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Evaluation of Genotoxicity by Comet Assay in Tissues of *Clarias gariepinus* Exposed to Cassava Effluent

Daniel I. Olorunfemi [a], Osuvwe C. Orororo [b*], N. E., Iloduba [a], Ejovi Osioma [c], Enyohwo D. Kpomah [c] and Ogheneovo L. Osio [d]

[a] Department of Environmental Management and Toxicology, University of Benin, Benin City, Nigeria.
[b] Department of Medical Biochemistry, Delta State University, Abraka, Nigeria.
[c] Department of Biochemistry, Federal University, Otuoke, Nigeria.
[d] Department of Science Laboratory Technology, Federal University of Petroleum Resources, Effurun, Nigeria.

Authors' contributions

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

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ABSTRACT

Untreated wastewater discharge into waterbodies poses a number of risks, including the potential for aquatic organisms' DNA to be damaged. Using the comet assay, this study assessed the genotoxic impact of cassava wastewater on the gonads, liver, and gills of post-juvenile *Clarias gariepinus*. The post-juvenile *C. gariepinus* fish were acquired from a fish farm in Edo State, Nigeria, and were subjected to different concentrations of cassava wastewater (0.2%, 0.3%, 0.5%,

**Corresponding author: E-mail: osuvwec@yahoo.com;*

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and 0.7%) for a duration of 96 hours. In contrast to the typical actions seen in the control groups, the fish exposed to the effluent exhibited restless, erratic movements, and gasping for air. The first set of repetitions showed that catfish mortality increased with concentration; after 96 hours, exposure to the lowest concentration (0.2%) and the highest concentration (0.7%) of cassava wastewater caused 10% and 40% of the fish to die, respectively. The second set of repetitions showed that catfish mortality increased with increasing concentration; after 96 hours, exposure to the lowest concentration (0.2%) and the highest concentration (0.7%) of cassava wastewater resulted in 20% and 50% catfish mortality, respectively. Following the exposure time, the fish were brought to the lab where their gonads, liver, and gills were removed in order to use the comet assay for genotoxic assessment. The genotoxins present in the wastewater can be the reason for the DNA impairment found in the genotoxic assessment result at different effluent concentrations. The investigation also showed that DNA damage increased with increasing concentration, indicating a dose-dependent genotoxic effect of cassava wastewater on post-juveniles of *C. gariepinus.* This research demonstrates the critical importance of treating wastewater before to discharge.

Keywords: Genotoxicity; comet assay; Clarias gariepinus; cassava effluent.

1. INTRODUCTION

The word "genotoxicity" in genetics describes the existence of chemicals that can damage a cell's DNA and RNA, thereby harming the integrity of the cell. Mutagens that cause genotoxicity, such as radiation and chemicals, can cause damage to DNA or chromosomes and consequent mutations. Damage to embryonic cells can result in inherited abnormalities that cause birth defects, while damage to somatic cells' genetic material may cause cancer in eukaryotic creatures [1].

Researchers examine DNA impairment in cells exposed to these dangerous substances so as to determine the genotoxicity of various substances. Single- or double-strand breaks, point mutations, loss of excision repair, cross-linking, and structural or numerical anomalies in chromosomes are examples of this type of DNA damage. It is acknowledged that genetic material compromise has a role in the development of malignant diseases marked by unchecked cell expansion. This result has led to the development of a variety of complex techniques, including in vitro and in vivo toxicological examinations, the Ames Test, and the Comet Assay, to evaluate a substance's potential to instigate damage to genetic material that could ultimately lead to the advancement of cancer [2-5].

One major global concern is the possibility of genotoxic chemical contamination of water. Mutagens in drinking water can have a negative impact on people's health, possibly leading to the development of cancer. Water ecosystems can also be harmed by environmental contamination [6]. Therefore, a great deal of study has been done to assess the mutagenic potential of drinking water as well as surface and groundwater sources. This research has revealed a wide range of materials that have the prowess to harm DNA [7].

Some refer to cassava (*Manihot esculenta*) as the "bread of the tropics," the "food of the poor," or even the "poverty fighter." It is a staple crop that is grown on a local scale by farmers in Nigeria and other tropical African countries. Because of its drought resistance and versatility in a range of soil types, including marginal ones, cassava is an important source of carbohydrates in tropical Africa. Numerous high-calorie foods, including garri (toasted granules), fufu (fermented cooked paste), starch, tapioca, and other confections, can be made from cassava tubers [8,9]. On the other hand, processing cassava generates a significant amount of liquid and solid waste. Leaves, stems, peels, effluent, and starch bagasse are some of these byproducts. Despite the obvious harm these methods inflict on the environment and public health, Nigerian farmers frequently dispose of these wastes carelessly in open spaces near major roads and streets, undeveloped land plots, unfinished buildings, and water bodies [8,10]. About 60–70% of raw cassava tubers are made up of water, much of which is converted into wastewater during processing. Furthermore, a significant amount of water is used in the processing of cassava roots into food items like starch and garri, which is subsequently released into the environment. Remarkably, depending on the intended end product, processing one ton of cassava tubers can produce nothing less than 250 kg of wastewater [8].

