various changes in the testes of the animals that were given varying concentrations of cassava effluent when compared to the negative control (Fig. 4A-D). Administration of the effluents caused histopathology lesions including presence of immature spermatic stages in the lumen of seminiferous tubules, tubular degeneration of the seminiferous tubules, atrophy and hypertrophy. This corresponds to the observation made by Agunbiade *et al.* [8] on *Mus musculus*.

Olorunfemi *et al.* [28] also reported that heavy metals-cyanide interaction in the cassava waste waters was responsible for the anomalies in cell division process and chromosome aberration induction in the *Allium cepa* root meristem. In this study, the histopathological implication of hypertrophy is the retention of spermatids during spermatogenesis and atrophy is the decrease in size of the testes. Likewise, the histopathological analysis of the liver also shows some lesions caused by the effluent in the test animals. Administration of the effluents caused mild histopathology lesions such as distortion and vacuolation in the liver tissue of the mice. These results is in consistence with related study conducted by Adeyemo, [15]. Similarly, histopathological examination of the kidney, gill and liver of the fingerlings of the Nile Tilapia, *Oreochromis niloticus* treated with cassava effluent indicated histological damage [19] but in contrast to the report of Avancini *et al.* [38], wherein the cassava starch fermentation wastewater was of low acute toxicity in mice, having shown slight histological lesions.

The histopathological analysis of the kidney in this study showed aberrations such as distortion of renal corpuscles, infiltrated glomerulus and abnormal parenchyma. This correlates with another study conducted by Adeyemo, [39] who assessed the histopathological effects of cassava effluent on adult female African catfish, *Clarias gariepinus***,** the fish was found to show signs of kidney and liver damage. The degree of this toxicity is directly proportional to the intensity of exposure as the negative control group that was treated with distilled water as it

showed a normal renal architecture. In a study by Agunbiade *et al*. [8], the genotoxic and histopathological effects of the effluents from carbonaceous bottling plant were established on the albino mice. The findings of the study showed that the histological lesions observed in the kidney were characterized by generalized occlusion of the tubular lumen, general tubular necrosis, with protein casts in the tubular lumen and multiple foci of haemorrhage in the parenchyma.

Spleen is a site of direct and indirect toxicity and a target for some carcinogens and also a site for metastasis of malignant neoplasms arising in other sites. In this study, the histopathological analysis of the spleen showed alterations in the splenic tissue in the exposed animals when compare to the negative control.

The histopathology of the lung examined in this study shows slightly distorted lung tissue with increased alveolar spaces, infiltrated lung tissue with reduced alveolar spaces when compared with the negative control. These histopathological lesions observed in the lung may be as a result of heavy metals such as Cd, Pb, Ag and Fe present in the cassava effluent. This result is in line with El-Refaiy and Eissa, [40] wherein the lung tissue of rats treated with Cd revealed severe inflammation and cell proliferation. Exposure to Pb has also been shown to affect cell types, tissues and organ system in animals [41]. Pb substance administered orally, cutaneously or intraperitoneally causes lung cancer in rodents and act synergestically with other carcinogen [42]. Also, Pb exerts multisystemic toxic effects through several mechanisms: by inhibiting enzyme activity, sometimes a consequence of binding to sulfhydryl group, also by altering the structure of cell membranes and receptors and by binding with proteins necessary for cellular functions [43, 44].

Our results show that cassava effluent is genotoxic (causing DNA damage) as well as cytotoxic causing different lesions to various cells and/or tissues. Since most of the physicochemical parameters analyzed especially the heavy metals were

present in high concentration when compared to the allowable limits in drinking water [45, 46], the sperm abnormalities as well as lesions observed in the testes may be thought to be as the result of the presence of cyanide and heavy metals present especially lead (Pb). Lead (Pb) has been reported to inhibit spermatogenesis and contributes to reduction of fertility and induction of congenital malformations in animals [47]. The heavy metals and cyanide present in the cassava effluent are also capable of generating ROS that can cause DNA, protein and lipid damage in eukaryotic [48]. These heavy metals have the capacity to bind to phosphate and base residues of DNA and alter its primary and secondary structures [49]. They can also interfere with protein structure and function to cause DNA damage [50]. Therefore, free radical generation and oxidative damage by these chemicals may be responsible for the observed cytogenotoxic damage in this study [34].

Our finding is therefore useful in further assessment of the toxic effects of chemicals present in the effluent samples on the environment if dumped indiscriminately.

