GENOTOXICITY BY COMET ASSAY IN TISSUES OF *Clarias gariepinus* EXPOSED TO CASSAVA EFFLUENT IN BENIN CITY

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AN UNDERGRADUATE PROJECT SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY.

SEPTEMBER, 2023

CERTIFICATION

This is to certify that this research titled **Assessment Of Genotoxicity By Comet Assay In Tissues Of Clarias Gariepinus Exposed To Cassava Effluent In Benin City** was carried out by **Nathalie Ebelechukwu Iloduba (Miss)** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City, in partial fulfilment of the requirements for the award of Bachelor of Science (B.Sc) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met requirements for the award of Bachelor of Science Degree in Environmental Management and Toxicology.

…… ……...

PROF D.I. Olorunfemi **Date**

Project Supervisor

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PROF Alex. A. Enuneku **Date**

Head of Department

DECLARATION

I **NATHALIE EBELECHUKWU ILODUBA** declare that **ASSESSMENT OF GENOTOXICITY BY COMET ASSAY IN TISSUES OF CLARIAS GARIEPINUS EXPOSED TO CASSAVA EFFLUENT IN BENIN CITY** is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

Nathalie Ebelechukwu Iloduba (Miss)

……..

Date

DEDICATION

I dedicate this report to almighty God, for his unceasing and unending grace to always push through. A special feeling of gratitude to my loving parents Mr and Mrs Iloduba. I also dedicate this report to my sisters and friends who have supported me throughout the process.

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ABSTRACT

The discharge of wastewater into waterbodies without proper treatment has led to several threats such as DNA damage to aquatic species. This study evaluates the genotoxic effect of cassava wastewater on the gonads, liver and gills of post juvenile *Clarias gariepinus* using comet assay. The post juveniles of *Clarias gariepinus* was purchased from fish farm located in Delta State and was exposed to various concentrations (0.2%, 0.3%, 0.5%, 0.7%) of cassava wastewater for 96 hours. Unlike normal behaviours observed in the control groups, the fish exposed to the effluent were restless, erratic in their movement and gasping for breath. After the exposure period, the fishes were transported to the laboratory and the cells of their liver, gills and gonads were extracted for genotoxic assessment using comet assay. The result obtained from the genotoxic assessment revealed DNA damage at varying concentrations of the wastewater. The study revealed that genotoxins present in the wastewater were obviously responsible for the DNA damage in the tissues in the organism. The study also revealed that the genotoxic effect of cassava wastewater on post juveniles of *Clarias gariepinus* is dose dependent as DNA damage increases with increased concentration. This study shows that the treatment of wastewater before disposal is very important.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Genotoxicity, a term within genetics, refers to the presence of substances that can harm the genetic material of cells (DNA and RNA), thereby compromising cell integrity. Genotoxins, which can include chemicals and radiation, are mutagens that can lead to genotoxicity, resulting in DNA or chromosomal damage and subsequent mutations. It's worth noting that genotoxicity is sometimes confused with mutagenicity.

While all mutagens possess genotoxic properties, it's important to note that not all genotoxic substances exhibit mutagenic effects. When the genetic material in somatic cells sustains damage, it can potentially trigger cancer in eukaryotic organisms, whereas damage to germ cells can result in hereditary mutations that lead to birth defects (Mohamed *et al*., 2017).

To assess the genotoxicity of substances, researchers investigate DNA damage in cells exposed to these harmful compounds. Such DNA damage can take the form of single or double-strand breaks, loss of excision repair, cross-linking, point mutations, and structural or numerical abnormalities in chromosomes. The compromised integrity of genetic material is recognized as a contributing factor in the development of cancer. As a result, various advanced techniques, including the Ames Assay, in vitro and in vivo Toxicology Tests, and the Comet Assay, have been developed to evaluate the potential of chemicals to induce DNA damage that can ultimately lead to cancer (Nagarathna *et al*., 2013).

1.2 STATEMENT OF PROBLEM

The global concern of water contamination with genotoxic substances is a significant issue. The presence of mutagens in drinking water can result in adverse health consequences for humans, including the potential development of cancer. Moreover, environmental pollution can disrupt aquatic ecosystems. As a result, numerous research studies have been conducted to

evaluate the mutagenic effects of drinking water, as well as surface and groundwater sources, uncovering a multitude of compounds that can damage DNA (Ferk *et al*., 2009).

Major sources of surface water pollution with various genotoxic compounds include industrial wastewater, sewage treatment plant effluents, and agricultural runoff. Given the presence of a wide array of chemical pollutants, effective monitoring of surface waters cannot rely solely on the analysis of a limited number of toxic compounds. Genotoxicity tests based on the enzymatic or metabolic activity of test organisms, while sensitive, lack specificity. Consequently, a combination of biotests involving both prokaryotic and eukaryotic organisms, such as bacteria, algae, Daphnia, mussels, and fish, becomes necessary to accurately assess the impact of genotoxic compounds on a diverse range of organisms (Lah *et al*., 2004).

The comet assay is a technique utilized to measure DNA strand breaks at the individual cell level. This assay has found widespread application in environmental research, particularly in evaluating the genotoxicity of pollutants and contaminants within aquatic ecosystems. It enables researchers to gauge how environmental stressors affect the DNA integrity of aquatic organisms (De Lapuente *et al*., 2015).

1.3 JUSTIFICATION OF PROBLEM

Fish play crucial roles in aquatic ecosystems, responding directly to low concentrations of toxic substances in the water and indirectly by consuming contaminated aquatic organisms. Therefore, fish can function as indicators for the early identification of pollution in marine environment (Cavas & ErgeneGözükara, 2005). Indiscriminate dumping of effluents into water bodies is a major cause of water pollution. Some research has been carried out on the genotoxic effect of cassava wastewater on the African Catfish (Ariyomo *et al*., 2017; Olufayo *et al*., 2016). The scope of this study is to determine whether comet assay can detect the genotoxic effect of cassava wastewater on post juvenile *Clarias gariepinus.*

1.4 AIM AND OBJECTIVES OF THE STUDY

This study aims to assess the genotoxic effect on post juvenile *Clarias gariepins* exposed to cassava wastewater using comet assay.

The objectives are to:

1. Expose the post juvenile *Clarias gariepinus* to various concentration of cassava wastewater.
2. Perform acute toxicity test on *Clarias gariepinus* using cassava wastewater
3. To assess using comet assay, the extent of genetic damage done on the test organisms after exposure to cassava wastewater

CHAPTER TWO

LITERATURE REVIEW

The assessment of genotoxicity in aquatic species exposed to wastewater has been a significant area of research. Wastewater's genotoxic effects on *Clarias gariepinus*, an African catfish species known for its sensitivity to toxic substances and pollutant accumulation, have garnered attention. The Comet assay, also referred to as single-cell gel electrophoresis (SCGE), is a widely employed approach to assess the harmful effects of toxicants on genetic material. This method is widely accepted for assessing DNA damage due to its sensitivity, simplicity, versatility, broad range of applications, and time efficiency. It is frequently used in environmental monitoring to evaluate the impact of various toxicants on aquatic organisms. Numerous investigations have employed the Comet assay to evaluate the harmful effects on the genetic material of *Clarias gariepinus* when subjected to compounds such as pesticides and wastewater. This literature review aims to provide up-to-date information and findings on this subject. It will enhance our understanding of how wastewater exposure affects *Clarias gariepinus* in terms of genotoxicity and provide insights into potential concerns for aquatic life and public health. Furthermore, it will assist in identifying knowledge gaps and areas requiring further investigation, ultimately aiding in the development of effective monitoring and mitigation measures.

Genotoxicity primarily relates to changes observed in genetic material, specifically in DNA or mRNA. These effects can appear as either changes in the genetic material or flexible adjustments in how genes are expressed (Lam, P.K.S., and Grey, J.S., 2003). Within pollution control and environmental monitoring, one of the key responsibilities is to evaluate the potential for genetic damage in aquatic environments.

Monitoring the harmful effects on the genetic integrity of water-dwelling organisms is of paramount importance for multiple factors. Firstly, from an ecological standpoint, Maintaining

the variety of genetic traits within natural populations is essential for their continued existence, and it is strongly preferable to avoid mutations induced by pollutants that disturb this genetic diversity. Secondly, it is imperative to identify the presence of cancer-causing effects in aquatic organisms in order to evaluate their health and prevent carcinogenic substances from entering the human food supply. Contact with detrimental substances has the potential to harm the DNA within live cells. When these DNA impairments are left unaddressed, they can initiate a cascade of biological effects that extend from the cellular level to structural changes, affecting entire organisms, and ultimately impacting broader communities and populations. As a result of the substantial number of chemicals with the capacity for genetic damage entering surface waters, the importance of evaluating genotoxicity is continuously increasing. Recognizing The dangers of genetic damage linked to water contamination became apparent in the late 1970s, and since then, several tests have been developed to evaluate changes in the genetic material of aquatic species.

The African catfish, *Clarias gariepinus*, is a widely cultivated freshwater fish in Egypt due to its rapid growth rate and environmental adaptability. It has been extensively used as a model for toxicological studies (Mahboub *et al*., 2021). African catfish is also a valuable protein source, making its well-being an indicator of habitat quality. Various researchers have chosen African catfish as a bio-indicator for genotoxicity and oxidative stress due to its responsiveness to ecological pollution (Osman *et al*., 2012; Arojojoye *et al*., 2018).

A study conducted by Zaghloul *et al*. (2020) in Fayoum Governorate, Egypt, investigated the genetic damage and tissue structure changes caused by water pollution in *Clarias gariepinus*. Water samples collected from the Nile River and main drains were assessed for quality. Genotoxic effects were evaluated using the micronucleus assay, comet assay, and chromosomal aberration analysis. The study revealed that exposure to water pollution resulted in genotoxic damage, as evidenced by increased micronucleus frequency, DNA damage, and chromosomal

abnormalities in *Clarias gariepinus*. Histopathological analysis also indicated various abnormalities and damage in the gills, liver, kidney, and muscles of the fish, highlighting the detrimental effects of pollution on organ health.