Due to its enormous cyanide content and significant chemical oxygen demand (COD), cassava wastewater poses significant environmental risks and if left untreated, can contaminate soil, rivers, and groundwater. Cassava is naturally high in cyanogenic glycosides, which can hydrolyze into hydrogen cyanide during processing, especially during pressing and washing. This release of cyanide into the environment, in the form of hydrocyanic acid, poses a significant environmental hazard [11]. High concentrations of cassava effluent negatively affect the physicochemical parameters of water, which in turn affect aquatic life. Using cassava effluent-contaminated water for irrigation can stunt plant growth or even cause plant death, depending on the concentration of cyanide.

The effluents from sewage treatment plants, industrial wastewater, and agricultural runoff are the main sources of surface water contamination that contain different genotoxic chemicals. A small number of harmful substances can not only be analyzed for surface water monitoring to be successful, given the diversity of chemical contaminants present. Although very sensitive, genotoxicity studies that rely on the metabolic or enzymatic activities of test organisms are not very specific. Therefore, in order to reliably determine the effects of genotoxic chemicals on a wide variety of organisms, a combination of biotests involving eukaryotic as well as prokaryotic organisms (fish, mussels, Daphnia, bacteria and algae) becomes required [12].

One method for quantifying DNA strand breakage at the individual cell level is the comet assay. Known also as single-cell gel electrophoresis (SCGE), this method is frequently used to evaluate the deleterious effects of toxins on genetic material. Because of its wide variety of uses, simplicity, sensitivity, adaptability, and time economy, this method is widely recognized for evaluating DNA damage. It is extensively employed in environmental monitoring to evaluate the effects of different toxicants on aquatic life. Environmental research has made extensive use of this assay, especially for assessing the genotoxicity of pollutants and toxins in aquatic environments. It makes it possible for scientists to assess how environmental stressors impact aquatic animals' DNA integrity [13]. To summarize, the comet assay involves applying single-cell suspensions in agarose to microscope slides, treating them with detergent and concentrated NaCl to break down cell membranes and extract histones, and then electrophoresizing the results. When DNA strands break and are observed under fluorescence microscopy, they travel toward the anode and resemble comets when dyed fluorescently [14].

The African catfish (*Clarias gariepinus*), is a popular freshwater fish farmed in Nigeria because of its quick growth and tolerance to a variety of environments. For toxicological research, it has been widely utilized as a model [15,16]. African catfish are a significant source of protein, and their health a good measure of the quality of their habitat. Because African catfish respond well to ecological contamination, many researchers have selected it as a bio-indicator for genotoxicity and oxidative stress [17-19].

Fish are important members of aquatic ecosystems since they can react to low levels of hazardous compounds in the water directly and indirectly through eating polluted aquatic creatures. Hence, according to Cavas and ErgeneGözükara [20], fish can serve as markers for the early detection of contamination in the maritime environment. A primary factor contributing to water contamination is the careless disposal of effluents into bodies of water. Given the economic significance of fish to humans and the high toxicity of unprocessed cassava products and effluents [21], more research evaluating the toxic effects of cassava waste water on fish is required, even though some studies have been carried out on the genotoxic effect of cassava wastewater on the African Catfish [18,22-23]. Additionally, there aren't many research using the comet assay to explain the genotoxic effects of cassava effluent on *Clarias gariepinus*. Therefore, this study evaluated the genotoxic effects of cassava effluent on selected tissues of *C. gariepinus* using the comet assay.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Iguedo, Usen district of Ovia South-West Local Government Area, Edo State, Nigeria. Iguedo is situated at Ovia Local Government Area at Latitude 6.72306 East and Longitude 5.38609 North (Fig. 1). The effluent was collected from a cassava processing industry at the point of discharge.