Conclusion

The cytogenotoxic and histopathological effects of the effluents established and heavy metals analyzed from the effluent in this study indicates that cassava effluent contain toxic substances which may constitute a risk to the environment and human health. Since the waste generated from cassava processing is not properly managed, this calls for a better policy option for cassava waste disposal management that will ensure the safety of human population residing around cassava production mill and those exposed through occupation in Nigeria and worldwide. More so, further study should be carried out on this work due to paucity of data on it on terrestrial animals and insignificant results on haematological profiles.



Figure 1: Map of Ilorin showing the point of location of the sample site (blue print)

Table 1: Physicochemical properties of cassava effluent sourced from Olorunsogo cassava mill

Parameters	Cassava effluent	WHO	USEPA	ISI	NESREA
pH	6.30	6.5-8.5	6.5-8.5	6.5-8.5	6-9
Appearance	Turbid	-	-	-	
Odour	Unpleasant	-	-	-	-
BOD	180.30	>6.0	-	-	30
DO	25.60	>30	-	-	
COD	145.60	-	-	-	80
Cyanide (mg/100g)	4.48	-	-	-	-
Total solids	96.80	-	-	-	500
Alkalinity (mg/L)	-		20		
Salinity	-	-	-	-	
TDS (mg/L)	54.10	-	500	-	2000
Lead(mg/L)	0.088	0.05	0.000	0.05	
Cadmium(mg/L)	0.118	0.005	0.005	0.01	
Zinc(mg/L)	0.261	5.0	5.0	5.0	
Iron(mg/L)	0.342	0.1	0.30	0.3	20

USEPA: United States Environmental Protection Agency (1996), NESREA: National Environmental Standards and Regulation Enforcement Agency, WHO: World Health Organization, ISI: Independent Schools Protectorate, BOD: Biochemical Oxygen Demand, COD: Chemical Oxygen Demand, DO: Dissolved Oxygen, TDS: Total Dissolved Solid.

Table 2: Summary of morphological abnormal sperm induced in Swiss albino male mice by different concentration of cassava effluent at 35days

Concentration levels (%)	Total no. of abnormalities	% of abnormalities	Mean ± S.E
Negative control (NC)	148	4.9%	49.33 ± 14.37
0.5%	290	9.6%*	96.66 ± 17.63
1.0%	243	8.1%*	81.00 ± 20.64
2.0%	294	9.8%*	98.00 ± 8.14
Positive control (PC)	373	12.4%*	124.0 ± 22.84

Total no. of sperm scored/concentration = 3000,
Significance level = 0.05, * = Significance difference

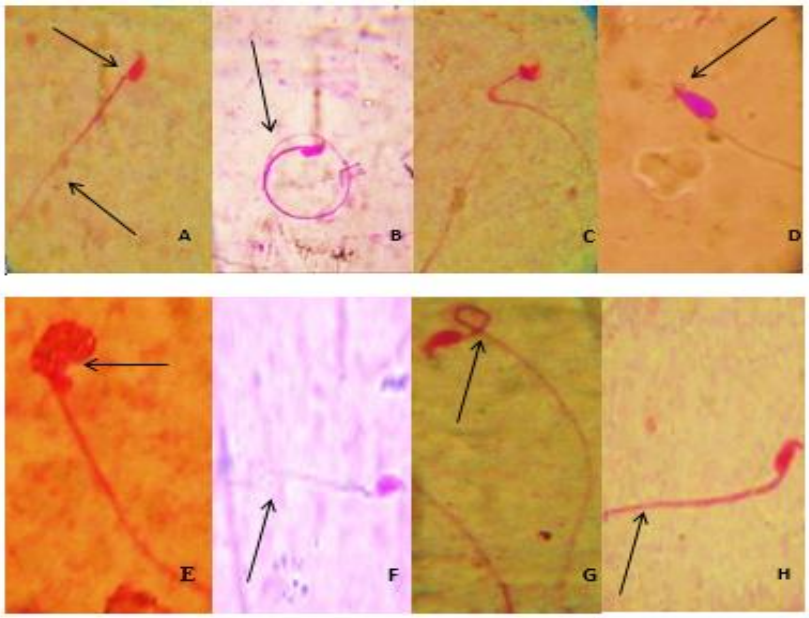


Figure 2: Abnormal sperm cells induced in mice exposed to different concentration of cassava effluent. (A)Normal sperm cell (B) Folded sperm (C) Wrong tail attachment (D) Hook at wrong angle (E) Amorphous head (f) Two tails (G) Long tail (H) Short tail (x1000).

Table 3: Frequencies of Micronucleus and other nuclear abnormalities observed in mice treated with cassava effluent.