Another study by Amaeze *et al*. (2020) in Lagos, Nigeria, compared the immediate harmful impact, blood-related changes, and genetic damage caused by ten frequently utilized pesticides on the African Catfish, *Clarias gariepinus* Burchell 1822. This research assessed the haematological and genotoxic effects of exposure to sublethal concentrations of these pesticides. Genotoxicity was evaluated using the comet assay and micronucleus test. The study found that exposure to pesticides induced genotoxic effects in *Clarias gariepinus*, evidenced by increased DNA damage and micronucleus formation.

Additionally, Ibor *et al*. (2020) conducted a study on the presence of pollutants and the impact on hormonal balance in *Clarias gariepinus* exposed to a simulated leachate from a landfill site in Calabar, Nigeria. This research investigated the hormonal disturbance caused by a simulated leachate on *Clarias gariepinus*, with genotoxicity assessed using the comet assay. The study demonstrated that exposure to the leachate induced genotoxic effects in *Clarias gariepinus*, as evidenced by increased DNA damage.

2.1 GENOTOXICITY IN AQUATIC ORGANISMS

The health of aquatic organisms is compromised by various hazards, encompassing chemical, physical, and biological contaminants present in their aquatic habitat. These hazards possess the potential to induce genetic modifications and even trigger the development of cancer in water-dwelling creatures. Due to the ongoing creation and discharge of these dangers into water environments, evaluating the genotoxic capacity of these environments has become a pivotal aspect of environmental pollution monitoring. The assessment of environmental genotoxicity serves as an early warning system for potential long-term adverse consequences of contamination, primarily focusing on responses observed within nucleic acids. These responses

can entail changes in genetic material in the form of either DNA or mRNA, which can appear as damage to the genetic material or flexible adjustments in how genes are expressed.

Monitoring the harmful effects on the genetic integrity of water-dwelling organisms is of paramount importance for multiple factors. Firstly, from an ecological standpoint, Maintaining the variety of genetic traits within natural populations is essential for their continued existence, and it is strongly preferable to avoid mutations induced by pollutants that disturb this genetic diversity. Secondly, it is imperative to identify the presence of cancer-causing effects in aquatic organisms in order to evaluate their health and prevent carcinogenic substances from entering the human food supply. Contact with detrimental substances has the potential to harm the DNA within live cells. When these DNA impairments are left unaddressed, they can initiate a cascade of biological effects that extend from the cellular level to structural changes, affecting entire organisms, and ultimately impacting broader communities and populations. As a result of the substantial number of chemicals with the capacity for genetic damage entering surface waters, the importance of evaluating genotoxicity is continuously increasing. Recognizing The dangers of genetic damage linked to water contamination became apparent in the late 1970s, and since then, several tests have been developed to evaluate changes in the genetic material of aquatic species. The feasibility of employing modifications in the stability of DNA as markers of exposure and the effects of genotoxicants has been explored. The presence of DNA adducts has been considered evidence of exposure to specific genotoxic agents. Currently, the sibling chromatid examination, chromosome abnormalities assessment, comet assay, and micronucleus assessment are the most commonly employed techniques for identifying DNA abnormalities in the study of environmental toxicity. While the sibling chromatid examination and chromosome abnormalities assessment are labor-intensive, resource-demanding, necessitate a growing group of cells, and may not be highly efficient because of the relatively abundant quantity of compact chromosomes in many marine species, these drawbacks have

prompted the advancement and application of the comet assay and micronucleus assessment. These newer methods offer simplicity, enhanced sensitivity, and time efficiency in assessing genotoxicity (Osman., 2014).

2.2 SOURCES OF ENVIRONMENTAL POLLUTANTS

Environmental pollutants can have adverse effects on aquatic organisms, including genotoxic impacts. One significant source of environmental pollutants known to cause genotoxic effects in aquatic organisms is pesticides. Experimental findings have demonstrated that various pesticides possess genotoxic or mutagenic properties, which represent initial risk factors in the development of long-term carcinogenic and reproductive effects (Cavalcante, Martinez, and Sofia, 2008).

Pesticides can enter aquatic systems through various pathways. For instance, when pesticides are applied to crops and there's subsequent rainfall, these chemicals can be washed from leaves and flow into aquatic habitats. The potential genotoxic effects of insecticides on non-target organisms are of widespread concern. Organophosphates, historically a commonly used group of pesticides, have been implicated in toxicity as alkylating agents and potential genotoxicant. Chlorpyrifos, one such organophosphate, is extensively utilized not only for pest control in agriculture but also for domestic pest management worldwide. Chlorpyrifos can enter water through runoff events, erosion, and leaching. Pyrethroids, another widely used pesticide, have also been reported to have genotoxic effects on organisms, including fish. Bifenthrin, a pesticide used in both agriculture and public health control programs (such as mosquito control), has been identified as carcinogenic and genotoxic (Bano *et al*., 2021).

Apart from pesticides, other sources of environmental pollutants capable of causing genotoxic effects in aquatic organisms encompass industrial effluents, pharmaceuticals, heavy metals, and organic pollutants.

Industrial activities can release a variety of pollutants into water bodies, including heavy metals, organic compounds, and chemicals. The release of industrial and household wastewater, along with the runoff from farming and urban regions, presents a significant danger to water environments and, as a result, to human well-being. The release of numerous chemicals into aquatic environments due to urbanization and industrial processes has led to contamination of streams and rivers. Of particular concern are metals, such as lead (Pb), chromium (Cr), zinc (Zn), copper (Cu), and mercury (Hg), which have the capacity to gather within living organisms. The pollution of water ecosystems with these metals has sparked international apprehension because of their possible harmfulness, prevalence, durability, and their capacity to jeopardize water organisms and human health through water consumption and fish consumption. Genotoxic pollutants display genetic mutations and/or structural chromosome damage, with their harm carrying over to future generations. DNA impairment can appear as mutations, inherited abnormalities, teratogenic impacts, and unregulated cell proliferation (De Mendonça Francisco *et al*., 2019).

Antibiotics, commonly used to prevent or treat bacterial infections and promote growth in various contexts, have also been detected in different environments, including surface water, groundwater, and sediment. Antibiotics are biologically active compounds that can inhibit various cellular processes, including protein and nucleic acid synthesis, cell wall formation, DNA replication, and cell division. These compounds are not completely removed during wastewater treatment, resulting in continuous exposure of aquatic environments to antibiotics. Even at low concentrations, antibiotics can exert nontarget toxicity on aquatic organisms, although the effects on fish are less frequently reported than on microorganisms. The presence of antibiotics in aquatic environments also raises concerns about the spread of antibiotic-resistant bacteria and antibiotic resistance genes. Even low concentrations of antibiotics, ranging from nanograms to micrograms per litre, can lead to the development of antibiotic-

resistant bacterial strains, posing a threat to human health and environmental ecosystems (Yang, Song, and Lim, 2020).

Like other aquatic organisms, fish can be exposed to antibiotics from multiple sources, including human and veterinary use as well as industrial discharges. Danner *et al*. (2019) conducted a comprehensive review of antibiotics, including quinolones, sulfonamides, tetracyclines, macrolides, penicillin, cephalosporins, and nitroimidazole, in surface freshwater worldwide. Quinolones, sulfonamides, tetracyclines, and macrolides were among the most frequently detected antibiotics in aquatic environments. Their aquatic toxicity has been extensively studied. These antibiotics, such as doxycycline, tetracycline, oxytetracycline, and sulfamethoxazole, can originate from aquaculture and domestic pollution, while sulfadiazine and sulfamethazine are primarily associated with animal husbandry. The types and concentrations of antibiotics found in aquatic environments vary by region, depending on local consumption patterns and antibiotic use. Hospitals represent a significant source of antibiotics entering aquatic environments. For antibiotics used in humans, wastewater from treatment plants is sampled to measure removal efficiency (Yang, Song, and Lim, 2020).

2.2.1 EXAMINE RESEARCH FOCUSED ON THE GENETIC DAMAGE INDUCED BY ENVIRONMENTAL POLLUTANTS IN WATER-DWELLING CREATURES, ENCOMPASSING *CLARIAS GARIEPINUS*

A study conducted by Oladokun, Sogbanmu, and Anikwe (2020) investigated the genotoxic and histological effects of exposure to sublethal concentrations of dichlorvos and paraquat in *Clarias gariepinus*. These pesticides, in liquid form and water-soluble, were obtained from a commercial vendor in Lagos, Nigeria. Fingerlings and juveniles of *C. gariepinus* were sourced from a local fish farm in Bariga, Lagos, Nigeria. The assessment of genetic damage was conducted through the micronucleus test. The research revealed that exposure to pesticides caused genetic harm, as there was a more significant elevated levels of micronuclei formation were detected in the red blood cells of *C. gariepinus* when they were exposed to increased

sublethal levels of pesticides. Histological analysis revealed alterations and damage in the gills at high pesticide concentrations, but no irregularities were noted in the liver, except for the presence of cytoplasmic fat droplets in the lowest non-lethal concentration of dichlorvos.

Another study by Ayanda, Tolulope, and Oniye (2021) investigated mutagenicity and genotoxicity in juvenile African catfish, *Clarias gariepinus*, exposed to formulations of glyphosate and paraquat. Juvenile *C. gariepinus* were obtained from a fish farm in Ota, Ogun State, Nigeria. Commercial formulations of glyphosate and paraquat were purchased from an outlet in Lagos, Nigeria. The genotoxic impact was evaluated using micronucleus and comet assays. The study found that exposure to glyphosate and paraquat induced micronuclei and other nuclear abnormalities in fish blood. These nuclear abnormalities included bean-shaped cells, fragmented apoptotic cells, and lobed nuclei. DNA damage in fish tissue was also observed, particularly in fish exposed to higher concentrations of glyphosate and paraquat. The extent of damage was concentration-dependent. The study concluded that glyphosate and paraquat exposure had genotoxic effects on *Clarias gariepinus*.