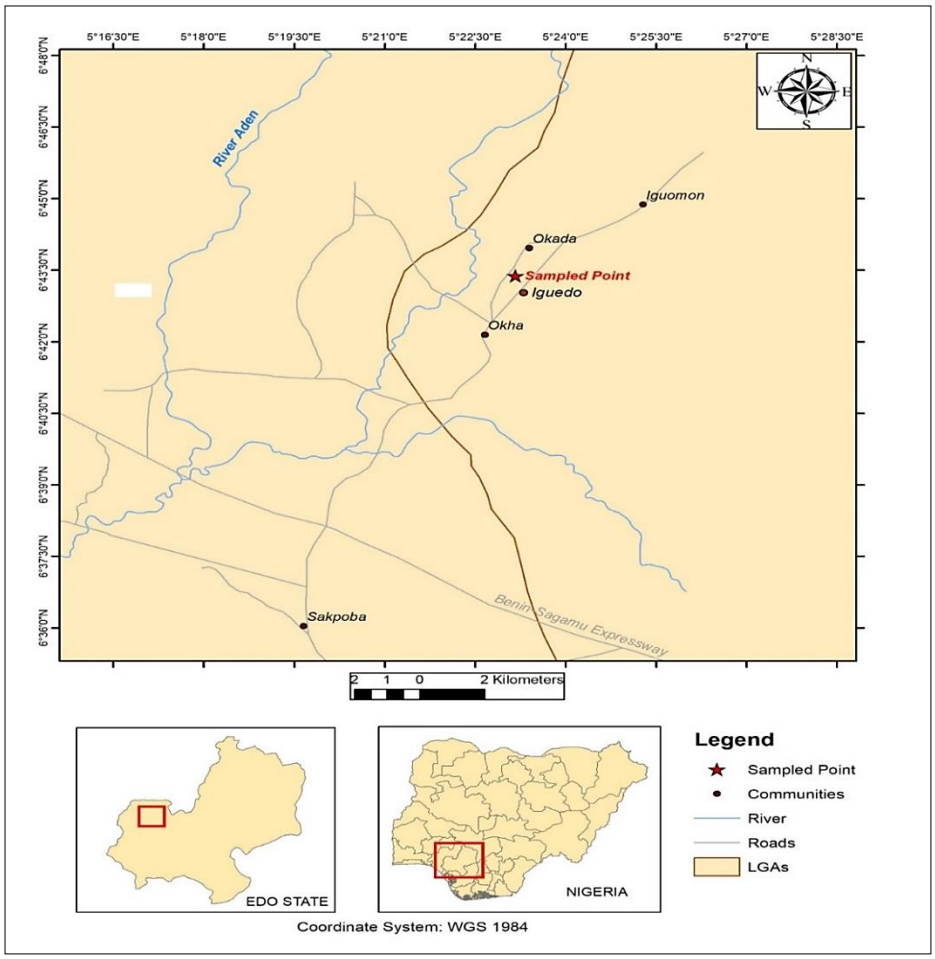

Fig. 1. Diagram illustrating the study's geographic region

Test animal: collection and acclimatization: One hundred post-juvenile *Clarias gariepinus* fish were acquired from Vinvok farms in Ororokpe, Delta State, Nigeria. They were then transported to the Wet Laboratory, Department of Fisheries, Faculty of Agriculture, University of Benin, Benin City, Edo state, Nigeria, under well-ventilated circumstances. Before the test, the fish were given five days to get used to the lab environment. The fish were fed commercial fish meal twice a day during the adaption period, and the water in the tanks where they were housed was changed daily. The fish were exposed to the cassava effluent after the adaptation phase.

Treatment fish samples and acute toxicity tests: After a series of tests, concentrations were chosen. Concentrations were selected following an array of experiments. For 96 hours, ten fish were exposed to every concentration. The fish were subjected to tests on five concentrations that were prepared by serial dilution: 0.2%, 0.3%, 0.5%, 0.7%, and control (distilled water). To prevent contamination of the test environment, the number of dead fish was counted at the commencement of the tests and then every 24 hours after that. The fish were then removed and disposed of appropriately [21]. When fish showed no indications of life and their carcasses were either sinking to the bottom of the test solution or floating horizontally close to the surface, they were declared dead. To prevent repeating the test if the first setup failed, a replicate per test concentration was employed, which also served to strengthen the statistical baseline.

2.2 Comet Assay Protocol

Extraction of organs and Slide Preparation: The fish samples are sacrificed and their gills, liver and gonads were excised and placed in separate well labelled eppendorf tubes. Comet assay slides are sterilized and allowed to dry. The slides are then labelled using a diamond pen and were then embedded in 0.5% low melting point agarose gel and thereafter placed on the

slides and covered with cover slips. The coverslip was gently removed after a while and 1% low melting point agarose was added. The slides were then dried and kept on a tray.

Lysing: The cells are lysed to release DNA. This was done using a solution containing sodium hydroxide, EDTA, Triton-X and DMSO for 24 hours. Sodium hydroxide was used for the osmotic pressure for the destruction of the cell membrane. EDTA chelates co-factors which are usually divalent metals that affect nuclear activities. DMSO helps in osmotic pressure. Triton-X acts as a detergent and breaks the phospholipid bilayer of animal cells.

Electrophoresis: The DNA samples were then subjected to electrophoresis, which caused the DNA to move through the agarose gel. It involved the use of an electrophoresis buffer that contains sodium hydroxide and EDTA. This allowed the DNA to separate into fragments. This was done by placing the slides into electrophoresis tank and passing current of 300 mA at 25V through it for thirty minutes. The process was conducted in the dark to avoid light-induced damage to the DNA samples.

Neutralization and Fixation: The slides were neutralized with Tris to keep the pH at 7.4. Neutralization was done three times every five minutes to stop the electrophoresis process. The slides were then fixed with cold ethanol and allowed to dry. Application of cold ethanol dehydrated any gel left on the slide and prevented contamination of the slide.

Staining: The slides were then stained with Giemsa stain and rinsed with distilled water. This allowed the DNA to be seen under a microscope.

Viewing and Scoring: The DNA fragments were then imaged with the aid of a light microscope fitted with a camera. The visual representations were thereafter analysed to determine the amount of DNA damage in the sample. A comet comprises a head and a tail, with the tail indicating the extent of DNA damage.

