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Evaluation of Genotoxicity and Antioxidant Defense System in the Brain of *Clarias Gariepinus* Exposed to Uproot, A Glyphosate Herbicide

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Abstract
Uproot, a glyphosate - based herbicide used to control weeds in farmlands may contaminate the aquatic habitat due to leaching and could pose a great risk to non – target aquatic organism like fish. This study evaluates the genotoxicity and antioxidant defense system in the brain of Clarias gariepinus exposed to Uproot, a glyphosate – based herbicide. Twenty juvenile Clarias gariepinus were divided into four groups (n = 5), with Group A (no glyphosate exposure); Groups B, C and D (30, 50 and 70 mg/L) of glyphosate exposure. Exposure to glyphosate lasted for 14 days. Fish were dissected and brain tissue was collected and analysed for some biochemical parameters and photomicrograph examinations using standard laboratory procedures. The results indicated that the activities of superoxide dismutase, catalase, peroxidase and nitric oxide concentration were reduced ($p < 0.05$) in glyphosate exposed fish as compared with the control fish. A significant elevation ($p < 0.05$) was observed in the activities of glutathione peroxidase, acetylcholinesterase, myeloperoxidase, levels of reduced glutathione, malondialdehyde and percentage DNA fragmentation in glyphosate induced fish as compared with the control. Glyphosate exposed Clarias gariepinus had comparable ($p > 0.05$) concentration of thiol protein and glutathione s- transferase activity. An elevated ($p < 0.05$) total protein concentration was also observed in fish exposed to 50 mg/mL glyphosate as compared to the control. Histopathological examination of the brain tissues revealed degenerated neurons, altered stratum with no visible Purkinje cell nucleus in glyphosate fish as compared with the control. Together, the findings of this study showed that exposure to glyphosate impaired the activities of antioxidant enzymes and could also lead to genotoxicity. Thus, these biochemical parameters could be employed as biomarkers for environmental contamination of glyphosate herbicide.
Keywords: *Glyphosate, Antioxidant defense, Clarias gariepinus, DNA fragmentation, Uproot*

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

The environment is becoming more and more threatened by urbanization, industrialization, increasing agricultural methods, and the rising human population. These environmental issues have gotten worse due to the intensive exploitation of natural resources. The aquatic ecology has been greatly influenced by these operations. Nonetheless, individuals have begun to become more cognizant in recent years and are making a concerted effort to stop the trend. The intent of employment of herbicides in agricultural practices is to control weeds and improve productivity of farm produce (Bhati, 2002).

Glyphosate which is a non-selective herbicide is among the widely used chemicals for weed control. The herbicidal potential of this chemical was first identified in 1970 (Annett *et al,* 2014). In 1974, it was processed and released into the agro-chemical market (Duke and Powel, 2008). The chemical has the potential to contaminate the environment. The aquatic habitat is polluted through water runoff and leaching from treated areas which constitutes a risk to non-target aquatic organisms. These toxic chemicals are sometimes sprayed directly on aquatic weeds, which eventually contaminate these bodies of water (Mishra, 2021).

The adverse effects of pollutants such as glyphosate in the aquatic ecosystem is of grave concern (Banaee and Taheri, 2019). Large scale and indiscriminate use of “Uproot”, a glyphosate-based herbicide in the Niger Delta and its environs is on the increase. The nearness of its application sites to natural water bodies and fish cultures in the locality could induce changes in several biochemical variables which could be employed to assess fish health. Changes in the biochemical variables could be alteration of cellular and tissue functions, changes in the physiology and the behaviour of the organism (Pervez and Raisuddin, 2005).

Glyphosate may exert its effect on fish system through radical formation which are high energy molecules that bring about oxidative stress. High level production of reactive oxygen species (ROS) on exposure to glyphosate in *Clarias gariepinus* may pose serious oxidative damage to nucleic acids, lipids and proteins leading to organelle damage and apoptosis (Bagchi *et al,* 1995), and (Citgo *et al,* 2009).