A study conducted by Alimba, Adekoya, and Soyinka (2019) investigated genetic, blood-related, and tissue structure changes caused by pharmaceutical wastewater in *Clarias gariepinus*. The pharmaceutical effluent was obtained from an industry in Satellite Town, Lagos State, Nigeria. Juvenile *C. gariepinus* sourced from a fish farm along Badagry, Lagos State, were used. Genotoxicity was assessed using the micronucleus assay. The study showed that the pharmaceutical effluent caused a concentration-dependent increase in micronuclei. Hematological analysis revealed that sub-lethal concentrations of the effluent significantly reduced red blood.

2.3 MECHANISM OF GENOTOXICITY

Genotoxicity is a comprehensive term encompassing "damage to the genome," and it constitutes a distinct and vital form of toxicity, with specific genotoxic events serving as

significant indicators of cancer. The genetic material sustains damage through interactions between genotoxic substances and DNA structure and sequence. These substances interact with specific locations or base sequences in DNA, resulting in various forms of damage such as lesions, breakages, fusions, deletions, mis-segregations, or non disjunctions, ultimately leading to genetic harm and mutations. For instance, high-valent oxidation state transition metals like chromium can interact with DNA, causing DNA lesions that contribute to carcinogenesis. Studies have established that the mechanisms underlying damage and base oxidation products resulting from the interaction between DNA and high-valent chromium are relevant to in vivo DNA damage formation, leading to cancer in populations exposed to chromate, establishing high valent chromium as a carcinogen. One of the most prevalent oxidative injuries in DNA provoked by reactive oxygen molecules is 8-hydroxydeoxyguanosine (8-OHdG), which represents an extremely mutagenic flaw. Oxidizing agents and unbound radicals, if present within cellular systems, can detrimentally influence and modify the configuration of lipids, proteins, and DNA. Reactive aliphatic aldehydes like 4-hydroxynonenal (4-HNE) emerge during the disintegration of lipid peroxyl radicals or primary free radical intermediates in lipid peroxidation. 4-Hydroxynonenal has been associated with various health conditions linked to oxidative stress, such as atherosclerosis, fibrosis, and neurodegenerative ailments, and it can trigger cell growth, differentiation, and cell-protecting reactions by affecting various signaling pathways (Mohamed *et al*., 2017).

In general, the harmful effects on genetic material can be categorized into "genetic damage caused directly" and "genetic damage caused indirectly," inside cells or within the cellular nucleus. Tiny particles at the nanoscale have the capability to penetrate the central core of the cell leading to harm inflicted upon the genetic material and causing direct harm to the genetic material in organisms. However, even when these tiny particles cannot directly access the central core of the cell, they can still have an indirect impact on genetic damage through

mechanisms such as oxidative strain, alterations in epigenetic marks, inflammatory responses, and cellular self-digestion (autophagy) (Wu *et al*., 2021).

3.3.1 Direct Genotoxicity:

Certain environmental pollutants have the capacity to directly harm the DNA of aquatic organisms. For example, exposure to pyrrolizidine alkaloids (PAs) found in specific plant species can induce direct DNA damage in animals, including humans. Similarly, metal pollution can lead to direct DNA damage by interfering with DNA repair mechanisms. Nuclear DNA is typically the primary target of genotoxicity. Furthermore, exposure to graphene family nanomaterials (GFNs) can result in direct physical membrane damage by cutting and penetrating cell membranes and cell walls. Additionally, small GFN fragments have the ability to penetrate the core center within a cell and directly engage with the DNA molecule. (Wu *et al*., 2021).

3.3.2 Indirect Genotoxicity:

Other environmental pollutants can cause indirect DNA damage by triggering oxidative stress and the generation of reactive oxygen species (ROS). For instance, exposure to herbicides like glyphosate and paraquat can induce indirect DNA damage by promoting oxidative stress and inhibiting DNA synthesis. Indirect genotoxicity encompasses various aspects, including oxidative stress, epigenetic toxicity, the impact of GFNs on DNA replication, repair and transcription, as well as inflammation and autophagy (Wu *et al*., 2021).

1. **Oxidative Stress:** Oxidative stress results from a disproportion in the generation and accumulation of chemical substances known as reactive oxygen species (ROS) and the capacity of a living organism to neutralize these reactive substances. The internalization of nanoparticles by organisms can induce the generation of intracellular ROS and trigger protection provided by antioxidants mechanisms. The production of ROS can

result in customary DNA harm induced by oxidative processes, such as breaks in single and double strands of DNA, connections between DNA strands, and alterations to DNA bases. Organisms possess robust protection provided by antioxidants mechanisms, including enzymes that neutralize reactive oxygen species like superoxide dismutase (SOD), peroxidase, and catalase, as well as control systems that shield them against the detrimental impacts of ROS. The production of ROS is influenced by the restriction of metabolism related to lipids, sugars, and proteins (Wu *et al*., 2021).

2. **Epigenetic Toxicity:** Epigenetic toxicity refers to the impact of chemical substances on epigenomes and their adverse effects on living organisms. This phenomenon can explain the long-term effects of chemical substances and the predisposition to diseases due to environmental factors, including chemicals. Exposure to nanoparticles can lead to changes in epigenetic regulation, this involves processes such as DNA methylation, changes to histones, control of non-coding RNA (ncRNA) gene activity, and modifications to chromatin structure. Compounds like graphene oxide can trigger microRNA (miRNA) protective regulatory mechanisms and prevent the adverse effects on the reproductive capacity of living organisms. Moreover, miRNAs can activate death receptor pathways by altering the expression of caspase-3 and tumour necrosis factor α receptor in cells exposed to graphene oxide, highlighting the role of epigenetic signals in protection mechanisms (Wu *et al*., 2021).

3. **Inflammation:** Inflammation, including acute and chronic inflammation, is a complex biological response to harmful stimuli like pathogens, toxins, or dead cells. Inflammation constitutes one of the responses associated with genetic damage indirectly caused by reactive oxygen species. Persistent or long-term inflammation can lead to indirect genetic damage, characterized by the buildup of molecules known as reactive oxygen species following exposure to certain toxicants. Toxicants such as

graphene oxide can prompt DNA fractures and trigger the nuclear factor kappa-B (NF-κB) pathway of cellular communication, potentially leading to inflammatory response within macrophages. Excessive cytokines related to inflammation have the potential to induce alterations to the genetic material. The connection or link between an inflammatory response and ROS is complex, and these mechanisms may possess distinct pathways for initiation (Wu *et al*., 2021).

4. **Autophagy:** Autophagy is a regulated catabolic pathway within cells that plays a crucial role in maintaining cellular homeostasis by degrading unnecessary or dysfunctional components. Autophagy manages the alteration of structures found in the nucleus, which include the nuclear lamina, chromatin, and DNA, playing a crucial role in maintaining the stability of the genetic material. The suppression of autophagic activity can impede the genetic material standard repair process and induce cell demise as a reaction to genetic harm. The process of autophagy and alterations in epigenetic markers work together to manage the viability of cells, with autophagy potentially serving as an outcome or subsequent process resulting from epigenetic modifications, representing an aspect of secondary genotoxicity. The connection between autophagy and DNA damage is intricate, as autophagy has the capacity to control the quantities of different proteins involved in damaged DNA detection and repair. The interplay between autophagy and other mechanisms of harm or toxic action, such as oxidative strain, modifications in epigenetic marks, programmed cell death (apoptosis), and inflammatory responses, are not completely comprehended. (Wu *et al*., 2021).

2.4 COMET ASSAY AS A TOOL FOR ASSESSING GENOTOXICITY

The comet assay is a technique used to evaluate DNA strand breaks in individual cells. It relies on the principle that fragmented DNA moves faster than intact DNA during electrophoresis through an agarose gel. While a detailed explanation of the method is beyond the scope of this

review, it is extensively described in other publications. In summary, single-cell suspensions in agarose are applied to microscope slides, treated with detergent and high-concentration NaCl to disrupt cell membranes and remove histones, and then subjected to electrophoresis. When there are strand breaks in the DNA, it moves toward the anode, creating a comet-like image when stained with a fluorescent dye and viewed under fluorescence microscopy (Cordelli, Bignami and Pacchierotti, 2021).

Scoring the comets can be done in several ways:

1. By visual inspection, using an ocular micrometre to measure comet length or length-to-width ratio or by categorizing cells into different classes based on the extent of migration.
2. By employing an image analysis system, which consists of a CCD camera attached to a fluorescent microscope along with specialized software and hardware designed for capturing and analysing images of fluorescent-stained nuclei (Lee and Steinert, 2003).

The comet assay is particularly valuable for detecting DNA strand breaks resulting from exposure to genotoxic substances or environmental stressors. It can also identify incomplete excision repair at the time of cell lysis, as incomplete repair introduces single-strand gaps in the DNA. Additionally, apoptosis (programmed cell death) leads to extensive double-strand breaks, and apoptotic cells display significant DNA fragmentation, with most of the DNA outside the comet head after the assay. Good correlations have been observed between the concentrations of apoptotic cells determined using the comet assay (Lee and Steinert, 2003). It's important to note that when conducting the comet assay on different tissues, normal DNA damage levels can vary significantly. Various factors, such as cell type diversity, cell cycle, cell turnover rate, and culture conditions, can influence DNA strand damage in a specific tissue. Homogeneous cell populations are often used to reduce inter-cell variability in comet assay

results. It's crucial to obtain cells with minimal disruption when working with different tissues. For instance, finely mincing tissues with scissors can yield a suitable cell suspension. Various tissues from marine and freshwater animals, such as gills, liver, and gonads, have been employed in the comet assay (Lee and Steinert, 2003).