Analysis: Using image analysis techniques on individual cells is the most versatile way to gather comet data, and there are a number of commercially available specialist software packages for this purpose. Individual comet photos were examined for a number of parameters using Graph Pad Prism A, such as DNA content (overall intensity), head and tail length, DNA percentage in tail, and tail moment (fraction of migrating DNA multiplied by a tail length measurement). The most often reported measurements of these are tail moment and/or tail length; the usage of percent DNA in the tail provides a clear indicator of the comet appearance and is also linearly related to the frequency of DNA breaks over a broad range of damage levels. Therefore, the comet tail moment parameter (TM) was employed in this investigation to ascertain the extent of DNA damage. It was computed by multiplying the comet tail length by the DNA proportion. The tail moment is the sum of the comet tail length, which represents the smallest observable size of moving DNA, and the tail DNA intensity, which represents the quantity of relaxed/broken bits [24].

3. RESULTS

3.1 Acute Toxicity Mortality Rate of *Clarias gariepinus* to Cassava Effluent Exposure

Table 1 displays the mortality rates of *C. gariepinus* when exposed to cassava wastewater. In the first set of replicates, exposure to the lowest concentration (0.2%) and the highest concentration (0.7%) of cassava wastewater resulted in 10% and 40% catfish mortality, respectively, after 96 hours, demonstrating an increase in mortality as the concentration increased. In the second set of replicates, exposure to the lowest concentration (0.2%) and the highest concentration (0.7%) of cassava wastewater led to 20% and 50% catfish mortality, respectively, after 96 hours, also indicating a rise in mortality with increasing concentration. Behavioural responses were observed to be dose dependent, and showed direct proportion with increased concentrations. As concentrations of the test effluent increased, the fish samples displayed initial disturbed swimming movements. This was accompanied with avoidance response manifested via surfacing behaviour. This was followed by sudden quick movement, unusual lethargy, and bleaching of the skin. It was also observed that the fish samples settled at the bottom of the aquaria. They were motionless and had slow opercula movements as they gulped for air. Fishes exposed to 100% concentration of cassava wastewater died in three minutes. Fishes in control (0% concentration) showed normal behaviour.

Table 1. Mortality rate of *Clarias gariepinus* exposed to varied concentrations of cassava wastewater

Concentration (%)	Duplicate mortality hour 1				Duplicate mortality hour 2			
	24	48	72	96	24	48	72	96
Control	0	0	0	0	0	0	0	0
0.2	0	0	1	0	0	1	1	0
0.3	0	1	0	1	0	0	1	1
0.5	2	1	0	1	1	0	0	1
0.7	2	1	1	0	2	2	0	1

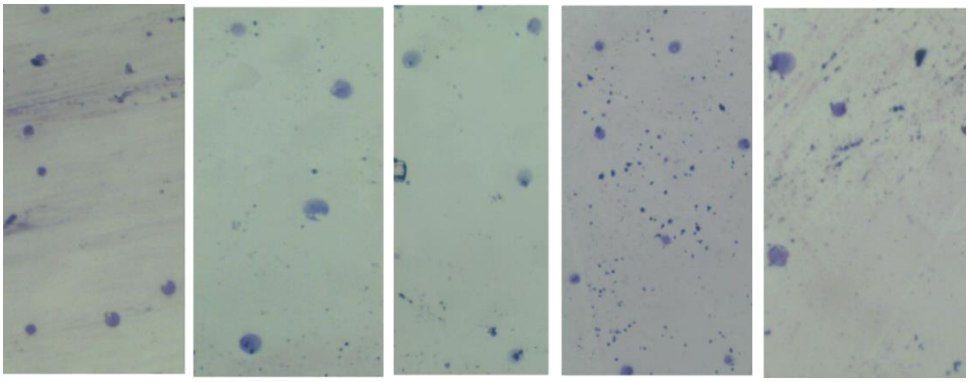

Plate 1. Micrographs of DNA damage in the gills of *Clarias gariepinus* dosed with cassava wastewater at concentrations 0 %, 0.2 %, 0.3 %, 0.5 % and 0.7 %

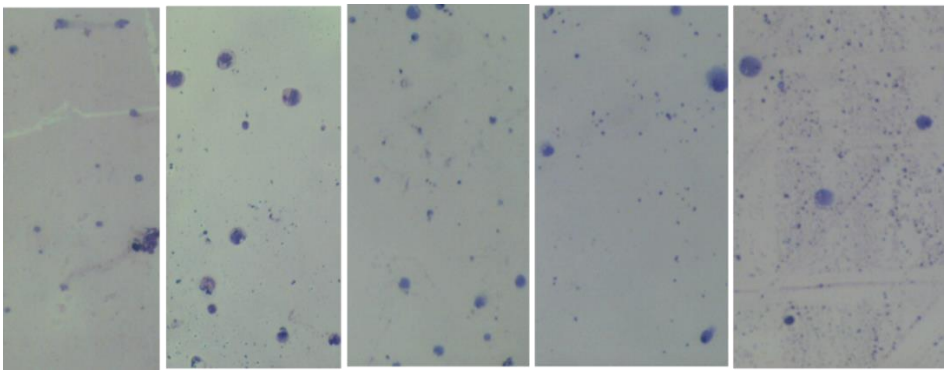

Plate 2. Micrographs of DNA damage in the gonads of *Clarias gariepinus* dosed with cassava wastewater at concentrations 0 %, 0.2 %, 0.3 %, 0.5 % and 0.7 %