Conc. Level	MN	MN Induction X ± SE	Binucleated	Enucleated	Nuclei bud	Polynucleated	Fragmented nucleus	Total NA induction	NA Induction X ± SE
NC	26 (0.87)	8.33 ± 0.33	0	0	0	0	0	0	0.00 ± 0.00
0.5%	234 (7.80)	78.00 ±14.74*	1	0	0	0	0	1 (0.03)	0.33± 0.33
1.0%	295 (9.8)	98.00 ± 6.01*	1	0	0	1	0	2 (0.06)	0.67± 0.33
2.0%	306 (10.20)	102.00 ± 8.89*	2	1	1	0	1	5 (0.16)	1.67 ± 0.33
PC	414 (13.80)	138.00±13.32*	5	2	3	2	1	13 (0.43)	4.33 ± 0.88*

Values in parentheses represent the percentage of occurrence

*Significant at $p<0.05$.

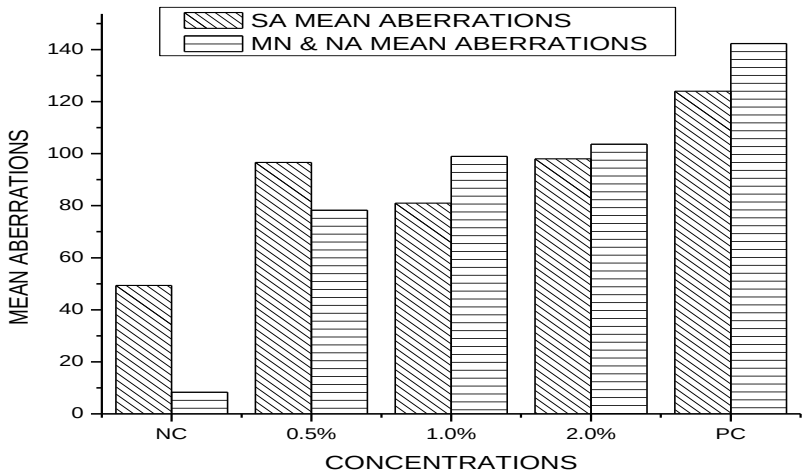


Figure 3: Histogram of mean abnormalities observed in mice exposed to different concentrations of cassava effluent.

SA: Sperm Abnormalities; MN & NA: Micronucleus and Nuclear Abnormalities

Table 4: Effects of cassava effluent on haematological profile.

CONC.	WBC (10^3/µL)	RBC (10^6/µL)	Hb (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (10^3/µL)
NC	2.19±1.3	6.64±1.5	11.50±0.6	44.75±2.5	53.20±1.4	14.50±0.7	27.45±1.3	2.92±4.0
0.5%	2.54±1.9	7.33±1.5	12.45±0.4	39.9±1.30	54.50±0.7	17.00±0.0	31.20±0.4	3.69±5.0
1.0%	1.72±3.5	4.40±3.4	10.40±3.0	38.80±2.7	54.10±0.8	12.60±4.9	23.35±6.8	2.88±1.3
2.0%	1.93±4.1	8.67±1.1	14.10±0.3	45.80±1.2	53.25±1.3	16.55±3.2	31.15±4.9	1.35±1.6

All the parameters are expressed in Mean ± S.E.

No significant at $p<0.05$

WBC: White Blood Cell, **RBC:** Red Blood Cell, **Hb:** Haemoglobin, **HCT:** Hematocrit, **MCV:** Mean Corpuscular Volume, **MCH:** *Mean* Corpuscular *Haemoglobin*, ***MCHC:*** *Mean* Corpuscular *Hemoglobin* Concentration and **PLT:** Platelet