The comet assay has found widespread use in environmental studies for assessing the genotoxicity of pollutants and contaminants in aquatic ecosystems. It allows researchers to assess the impact of environmental stressors on the DNA integrity of aquatic organisms (De Lapuente *et al*., 2015).

The comet assay is not only cost-effective but also highly sensitive for measuring genetic harm to sperm. The extent of genetic injury to sperm assessed using the comet assay is closely correlated with other immediate or straightforward methods like the terminal deoxynucleotidyl transferase dUTP nick end labeling assay. This technique can be applied to various types of cell including sperm, and needs only a small number of cells for analysis. Data obtained from the comet assay are accurate and less susceptible to experimental prejudice since it involves measurement using specialized software. Genetic injury assessed through single cell gel electrophoresis is strongly linked to male reproductive system (ART) outcomes (Simon *et al*., 2018). Additionally, the comet assay can be applied to a wide range of animal models, without specific requirements for rodent strains, and can be integrated with other complementary short-term genotoxicity assays in the same animals. Furthermore, it is a cost-effective method. However, some limitations include the relative increase in readouts compared to controls rather than absolute mutation numbers, limited standardization of assay procedures, sensitivity to indirect genotoxicity mechanisms related to toxicity and cellular stress, and insensitivity to certain genotoxicity modes of action, such as aneugenicity and DNA crosslinking (Cordelli, Bignami, and Pacchierotti, 2021b).

2.4.1 REVIEW OF STUDIES ON THE APPLICATION OF THE COMET ASSAY IN GENOTOXICITY RESEARCH

Naguib *et al*. (2022) conducted a study titled "Assessment of Genotoxic Effects of Silver Nanoparticles on Catfish (*Clarias gariepinus*) Erythrocytes Using the Comet Assay." Their research employed the comet assay to evaluate the genotoxic impact of silver nanoparticles (AgNPs) on catfish. Silver nanoparticles with sizes of 20 and 40 nanometres were acquired from materials that are nanostructured and lack a defined crystalline form in Houston. *Clarias gariepinus* samples were obtained from a privately-owned fish farm, they were later relocated to the Fish Biology and Pollution Laboratory within the Department of Zoology at the Faculty of Science, Assiut University. The study involved conducting a neutral comet assay based on a specified protocol. Exposure of *Clarias gariepinus* to nanoparticles composed of silver (AgNPs) and a compound called silver nitrate (AgNO3) caused harm to the genetic material, as indicated by parameters related to the comet assay that indicate a neutral environment, measuring the fragmentation of DNA's double helix. The extent of damage was dependent on the levels and dimensions of the nanoparticles.

In another investigation conducted by Moussa, Mohamed, and Abdel-Khalek (2022), titled "Metal Accumulation and DNA Damage in *Oreochromis niloticus* and *Clarias gariepinus* After Prolonged Exposure to Discharges from the Batts Drain: Potential Human Health Risks," the comet assay was employed to detect metal accumulation and DNA damage in *Oreochromis niloticus* and *Clarias gariepinus* after extended exposure to discharges from the Batts Drain. During the summer season, *O. niloticus* and *C. gariepinus* specimens were collected from the study sites with the assistance of experienced fishermen. A total of hirty-six male fish in good health and maturity were selected for the study. The collected fish samples were transported to the ecology laboratory at the Zoology Department, Faculty of Science, Cairo University, in large plastic containers equipped with two portable oxygen pumps each. The study focused on determining the concentrations of Cu, Zn, Fe, Cd, Pb, and Al metals in various tissues,

including liver, kidney, gills, skin, and muscle, in both species. The results indicated significant DNA damage and metal accumulation in both fish species. Furthermore, the study highlighted the presence of several metals in their massive form within edible tissues, which could pose significant health risks to consumers. Consequently, the combined risk linked to the existence of these various metals should not be underestimated.

Osman et al. (2008) conducted a study titled "Monitoring DNA Breakage in Embryonic Stages of the African Catfish *Clarias gariepinus* (Burchell, 1822) After Exposure to Lead Nitrate Using the Alkaline Comet Assay." This study utilized the alkaline comet assay to monitor DNA breakage in the embryonic stages of the African catfish *Clarias gariepinus* after exposure to lead nitrate. The artificial reproduction of African catfish was carried out in the hatchery of the Department of Fish Culture and Fisheries at Wageningen Agricultural University in the Netherlands. Fertilized eggs were divided into four groups, including one control group exposed to tap water and three groups exposed to lead nitrate at concentrations of 100, 300, and 500 micrograms per litre. The comet assay was conducted according to a modified protocol. Exposure to lead nitrate at various concentrations and exposure durations resulted in DNA damage in terms of DNA strand fractures occurring in the early developmental stages of African catfish known as *Clarias gariepinus*.

2.5 CASSAVA EFFLUENT AS AN ENVIRONMENTAL HAZARD

Cassava (Manihot esculenta), a vital staple crop cultivated by small-scale farmers across Nigeria and other tropical African regions, is commonly known as the "bread of the tropics," the "food of the poor," or even the "poverty fighter." Its resilience to drought and adaptability to various soils, including marginal ones, make cassava a significant source of carbohydrates in tropical Africa. Notably, it's a famine-reserve crop, offering subsistence farmers the flexibility of harvesting schedules due to its non-seasonal nature. Cassava tubers can be processed into a wide range of high-calorie foods, such as garri (toasted granules), fufu

(fermented cooked paste), starch, tapioca, and other confectioneries. Some low-cyanide cassava varieties even allow direct consumption without further processing. This versatility makes cassava an ideal, low-cost, and high-calorie food source for many underprivileged Nigerians (Oghenejoboh *et al*., 2021).

However, cassava processing generates substantial amounts of solid and liquid waste. These by products include leaves, stems, peels, wastewater, and starch bagasse. In Nigeria, farmers often dispose of these wastes indiscriminately in water bodies, unfinished buildings, undeveloped land plots, and open spaces near major roads and streets, despite the evident harm these practices inflict on both the environment and public health (Oghenejoboh *et al*., 2021).

Raw cassava tubers consist of approximately 60–70% water, a significant portion of which transforms into wastewater during processing. Moreover, processing cassava roots into edible products like garri and starch involves using substantial amounts of water, which is then discharged into the environment. It is noteworthy that processing just one ton (1000 kg) of cassava tubers can yield between 250 and 600 kg of wastewater, depending on the desired final product. This wastewater is frequently released into the environment in Nigeria with little regard for its adverse effects. Apart from contaminating soil, streams, and groundwater, cassava wastewater becomes a breeding ground for mosquitoes and other parasitic insects, often resulting in unpleasant odours when left stagnant in open channels (Oghenejoboh *et al*., 2021).

Cassava wastewater poses significant environmental risks due to its high cyanide content and substantial chemical oxygen demand (COD). If not properly treated, its discharge can pollute soil, rivers, and groundwater. Cassava inherently contains cyanogenic glycosides, which can hydrolyse into hydrogen cyanide during processing, particularly during pressing and washing.

This release of cyanide into the environment, in the form of hydrocyanic acid, poses a considerable environmental hazard (Fawole *et al*., 2014).

Cyanide exposure from cassava wastewater can be lethal, even in relatively small concentrations, to various animal species, including mammals. High concentrations of cassava effluent adversely affect the physicochemical parameters of water, which in turn impact aquatic life. The use of cassava effluent-contaminated water for irrigation can inhibit plant growth or even lead to plant death, depending on the cyanide concentration (Fawole *et al*., 2014).

Research has shown that cassava mill effluents induce mortality and cause changes in behaviour, enzymatic activity, haematological parameters, and histopathological conditions, especially in fish species like *Clarias gariepinus*. Furthermore, the presence of nitrogenous compounds, such as nitrite and nitrate, in cassava effluents adversely affects fish in aquatic ecosystems. Reports have also documented the toxicity of cassava mill effluents on vegetation, domestic animals, and livestock, such as goats and sheep (Izah, Bassey, and Ohimain, 2017). The high concentration of cyanide in domestic water bodies intended for fish farming poses a severe risk to human health, as cyanide exposure can impact the thyroid and central nervous system, potentially leading to paralysis (Oghenejoboh, 2015).

Cyanide is highly toxic to fish, and fish are more sensitive to cyanide than humans. Increased industrial and agricultural waste discharges have resulted in aquatic environmental pollution, with cassava processing, especially in areas highly concentrated with cassava industries, being recognized as a significant contributor to environmental damage and water scarcity. Various studies have investigated the effects of cassava effluent on non-target organisms, emphasizing the need for toxicant concentrations to remain below levels causing fish mortality. The continuous use and unregulated discharge of untreated cassava effluents into natural waterways

have harmful consequences for fish populations and other aquatic life forms, potentially leading to long-term environmental effects (Olufayo *et al*., 2016).

Given the severe impact of cassava mill effluents on the environment and biodiversity, including fisheries, livestock, microorganisms, and vegetation, there is an urgent need for effluent treatment before discharge (Izah, Bassey, and Ohimain, 2017). Various treatment technologies, encompassing chemical, physical, and biological methods, are available and should be selected based on effluent constituents and characteristics. Microbes, particularly yeast such as *S. cerevisiae* and *Torulaspora delbrueckii*, have demonstrated the ability to effectively reduce inorganic compounds commonly found in wastewater from tannery operations (Izah, Bassey, and Ohimain, 2017).

Treating cassava mill effluents before discharge is crucial to mitigate their impact on the receiving environment. Therefore, the objective of this study is to isolate S. cerevisiae from palm wine and employ it in the treatment of cassava mill effluents (Izah, Bassey, and Ohimain, 2017).