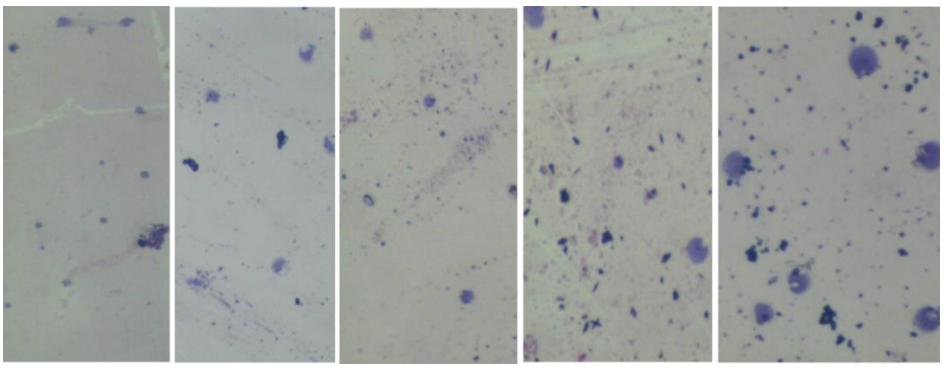

Plate 3. Micrographs of DNA damage in the liver of *Clarias gariepinus* dosed with cassava wastewater at concentrations 0 %, 0.2 %, 0.3 %, 0.5 % and 0.7 %

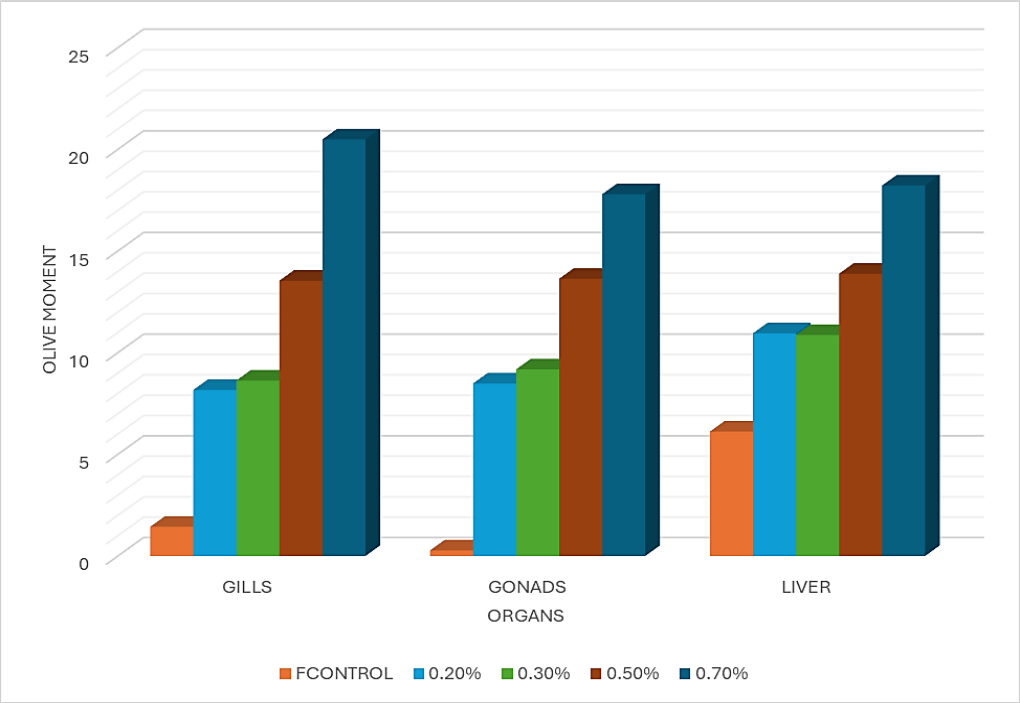

Fig. 2. Genotoxic potential of cassava on *Clarias gariepinus*

3.2 Genotoxicity in Tissues of *Clarias gariepinus* Exposed to Cassava Effluent by Comet Assay

The genotoxic effects of Cassava Effluent in tissues of *Clarias gariepinus* is displayed in Plates 1 (gills), 2 (gonads) and 3 (liver), while Fig. 2 shows the total genotoxic potential of Cassava effluent on *Clarias gariepinus.* The result showed that cassava wastewater caused DNA damage in the tissue of African Catfish. The genotoxic effect of the wastewater increased at higher concentration indicating that the effect was concentration dependent. The outcome indicated a higher degree of DNA damage in both the gonads and liver at lower concentration of 0.2% and 0.3% respectively. The gills showed higher DNA damage at higher concentration of 0.7% compared to other organs.