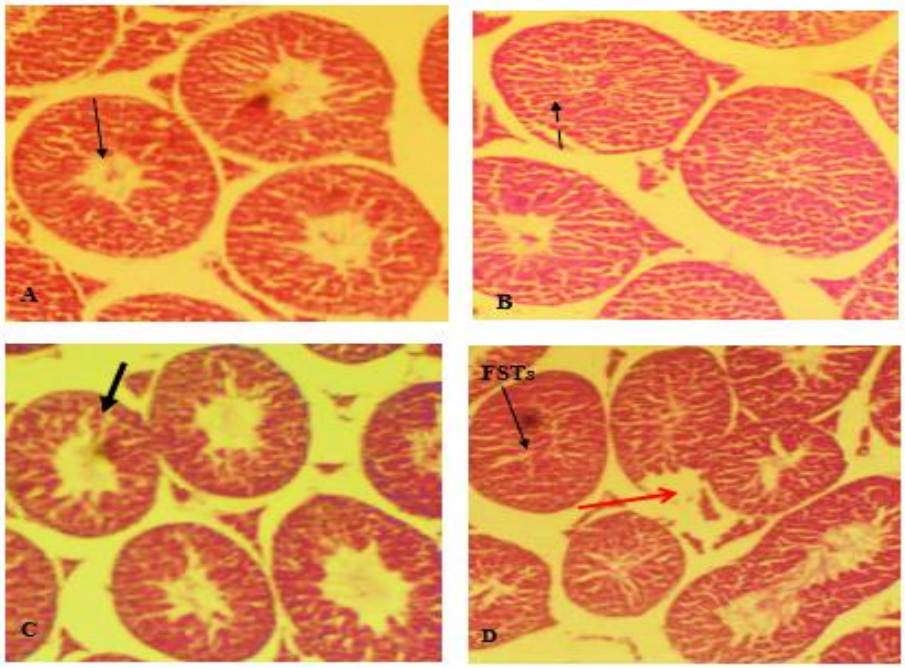


Figure 4: Histopathological analysis of the testes of mice exposed to different concentrations of cassava effluents (Mag. X400).

(A) Negative control-shows closely packed uniformly seminiferous tubules (STs) of regular outlines (Thin arrow). (B) 0.5% of cassava effluent-shows shrinking of STs resulting into atrophy (Dash arrow). (C) 2.0% of cassava effluent-there is slight hypertrophy with well differentiated germ cells. There is also slight degree of degeneration of STs (Thick arrow). (D) Positive control (20mg/kg, cyclophosphamide)-degeneration of the walls of the epithelial (red arrow), fewer leydig cells, shrinking (atrophy) and fusion of the STs (FSTs)

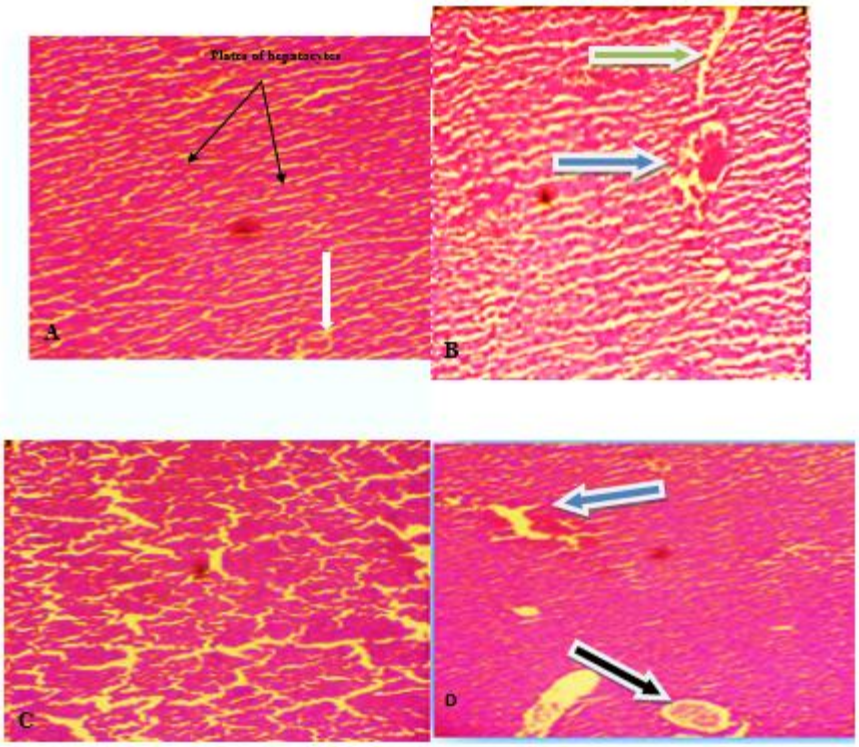


Figure 5: Histopathological analysis of the liver of mice exposed to different concentrations of cassava effluents (Mag. X1000). Green arrow: Infiltration; Blue arrow: Distortion, white arrow: central vein; black arrow: vacuolation.