2.5.1 REVIEW STUDIES ON CASSAVA EFFLUENT TOXICITY AND ITS EFFECTS ON CLARIAS GARIEPINUS

A study conducted by Ariyomo *et al*. (2017) in Ekiti State, Nigeria, investigated the toxicity of cassava effluents on catfish, *Clarias gariepinus*, and its effects on certain target organs. They used 200 healthy *Clarias gariepinus* juveniles sourced from the Federal Polytechnic Ado Ekiti fish farm, transporting them to the Fisheries Laboratory of the Federal University Oye-Ekiti, Ekiti State. The cassava effluent, used as a toxic substance, was collected from the Ikole cassava processing factory in Ikole-Ekiti. The experiments followed standard bioassay procedures. Fish subjected to different levels of the wastewater discharge exhibited varying degrees of damage to their gills and livers. Histological analysis of the liver revealed signs of

necrosis at high concentrations, with disrupted cellular arrangements and lesions present in liver tissues.

Olufayo *et al.* (2016) conducted a study to examine how cassava mill effluent affects the histology of juvenile *Clarias gariepinus*. Their research took place in a laboratory setting, and the objective was to evaluate the influence of cassava mill wastewater on the histological features of various organs, including the gills, liver, and kidneys, in these juvenile fish.

The juvenile *Clarias gariepinus* specimens used in the study were sourced from the Federal University of Technology Teaching Farm. To simulate exposure to cassava mill effluent, 150 litre of fresh cassava effluents were collected from small-scale cassava processing mills in Akure, Nigeria. These effluents were then transported to the Limnology Laboratory of the Fisheries and Aquaculture Department at the Federal University of Technology in Akure, Nigeria, for the experiment.

During the experiment, the researchers observed histological alterations in the structures of the gills, kidneys, and liver of the juvenile *Clarias gariepinus* exposed to cassava mill effluents. These changes included vacuolation, congestion, and filament degeneration in the gills when exposed to lower concentrations of cassava mill effluent. In contrast, higher concentrations of the effluent led to more severe filament degeneration and lamellae degeneration in the gills.

Regarding the kidneys, exposure to lower concentrations of cassava effluents resulted in observable cellular changes, including tubule fusion, pyknosis, tubule pyknosis, and kidney cell degeneration. Higher concentrations of cassava mill effluents caused more severe effects, such as karyolysis, rupture of nuclear membranes, fragmentation of nuclear chromatin, shrinkage, dense nuclei, and vacuole formation in the kidneys.

In the case of the liver, exposure to lower concentrations of cassava mill effluents led to observable fibrosis and nucleus dissolution. However, exposure to higher concentrations

resulted in more severe effects, including complete dissolution of the nucleus, loss of chromatin materials, vacuolation, and ultimately, the complete absence of the nucleus.

This study highlighted the significant impact of cassava mill effluent on the histological structures of various organs in juvenile *Clarias gariepinus*, emphasizing the potential harm posed by the effluent to aquatic organisms.

2.6 GENOTOXICITY BIOMARKERS FOR ASSESSING CHEMICAL HAZARDS IN AQUATIC ECOSYSTEMS

Genotoxicity biomarkers serve as valuable tools for evaluating chemical risks within aquatic environments due to the presence of chemical substances capable of damaging the genetic material, even when present in small amounts, can have profound effects on ecological systems. Several DNA structural changes can be used as indicators of genotoxicity, including strand fractures, single-point alterations, and abnormalities in chromosomes, micronucleus formation, changes in the mechanisms responsible for DNA repair and disruption in the progression of the cell cycle. Alterations to the genetic material can trigger diverse cellular reactions, such as halting the cell cycle, triggering programmed cell death (apoptosis), and disrupting DNA repair mechanisms. Unrepaired or incorrectly repaired DNA damage can lead to genomic mutations, which have the potential to induce cell transformation or cell death (Mahaye *et al*., 2017).

Chemical and physical pollutants can induce DNA alterations such as single and double strand breaks, DNA-DNA crosslinks, and DNA-protein crosslinks, either directly or indirectly through interactions with oxygen radicals (Bolognesi and Cirillo, 2014).

Various methods have been developed to assess DNA alterations induced by pollutants. For example, the 32P-postlabelling assay is highly sensitive and can detect bulky aromatic adducts resulting from complex mixtures of environmental pollutants. However, its complexity limits its use in large-scale environmental biomonitoring programs (Bolognesi and Cirillo, 2014).

The alkaline elution assay is based on the rate at which DNA single strand fragments pass through a membrane filter under alkaline conditions, correlating with the length of the DNA strand. This assay measures single and double strand breaks, DNA-DNA and DNA-protein cross-linking and has been effectively applied to aquatic test organisms exposed to chemical compounds in aquatic environments (Bolognesi and Cirillo, 2014).

The DNA alkaline unwinding assay detects DNA damage at the individual cell level. In this assay, cells or crude DNA extracts are subjected to alkaline conditions to allow controlled "unwinding" of double-stranded DNA into single-stranded DNA at each strand break. Quantifying strand breaks uses fluorescent dyes with high affinity for intact double-stranded DNA (Bolognesi and Cirillo, 2014).

The Comet assay, more recently developed, is widely used for detecting DNA damage at the individual cell level and is considered one of the most promising genotoxicity biomarkers. It can detect a broad range of DNA lesions with high sensitivity in aquatic species (Bolognesi and Cirillo, 2014).

Chromosomal damage, reflecting accumulated effects from long-term exposure to genotoxic chemicals, can be assessed through techniques like the micronucleus assay, which is applicable to any proliferating cell population regardless of the karyotype (Bolognesi and Cirillo, 2014).

CHAPTER THREE

3.1 MATERIALS AND METHODS

3.2 STUDY AREA

The study area is located at 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. The effluent was collected from a cassava processing industry at the point of discharge.

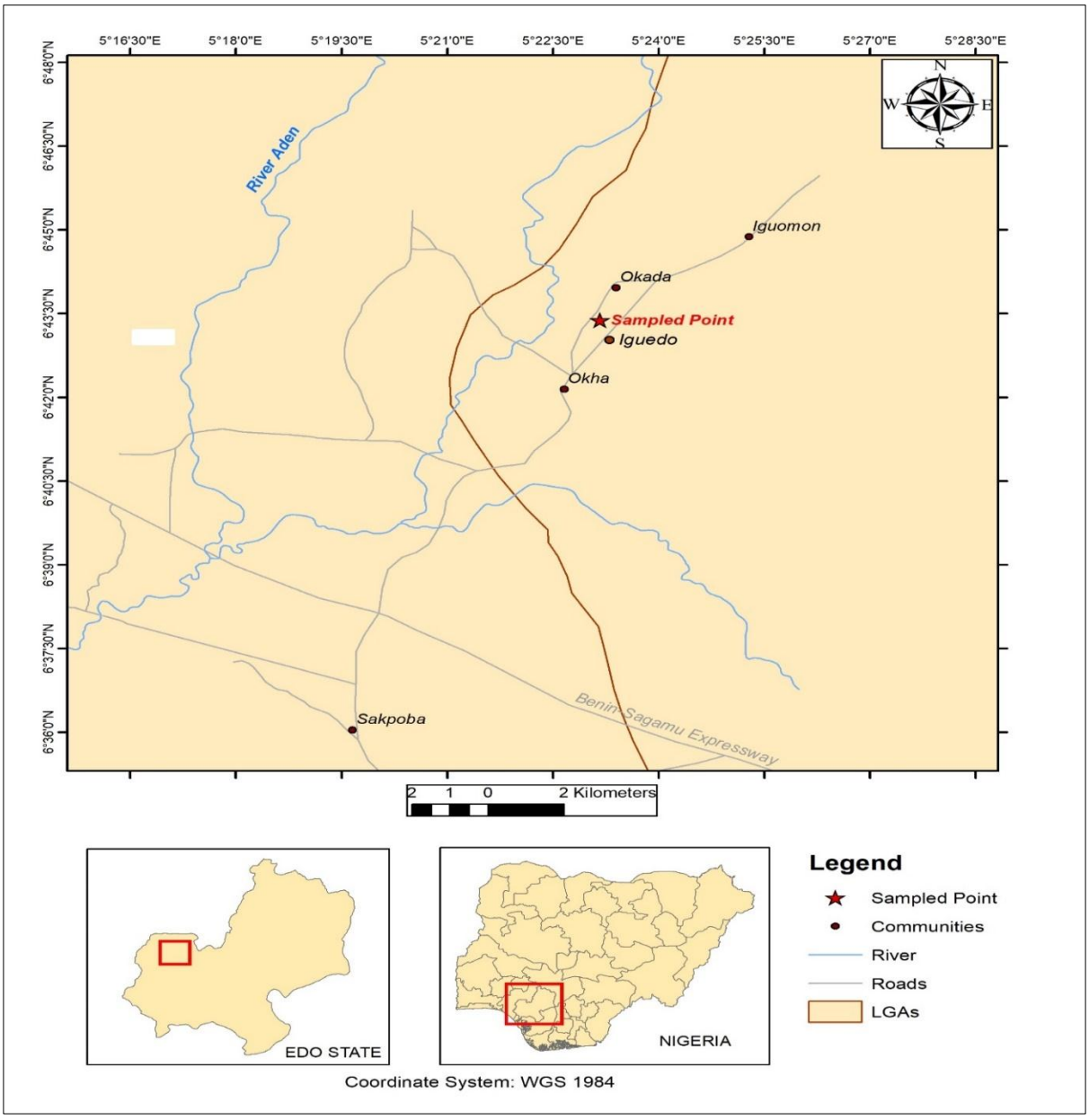

Fig 3.1: Diagram illustrating the study's geographic region

3.2.1 Test animal: collection and acclimatization

A total of one hundred post juvenile catfish (*Clarias gariepinus*) were purchased from Vinvok farms located at Orerokpe, Delta State, Nigeria. They were subsequently conveyed under well-ventilated conditions to the Wet Laboratory, Department of Fisheries, Faculty of Agriculture, University of Benin, Benin city, Edo state, Nigeria. The fishes were allowed to acclimatize to the laboratory conditions for a day prior to the test. During the adaptation period, the fish received two daily feedings of commercial fish food, and the water in the water within the tanks where the fish were kept was replaced every day. Following the adaptation phase, the fish were exposed to the experimental toxic substances.