4. DISCUSSION

This study used the comet assay to assess the genotoxic effects of cassava effluent on *Clarias gariepinus* tissues. Fish are essential to aquatic habitats because they are multifunctional members of the food chain. They react indirectly by eating contaminated aquatic creatures and show direct susceptibility to even very small quantities of harmful compounds in the water [20]. Fish can therefore act as early warning signs of pollution of aquatic ecosystems. Fish are good indicators of wastewater contamination in aquatic environments because of their frequent metabolic oscillations in reaction to wastewater [25]. Wastewaters and effluents are a substantial contribution to water pollution.

Death was caused by exposure to both low and high concentrations of cassava effluent in a dose-dependent manner (Table 1). Behaviors like erratic swimming patterns, avoidance-related surfacing behaviors that resulted in skin bleaching, abrupt, rapid movements, uncommon lethargy, and fish that settled at the lowermost part of the aquarium in a motionless state with sluggish opercula movements and gasping for air were also dose-dependent. Additionally, they are comparable to other related investigations [25]. The outcome provided a clear picture of how the effluent affected the aquatic habitat and aquatic organisms. Since we all come into contact with water and depend on it for living, this effect extends beyond aquatic habitats to other ecosystems and organisms.

The comet assay is used to quantify DNA strand breaks, an important indicator of genotoxicity in fish [26]. As a result of exposure to a variety of pollutants present in aquatic settings, the comet test is a commonly used, simple, and highly sensitive tool for evaluating DNA damage in

several fish organs, including gills, liver, gonads, kidney, and blood [27]. Under alkaline circumstances, the comet assay can detect multiple types of DNA damage, such as single-strand breaks, alkaline labile sites, and wastewater-induced DNA cross-links [28]. (Kumar et al., 2010). Since the comet assay does not require cells to be dividing mitotically, it has significant advantages over conventional cytogenetic techniques [29-31].

The study's findings demonstrate that African catfish tissue suffers DNA damage from cassava wastewater. Higher concentrations of the wastewater had a greater genotoxic effect, suggesting that the effect was concentration dependent. The results showed that the liver and gonads had more DNA damage at lower concentrations of 0.3% and 0.2%, respectively. Elevated levels of DNA damage in gill cells may be caused by the gills' ongoing exposure to water contaminants that can harm DNA. Fish gills are essential organs that help in breathing, osmoregulation, and excretion. Gills are the main point of entry for effluents, hence they are good candidates for environmental impact studies since they are frequently regarded as great indicators of water quality. Respiratory distress can result from any damage to the gills caused by xenobiotic substances or modifications in membrane permeability, which can have a substantial impact on oxygen absorption rates [22].

It is well known that a variety of genotoxic chemicals generate electrophilic free-radical metabolites and reactive oxygen species, which interact with DNA and cause disruption [32-34]. Research conducted by Chandra and Khuda-Bukhsh [35] has demonstrated that the metabolism of azadirachtin generates free radicals and electrophilic ions that interact with nucleophilic sites in DNA, causing breaks and associated damage. According to Moore, et al. [36], oxidative stress occurs naturally in an organism and is a major factor in the induction of cytotoxicity and genotoxicity in a number of critical tissues. According to Olourunfemi et al. [23], the oxidative stress caused by the effluent's constituents in the fish tissues may be the cause of the genotoxic effects of cassava effluent that were reported in the study.

Fish samples showed indications of liver and gill damage in a similar investigation examining the haematological and histological effects of cassava effluent on adult female African catfish, *Clarias gariepinus*. According to previous studies, histological analyses of the kidney, gills, and liver of Nile Tilapia fingerlings (*Oreochromis niloticus*) and *Clarias gariepinus* subjected to cassava effluent and other agro-industrial waste waters revealed damage [37-39]. It is clear that cassava wastewater can be dangerous to people if it is not properly cleaned before being released into water bodies. It is also harmful to aquatic life.

The inhibition of enzymes involved in DNA replication or repair may also be a significant contributing factor to DNA damage [40]. Normal cellular functions depend on the integrity of DNA, and changes to DNA can cause tissue development, cell division, loss of cell structure, and degradation, which can ultimately lead to the loss of cellular regulatory systems. According to studies [31,40], wastewater and cellular DNA interact physicochemically to cause a variety of primary alterations in DNA, such as single-strand breaks, double-strand breaks, DNA-protein crosslinks, and damage to purine and pyrimidine bases. These findings are consistent with the results obtain from this investigation.