The African catfish, *Clarias gariepinus* is consumed on daily basis in the Niger Delta. It is widely used for toxicity studies. Therefore, this study is aimed to examine the toxicity of acute concentrations of this chemical on *Clarias gariepinus* using DNA fragmentation as a basis for genotoxicity.

The biochemical responses of the African catfish on exposure to glyphosate may be similar to those of mammals and other vertebrates (Sancho *et al*, 2000). This species of catfish is relatively abundant in the region of this research which provides a source of protein, and it is thought to be a potential bio-indicator for toxicity studies (Osioma and Iniagha, 2019).

Studies of have shown the induction of oxidative stress, neurological dysfunction and neurotoxic effects in various species of aquatic organisms exposed to glyphosate (Singh *et al*, 2018). The brain being a delicate organ is vulnerable to glyphosate induced toxicity (Soloneski and Larramendy, 2017). However, the genotoxic and antioxidant responses in the brain of *Clarias gariepinus* exposed to uproot is unclear. This study however will evaluate genotoxicity and antioxidant defense system in the brain of *Clarias gariepinus* exposed to Uproot which will provide valuable insights into genotoxic and neurotoxic effects of glyphosate on aquatic organisms.

To ascertain the impact of this chemical exposure on aquatic ecosystems, the activity of several antioxidant enzymes in the brain tissue of *Clarias gariepinus* subjected to different doses of glyphosate will be evaluated.

The activities of some anti-oxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), as well as reduced glutathione (GSH), malondialdehyde (MDA) and nitric oxide concentration in brain tissue of *Clarias gariepinus* exposed to different concentrations of Uproot, a glyphosate-based herbicide will be investigated. Protein Thio and total brain protein of the fish and other associated parameters will be will also be assessed.

1.2 Statement of Problem

This research aims to determine if glyphosate exposure might cause:

i. Brain genotoxic effects such as DNA fragmentation which could potentially impact on the health and survival of *Clarias gariepinus.*
ii. Modifications in the antioxidant system of the brain, including reduced glutathione, glutathione-S-transferase, catalase, and superoxide dismutase, which may be signs of oxidative stress.
iii. Changes in histopathological parameters of the brain tissue of *Clarias gariepinus.*

These findings will give a better understanding of the impacts of glyphosate exposure on the well- being of *Clarias gariepinus.* The data will inform and expand understanding of how pesticides and herbicides influence ecosystem health and function.

1.3 Aim and Objectives of the study

The primary aim and objective of this study is to evaluate genotoxicity and antioxidant defense system in the brain of *Clarias gariepinus* exposed to Uproot, a glyphosate-based herbicide.

The specific objectives are to;

i. Assess genotoxic effects of Uproot on *Clarias gariepinus* brain cells using DNA fragmentation as potent biomarker.
ii. Examine the impact of Uproot exposure on some antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and non-enzymatic antioxidant such as reduced glutathione (GSH) in the brain of *Clarias gariepinus*.
iii. Investigate histopathological changes in the brain of *Clarias gariepinus* exposed to Uproot.

iv. Determine the activity of acetylcholinesterase (AChE), myeloperoxidase (MPO), glutathione-S-transferase (GST), in the brain of *Clarias gariepinus* exposed to Uproot.
v. Assess lipid peroxidation, level of malondialdehyde (MDA) and nitric oxide in the brain tissue of *Clarias gariepinus.*

1.4 Significance of the study

The significance of this study is multifaceted which include;

i. Provision of insights into genotoxic effects of glyphosate-based herbicide on aquatic lives.
ii. Evaluation of the impact of glyphosate on antioxidant defense system in fish brain tissue.
iii. Enabling mechanistic understanding of glyphosate toxicity in non-target aquatic organisms.
iv. Assessment of environmental risk posed by glyphosate-based herbicides in aquatic ecosystems
v. Inform strategies for mitigating pesticide pollution in water bodies.
vi. Information might help aquaculture become more sustainable
vii. Educate policymakers and regulators about pesticides
viii. Awareness creation on pesticide impacts on aquatic ecosystems and human health.
ix. Support for evidence-based environmental conservation decision-making