3.2.2 Bioassay for acute toxicity

After a series of tests, concentrations were chosen. Ten fish were subjected to each concentration for a period of 96 hours. Five concentrations were prepared through serial dilution and tested on the fish. The fishes were then exposed to varying concentrations: 0.2%, 0.3%, 0.5%, 0.7%, and control (distilled water). At the commencement of the experiments and every 24 hours thereafter, the count of deceased fish was recorded, and they were promptly removed and properly disposed of to avoid contaminating the test environment (Sogbanmu *et al.,* 2018). Fish were deemed deceased when they exhibited no signs of movement and their bodies were either floating horizontally near the surface or sinking to the bottom of the test solution. A replicate per test concentration was used to avoid repetition of the test due to failure of the initial setup and to provide a stronger statistical baseline.

3.2.3 Acute toxicity tests

The assessments for immediate toxicity were conducted employing post juvenile catfish (*Clarias gariepinus*). The studies were conducted using plastic tank shielded with a plastic lid to deter the fish from escaping. Ten fish were chosen arbitrarily and placed into the test tanks for toxicity evaluation. Following a preliminary test to determine the appropriate range, the

juvenile fish were subjected to different concentrations; 0.2%, 0.3%, 0.5%, 0.7%, and control (distilled water).

3.2.3 Mortality

Fish deaths were documented every 24 hours for a duration of four days, totaling 96 hours. Deceased fish were promptly eliminated and properly disposed of to avoid contaminating the testing environment (Sogbanmu *et al* 2018).

3.3 PROTOCOL FOR COMET ASSAY

3.3.1 Extraction of organs

The fish samples are sacrificed and their gills, liver and gonads are placed in separate well labelled eppendorf tubes.

Plate 3.1: extraction of gills, liver and gonads from *Clarias gariepinus*

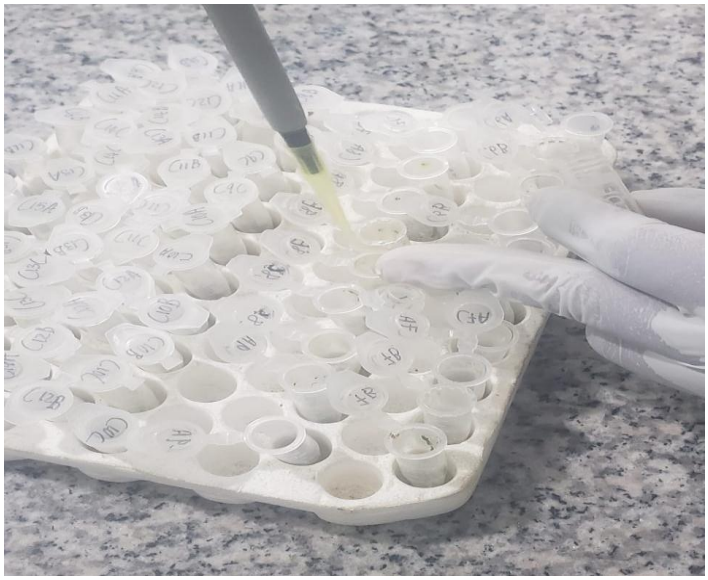

Plate 3.2: labelled eppendorf tubes with vital organs in them

3.3.2 Slide preparation

Comet assay slides are sterilized and allowed to dry. The slides are then labelled using a diamond pen and are embedded in normal agarose. The cells are then embedded in 0.5% low melting point agarose gel and then 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.

Plate 3.3: embedding slides in normal agarose

3.3.3 Lysing

The cells are lysed to release DNA. This is typically 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.

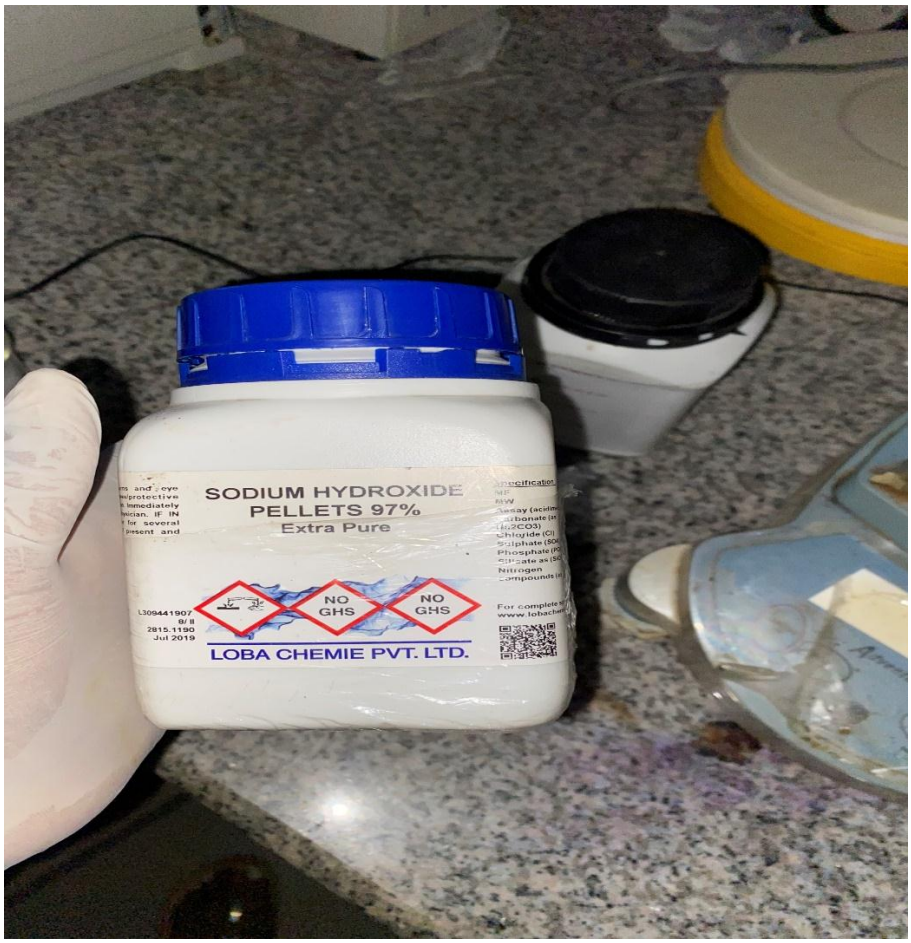

Plate 3.4: sodium hydroxide for making the lysing solution

3.3.4 Electrophoresis

The DNA is then subjected to electrophoresis, which causes the DNA to move through the agarose gel. It involves the use of an electrophoresis buffer that contains sodium hydroxide and EDTA. This allows the DNA to separate into fragments. This is done by placing the slides into electrophoresis tank and passing current of 300 amperes at 25V through it for thirty minutes. Electrophoresis process is done in the dark as light could damage the DNA.

Plate 3.5: electrophoresis setup

3.3.4 Neutralization

The slides are neutralized with tris. Tris is to keep the pH at 7.4. Neutralization is done three times every five minutes to stop the electrophoresis process.

Plate 3.6: neutralization of slides with tris

3.3.5 Fixation

The slides are then fixed with cold ethanol and allowed to dry. Cold ethanol dehydrates any gel left on the slide and prevents contamination of the slide.

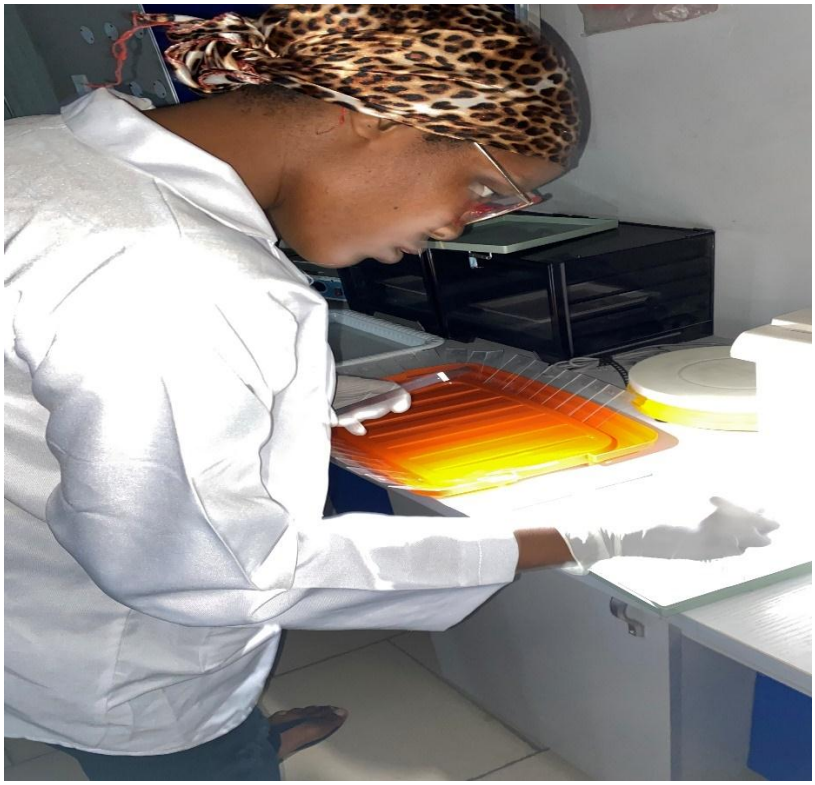

Plate 3.7: fixation of slides with cold ethanol

Plate 3.8: drying of fixed slides

3.3.6 Staining

The slides are then stained with giemsa stain and rinsed with distilled water. This allows the DNA to be seen under a microscope. Giemsa stain is powdered and is prepared three months prior to use. Staining is done in a coplin jar for five minutes and then rinsed with distilled water and allowed to dry.