A: Negative control

B: 0.5% of cassava effluent

C: 2.0% of cassava effluent

D: Positive control (20mg/kg, cyclophosphamide)

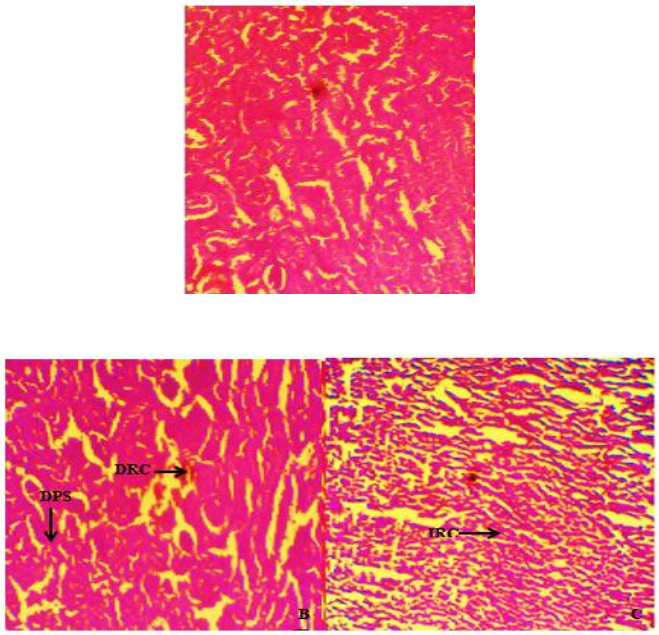


Figure 6: Histopathological analysis of the kidney of mice exposed to different concentrations of cassava effluents (Mag. X1000). A Negative control micrograph shows a grossly normal renal architecture B. 2.0% cassava effluent micrograph shows slightly distorted renal corpuscles (DRC) and distorted parenchyma surrounding (DPS). C. Positive control micrograph shows infiltrated renal cortex (IRC) with irregular tubules

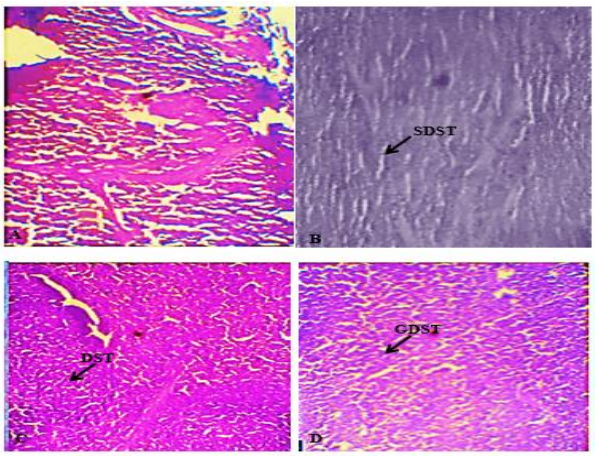


Figure 7: Histopathological analysis of the spleen of mice exposed to different concentrations of cassava effluents (Mag. X1000).

A. Negative control (distilled water): Micrograph shows fairly normal architecture of splenic tissue. B. 0.5% cassava effluent shows a slight distortion of splenic tissue (SDST). C. 1.0% cassava effluent shows distorted splenic tissue (DST). D. Positive control (cyclophosphamide) shows grossly distorted splenic tissue (GDST) with unidentifiable features

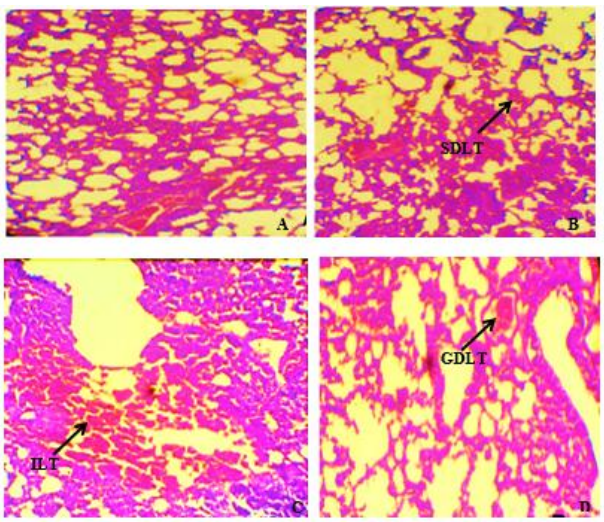


Figure 8: Histopathological analysis of the lung of mice exposed to different concentrations of cassava effluents (Mag. X1000).

A. Negative control (distilled water): Micrograph shows apparently normal lung architecture
B. 0.5% cassava effluent photomicrograph showing slightly distorted lung tissue (STLT) with increased alveolar spaces.
C. 2.0% cassava effluent photomicrography showing an infiltrated lung tissue (ILT) with reduced alveolar spaces.
D. Positive control (cyclophosphamide) photomicrography showing grossly distorted lung tissue (GDLT)

Conflict of Interest

The authors declare no conflict of interest

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