5. CONCLUSION

According to the study, the comet assay method may be used to determine the genotoxic effects of cassava effluent in the tissues of the African cat fish, *C. gariepinus.* It's possible that the hazardous chemical cyanide is what causes the genotoxic consequences of cassava effluent. Therefore, it's crucial to adequately treat effluent from the cassava processing companies before releasing it into bodies of water. Regulatory organizations should also be established to keep an eye on the effluent discharge procedure and production process in order to protect the aquatic ecology.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mohamed SAKS, Upreti S, Rajendra SV, Dang R. Genotoxicity: Mechanisms, testing

guidelines and methods. Global J Pharm Pharma Sci. 2017;1(5):133-138.
2. Nagarathna PKM, Wesley MJ, Reddy PS, Reena K. Review on genotoxicity, its molecular mechanisms and prevention. Int J Pharm Sci Rev Res. 2013;22(1):236-243.
3. Turan F, Eken M, Ozyilmaz G, Karan S, Uluca H. Heavy metal bioaccumulation, oxidative stress and genotoxicity in African catfish *Clarias gariepinus* from Orontes river. Ecotoxicology. 2020, Nov;29:1522-37.
4. Nwani CD, Somdare PO, Ogueji EO, Nwani JC, Ukonze JA, Nwadinigwe AO. Genotoxicity assessment and oxidative stress responses in freshwater African catfish *Clarias gariepinus* exposed to fenthion formulations. Drug and Chemical Toxicology. 2017, Jul 3;40(3):273-80.
5. Nnaji KC, Nwamba HO, Nwani CD. Behavioral, Biometric and oxidative stress-induced alterations in *Clarias gariepinus* juveniles exposed to chlorpyrifos. Asian J. Fish. Aqu. Res. 2024, Jan. 13;26(1):52-60. [cited 2024 Jun. 19] Available:https://journalajfar.com/index.php/AJFAR/article/view/726
6. Efekemo O, Davies IC, Orororo CO. Water quality assessment and heavy metal levels in mudskipper (Periophthalmus Papilio), sediments and water of mangrove swamps, Rivers State, Nigeria. Afri J Environ Nat Sci Res. 2024;7(1):128-145.
7. Ferk F, Mišík M, Grummt T, Majer B, Fuerhacker M, Buchmann C, Vital M, Uhl M, Lenz, K, Grillitsch B, Parzefall W. Genotoxic effects of wastewater from an oncological ward. Mut Res/Genetic Toxic Environ Mut. 2009; 672(2):69-75.
8. Oghenejoboh KM. Value added cassava waste management and environmental sustainability in Nigeria: A review. Environ Challen 2021;4:100127.
9. Tonukari NJ, Egbune EO, Avwioroko OJ, Orororo OC, Anigboro AA. A novel pig feed formulation containing *Aspergillus niger* CSA35 pretreated cassava peels and its effect on growth and selected biochemical parameters of pigs. Afri J Biotech. 2016;15(9):776-785.
10. Orororo OC, Tonukari NJ, Avwioroko OJ, Ezedom T. Effect of supplementation of animal feed with dried cassava (*Manihot esculenta*) peels, and stems of *Vernonia amygdalina* and *Pennisetum purpereum* on some biochemical parameters in Pigs. Nig Soc Exp Bio (NISEB) J. 2014;14(4):177-183
11. Fawole O, Yekeen T, Ayandele A, Ajayi O, Aina O, Omosehin O, Odebunmi A. Cassava mill effluent toxicity assessment using two bioassays and bacterial attributes. Poll Res. 2014;32:243-250.
12. Lah B, Malovrh S, Narat M, Cepeljnik T, Marinsek-Logar R. Detection and quantification of genotoxicity in wastewater- treated Tetrahymena thermophila using the comet assay. Environ Toxico. 2004;19(6):545-553.
13. De Lapuente J. The comet assay and its applications in the field of ecotoxicology: A mature tool that continues to expand its perspectives. Frontiers Gen. 2015;6(180).
14. Cordelli E, Bignami M, Pacchierotti F. Comet assay: A versatile but complex tool in genotoxicity testing. Toxico Res. 2021;10(1):68-78.
15. Mahboub HH. Silica nanoparticles are novel aqueous additive mitigating heavy metals toxicity and improving the health of African catfish, *Clarias gariepinus*. Aqua Toxico. 2022;249:106238.
16. Busari AA, Efejene IO, Olayemi SO, Orororo OC, Egbune EO. Evaluation of antibiotic use and analysis of ciprofloxacin and gentamicin residue in fish samples from farms in Lagos, Nigeria. Environ Mon Assess. 2024;197(2):127.
17. Osman AGM. Genotoxicity tests and their contributions in aquatic environmental research. J Environ Protect. 2014;5(14): 1391–1399.
18. Ariyomo TO, Jegede T, Adeniran AF, Omobepade BP. Toxicity of cassava effluents to catfish, *Clarias gariepinus*, and the effect on some target organs. Ann Aqua Res. 2017;4(2):1034.
19. Zaghloul K. Genotoxic and histopathological effects of water pollution on *Clarias gariepinus* fish at fayoum governorate, Egypt. Nat Res. 2020;11(11): 499–519
20. Cavas T, Ergene-Gözükara S. Induction of micronuclei and nuclear abnormalities in Oreochromis niloticus following exposure to petroleum refinery and chromium processing plant effluents. Aqua Toxic. 2005;74:264–271.
21. Egbune OU, Egbune EO, Orororo OC, Ezedom T, · Onojakpor O, Sabo AM, Amadi K. Chronic cassava meal modulates body weight, histology and weight of

reproductive organs in male albino rats. Toxico Environ Health Sci; 2023. Available:https://doi.org/10.1007/s13530-023-00179-4