Plate 3.9: coplin jar with giemsa stain in it

3.3.7 Viewing and Scoring

The DNA fragments are then imaged with the aid of a light microscope fitted with a camera. The visual representations are then 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.

3.3.8 Statistical Analysis

Slides were stained with Giemsa stain, viewed under a light microscope (magnification X160), scored using imagej_v_1.3.1; derived excel sheets were cleaned. 2-way ANOVA (analysis of variance) statistical analysis was done on the derived mean and standard deviation of each group using GraphPad prism 8, Turkey multiple test was carried out.

CHAPTER FOUR

RESULTS

4.1 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 dose dependent, it increased with increase concentrations. In higher concentrations of the test effluent, fish showed initial disturbed swimming movements and surfacing behaviour indicative of avoidance response. This was followed by bleaching of the skin, sudden quick movement, unusual lethargy and fish settling at the bottom of the aquaria motionless with slow opercula movements, gulping for air. Fishes exposed to 100% concentration of cassava wastewater died in three minutes. Fishes in control (0% concentration) showed normal behaviour.

Table 4.1: 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

4.2 GENOTOXICITY RESULT

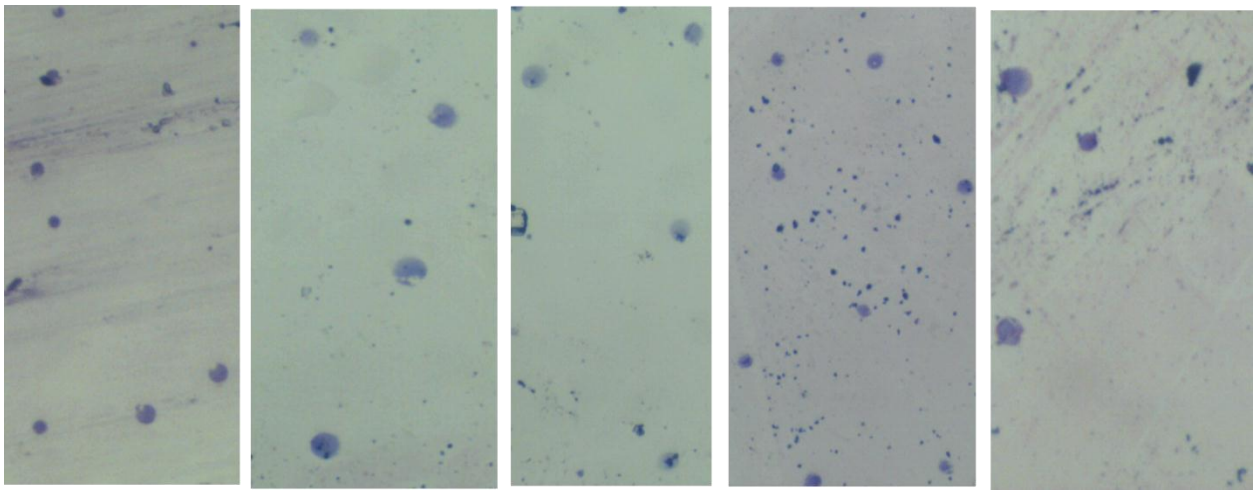

Plate 4.1: Micrographs of DNA damage shown by gills of *Clarias gariepinus* dosed with cassava wastewater at concentrations 0 %, 0.2 %, 0.3 %, 0.5 % and 0.7 %

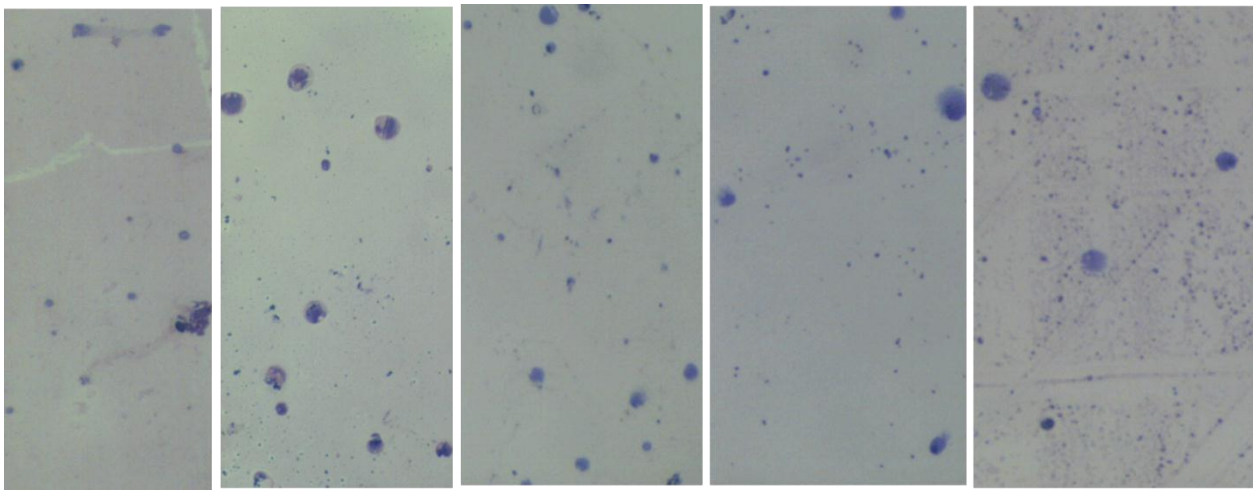

Plate 4.2: Micrographs of DNA damage showed by gonads of *Clarias gariepinus* dosed with cassava wastewater at concentrations 0 %, 0.2 %, 0.3 %, 0.5 % and 0.7 %

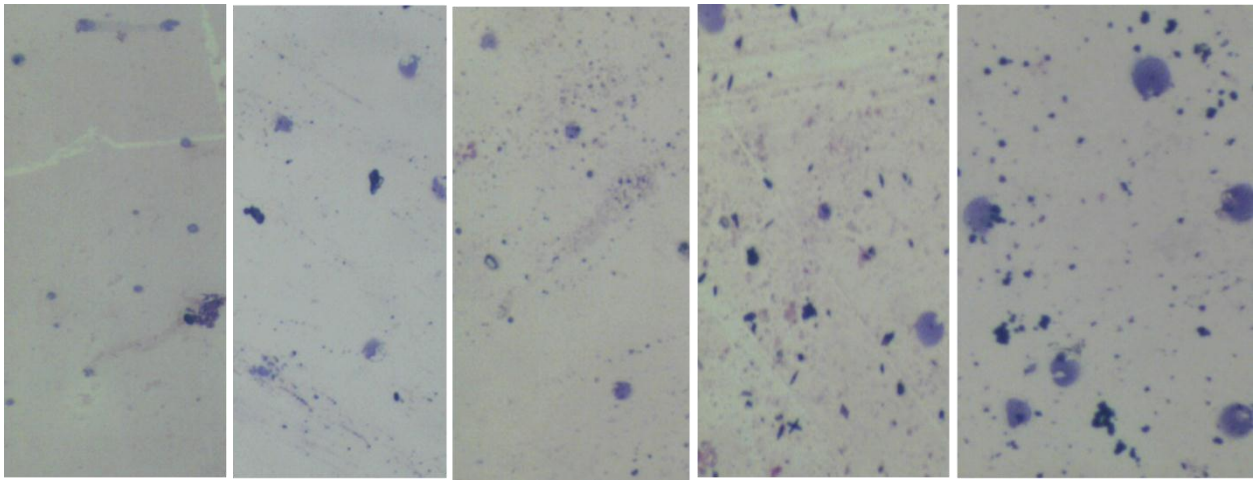

Plate 4.3: Micrographs of DNA damage showed by liver of *Clarias gariepinus* dosed with cassava wastewater at concentrations 0 %, 0.2 %, 0.3 %, 0.5 % and 0.7 %