1.5 Justifications of the study

This study's strong arguments include the following:

i. Glyphosate based herbicides are used and applied widely, leading to potential environmental contamination.
ii. The African catfish especially *Clarias gariepinus* is an important species that is sensitive to water pollution.
iii. Research gaps on the genotoxic and antioxidant effects of glyphosate in fish brain tissue exist.
iv. Herbicide pollution threatens aquatic ecosystem and biodiversity.
v. Glyphosate contamination affect non-target aquatic organisms including *Clarias gariepinus*.
vi. Understanding pesticides impact on organisms informs conservation decision.
vii. Fish and other aquatic organisms contribute to food security and economy.
viii. Aquatic livelihoods and productivity are impacted by herbicide contamination
Public concern about herbicide impact on environment and health.
ix. Findings make evidence-based decision.

CHAPTER TWO

LITERATURE REVIEW

2.1 Conceptual Framework

2.1.1 Agrochemicals and the Environment

With the rise in fish farming and aquaculture, there is an increase in the use of agrochemicals. Agrochemicals are pesticides which include; insecticides, herbicides, and fungicides. These chemicals are marketed as harmless, with little or no side effects as usually described by the manufacturers. Despite the rapidly increasing use of these agrochemicals, researchers have viewed some of these chemicals as materials whose environmental fate is poorly understood (Dedeke, *et al,* 2018).

The use of agrochemicals, such as herbicides in crop and fish farming, most especially around fish ponds has raised a lot of questions regarding public health. Some of these chemicals with negative effects on target organisms in aquaculture include Copper sulphate, Malachite green, Formalin, Potassium permanganate, etc. According to (Alarape, *et al*, 2013), *Clarias gariepinus* exposed to copper sulphate resulted in necrotic ovaries, matted lamellae of the gills, and multifocal severe degeneration of the seminiferous tubules, while those exposed to Malachite green resulted in disrupted and depleted seminiferous tubules, focal localized vacuolation of skin, and generalized fatty degeneration of liver (Adeyemi, *et al,* 2011).

The use of agrochemicals can easily be washed into ponds and other water bodies by rain, altering the physicochemical parameters of the water and the chemicals could also build up in the fish systems, and this would pose a threat to human health and public health in general.