22. Olufayo MO, David OT. The effect of cassava mill effluent on histology of *Clarias gariepinus* juveniles. Revue Scientifique Et Technique Forêt Et Environnement Du Bassin Du Congo. 2016;7:10–17.
23. Olorunfemi DI, Oteri O, Orororo OC, Tina-Bayoko BE, Pere C, Osioma E. Genotoxic effects of cassava effluent on the expression of selected genes in the african catfish, *Clarias gariepinus.* J Adv Bio Biotech. 2024;27(5):352-364.
24. Sogbanmu TO, Osibona AO, Oguntunde AO, Otitoloju AA. Biomarkers of toxicity in *Clarias gariepinus* exposed to sub-lethal concentrations of polycyclic aromatic hydrocarbons. Afr J Aquat Sci. 2018;43: 281-92
25. Cattaneo R, Clasen B, Loro VL, de Menezes CC, Pretto A, Baldisserotto B, De Avila LA. Toxicological responses of Cyprinus carpio exposed to a commercial formulation containing glyphosate. Bull Environ Contam Toxi. 2011;87:597–602.
26. Mitchelmore CL, Chipman JK. Detection of DNA strand breaks in brown trout (*Salmo trutta*) hepatocytes and blood cells using the single cell gel electrophoresis (comet) assay. Aqua Toxico. 1998;41:161–182.
27. Dhawan A, Bajpayee M, Parmar D. Comet assay: A reliable tool for the assessment of DNA damage in different models. Cell Bio Toxico. 2009;25:5–32.
28. Kumar R, Nagpure NS, Kwshwaha B, Srivastava SK, Lakra WS. Investigation of the genotoxicity of malathion to freshwater teleost fish Channa punctatus (Bloch) using the micronucleus test and comet assay. Arch Environ Contam Toxico. 2010; 58:123–130.
29. Ali D, Kumar S. Long-term genotoxic effect of monocrotophos in different tissues of freshwater fish Channa punctatus (Bloch) using alkaline single cell gel electrophoresis. Sci Total Environ. 2008; 405(1):345– 350.
30. Ali D, Nagpure NS, Kumar S, Kumar R, Kushwaha B, Lakra WS. Assessment of genotoxic and mutagenic effects of chlorpyrifos in freshwater fish Channa punctatus (Bloch) using micronucleus assay and alkaline singlecell gel electrophoresis. Food Chem Tox. 2009; 47(3):650–656.
31. Nwani CD, Lakra WS, Nagpure NS, Kumar R, Kushwaha B, Sirvastava SK. Mutagenic and genotoxic effects of carbosulfan in freshwater fish Channa punctatus (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. Food Chem Toxico. 2010; 48(1):202–208.
32. Orororo OC, Asagba SO, Tonukari NJ, Okandeji OJ, Mbanugo JJ. Cadmium-induced testicular damage in wistar rats: Protective effects of *Hibiscus sabdariffa* L. anthocyanins. Int J Biochem Res Rev. 2018;21(4):1-8.
33. Orororo OC, Efekemo O, Udi OA. Changes in liver histology, hematological parameters and lipid profile of cadmium-exposed rats treated with combined leaf extract of *Vernonia amygdalina* and *Occimum gratissimum.* Asian J Med Health. 2022;20(11):195-203.
34. Efekemo O, Orororo OC. Effect of mixture of leaf extract of *Ocimum gratissimum* and *Vernonia amygdalina* on cadmium induced hepatotoxicity in wistar rats. J Biochem Int. 2022;9(4):73-81.
35. Chandra P, Khuda-Bukhsh AR. Genotoxic effects of cadmium chloride and azadirachtin treated singly and in combination in fish. Ecotox Environ Safety. 2004;58:194–201.
36. Moore PD, Yedjou CG, Tchounwou PB. Malathion-induced oxidative stress, cytotoxicity and genotoxicity in human liver carcinoma (HepG2) cells. EnvironToxico. 2010;25(3):221–226.
37. Amaeze NH. Comparative assessment of the acute toxicity, haematological and genotoxic effects of ten commonly used pesticides on the African Catfish, *Clarias gariepinus* Burchell 1822. Heliyon. 2020; 6(8):e04768.
38. Alimba CG, Adekoya KO, Soyinka OO. Exposure to effluent from pharmaceutical industry induced cytogenotoxicity, hematological and histopathological alterations in *Clarias gariepinus* (Burchell, 1822). PubMed. 2019;18:63–78.
39. Ayanda OI, Tolulope A, Oniye SJ. Mutagenicity and genotoxicity in juvenile African catfish, *Clarias gariepinus* exposed to formulations of glyphosate and paraquat. Sci Prog. 2021;104(2):1-5.

40. Guilherme S, Santos MA, Barroso C, Gaivao I, Pacheo M. Differential genotoxicity of roundup formulation and its constituents in blood cells of fish (*Anguilla anguilla*): Considerations on chemical interactions and DNA damaging mechanisms. Ecotoxico. 2012;21:1381–1390.

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