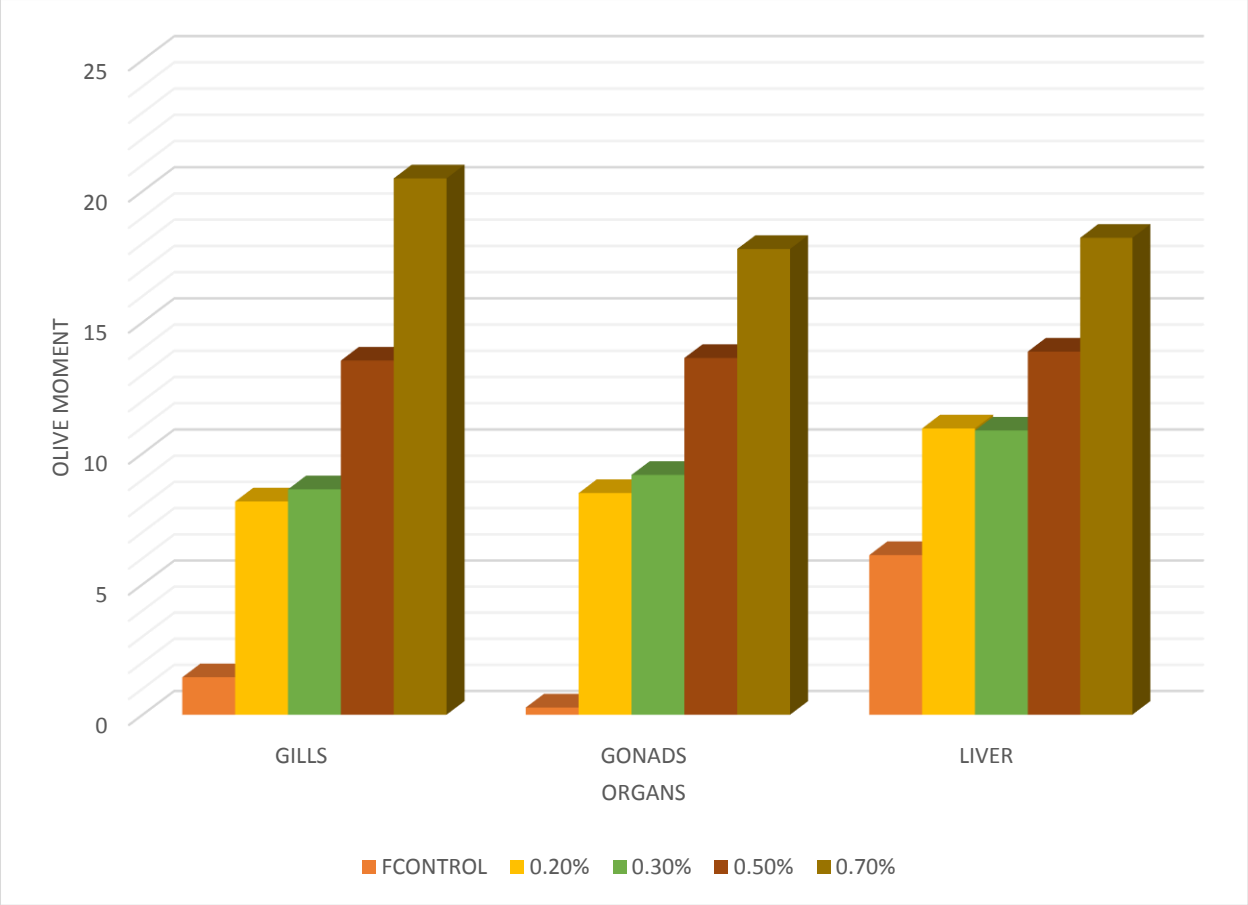

Fig. 4.1: Genotoxic potential of Cassava on *Clarias gariepinus*

CHAPTER FIVE

DISCUSSION AND RECOMMENDATION AND CONCLUSION

5.1 Discussion

In the current study, cassava wastewater caused a noticeable decrease in optical density in comparison to the control group. Fish hold a pivotal role in aquatic ecosystems, playing multiple roles in the trophic chain. They exhibit direct sensitivity to even minute concentrations of toxic substances in the water and indirectly respond by consuming contaminated aquatic organisms (Cavas & ErgeneGözükara, 2005). As a result, fish can serve as early indicators of aquatic ecosystem contamination. Wastewaters and effluents are a significant contributor to water pollution, and fish, due to their frequent metabolic fluctuations in response to wastewater, are excellent indicators of wastewater pollution in aquatic environments (Cattaneo *et al*., 2011).

Various species of freshwater and marine fishes have been employed as models for genotoxicity assessments in aquatic environments. DNA strand breaks, a crucial biomarker of genotoxicity in fish, are measured using the comet assay (Mitchelmore & Chipman, 1998). The comet assay is a widely used, straightforward, and highly sensitive technique for assessing DNA damage in various fish tissues, such as gills, liver, gonads, kidney, and blood, following exposure to diverse pollutants found in aquatic environments (Dhawan, Bajpayee, & Parmar, 2009). Among these tissues, blood is particularly convenient for collection since it comprises approximately 97% of the total fish blood and is readily accessible. Consequently, red blood cells (RBCs) are frequently utilized in fish to evaluate DNA damage using the comet assay method. For solid tissues like gills, liver, and kidney, cellular dissociation and isolation are required before proceeding with the comet assay, employing methods that do not induce DNA damage themselves.

The comet assay under alkaline conditions can identify various DNA damage types, including single-strand breaks, alkaline labile sites, and DNA cross-links induced by wastewater (Kumar,

Nagpure, Kwshwaha, Srivastava, & Lakra, 2010). The comet assay offers substantial advantages over other cytogenetic methods because it does not necessitate cells to be mitotically dividing (Ali *et al*., 2009; Ali & Kumar, 2008; Nwani *et al*., 2010).

The result of the study shows that cassava wastewater cause 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. Abnormal behaviour such as loss of balance, resting at the bottom of the tank, consistent jumping, pale skin were observed in this study and were similar other related studies.

Higher levels of DNA damage in gill cells could be attributed to the constant exposure of gills to water pollutants capable of causing DNA damage. Gills are vital organs for fish, serving critical functions in respiration, osmoregulation, and excretion. Gills are often considered excellent indicators of water quality, making them suitable for environmental impact studies, as they serve as the primary entry point for effluents. Any damage to gills due to xenobiotic chemicals or changes in membrane permeability can significantly impact oxygen uptake rates, leading to respiratory distress (Olufayo *et al*., 2016).

Many genotoxic substances are known to produce reactive oxygen species and electrophilic free-radical metabolites that interact with DNA, leading to DNA disruption. Studies by Chandra and Khuda-Bukhsh (2004) have shown that the metabolism of azadirachtin produces electrophilic ions and free radicals that interact with DNA's nucleophilic sites, resulting in breaks and related DNA damage. Oxidative stress within an organism is also reported to play a pivotal role in inducing cytotoxicity and genotoxicity in various vital tissues (Moore, Yedjou, & Tchounwou, 2010).

The current study has observed a time-dependent increase in DNA damage. In a related study investigating the haematological and histopathological effects of cassava effluent on adult female African catfish, *Clarias gariepinus*, the fish displayed signs of gill and liver damage. Similarly, histopathological examinations of the kidney, gills, and liver of Nile Tilapia fingerlings, *Oreochromis niloticus*, exposed to cassava effluent indicated damage (Olorunfemi *et al*., 2011). It is evident that cassava wastewater is toxic to aquatic organisms and can pose a risk to humans if not adequately treated before discharge into water bodies.

The nucleic acid content of an organism is considered an indicator of its protein synthesis capacity. Fish growth and development rely on DNA content, which plays a crucial role in biochemical processes (Buckley, 1980). Active protein synthesis and cell growth are contingent on DNA content, and exposure to wastewater leads to changes in nucleic acid content.

The substantial reduction in DNA content observed in various fish organs exposed to cassava wastewater in this study may be attributed to reduced protein synthesis, impaired nucleic acid metabolism, and cellular degradation. Another key factor in DNA damage could be the inhibition of enzymes responsible for DNA replication or repair (Guilherme *et al.*, 2012). The integrity of DNA is a crucial component of normal cellular processes, and alterations in DNA can lead to cell structure loss, proliferation of new cells, tissue formation, and degradation, ultimately resulting in the loss of cellular control mechanisms. Wastewaters have been shown to induce various primary changes in DNA, including single-strand breaks, double-strand breaks, DNA-protein crosslinks, and damage to purine and pyrimidine bases, as a result of physicochemical interactions between wastewater and cellular DNA.

5.2 CONCLUSION

The study showed that cassava wastewater is highly toxic to aquatic species even in small concentrations. Cassava effluent is able to induce genetic damages in in aquatic species even in small concentrations.

5.3 RECOMMENDATION

Cassava wastewater is a highly toxic effluent. This is mainly due to the presence of cyanide in it. Cyanide is a toxic chemical and fishes are more toxic to it than humans. Its is therefore important that that wastewater gotten from cassava processing industries be treated properly before discharging it into waterbodies.

Also, regulatory agencies should be put in place to monitor the production process and discharge of effluent in order to safeguard the aquatic ecosystem.

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APPENDIX

Tukey Multiple Comparisons test on the harvested organs from (species name) fish treated with cassava.

Tukey's multiple comparisons test	Significant?	Summary	Adjusted P Value
GILLS			
FCONTROL vs. 0.20%	Yes	****	<0.0001
FCONTROL vs. 0.30%	Yes	****	<0.0001
FCONTROL vs. 0.50%	Yes	****	<0.0001
FCONTROL vs. 0.70%	Yes	****	<0.0001
0.20% vs. 0.30%	No	ns	0.8023
0.20% vs. 0.50%	Yes	****	<0.0001
0.20% vs. 0.70%	Yes	****	<0.0001
0.30% vs. 0.50%	Yes	****	<0.0001
0.30% vs. 0.70%	Yes	****	<0.0001
0.50% vs. 0.70%	Yes	****	<0.0001
GONADS			
FCONTROL vs. 0.20%	Yes	****	<0.0001
FCONTROL vs. 0.30%	Yes	****	<0.0001
FCONTROL vs. 0.50%	Yes	****	<0.0001
FCONTROL vs. 0.70%	Yes	****	<0.0001
0.20% vs. 0.30%	No	ns	0.4764
0.20% vs. 0.50%	Yes	****	<0.0001
0.20% vs. 0.70%	Yes	****	<0.0001
0.30% vs. 0.50%	Yes	****	<0.0001
0.30% vs. 0.70%	Yes	****	<0.0001
0.50% vs. 0.70%	Yes	****	<0.0001
LIVER			
FCONTROL vs. 0.20%	Yes	****	<0.0001
FCONTROL vs. 0.30%	Yes	****	<0.0001
FCONTROL vs. 0.50%	Yes	****	<0.0001
FCONTROL vs. 0.70%	Yes	****	<0.0001
0.20% vs. 0.30%	No	ns	0.9997
0.20% vs. 0.50%	Yes	****	<0.0001
0.20% vs. 0.70%	Yes	****	<0.0001
0.30% vs. 0.50%	Yes	****	<0.0001
0.30% vs. 0.70%	Yes	****	<0.0001
0.50% vs. 0.70%	Yes	****	<0.0001