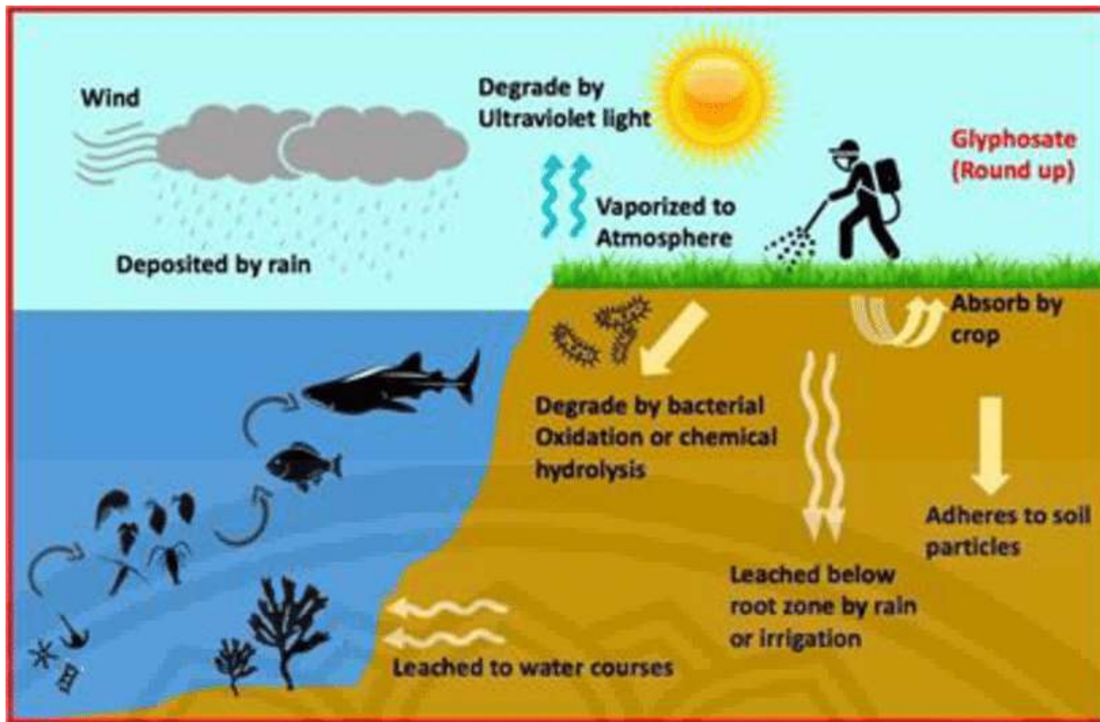


FIG 1: Distribution and transportation of glyphosate into aquatic organisms and environment. Source; Amnuay Wattanakornsiri (2020).

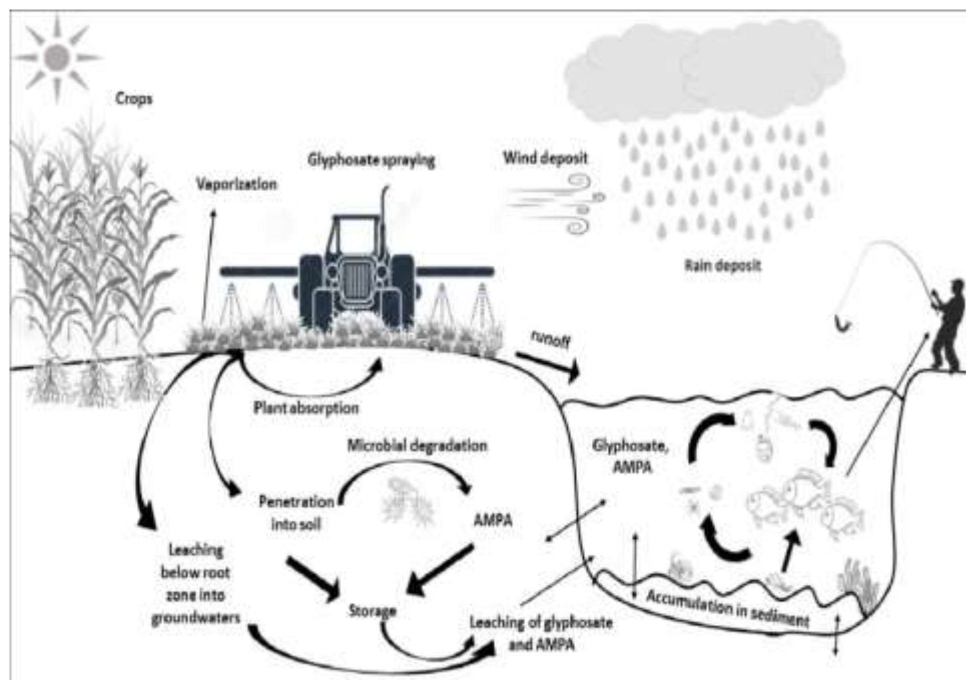


FIG 2: Distribution of glyphosate and its metabolite AMPA. **Source:** Nikola Mikuskova (2021)

2.1.1.1 Uproot

Uproot is a commercial formulation of glyphosate (N-phosphonomethyl glycine). It is a biocide with a broad-spectrum of activity introduced for weed control in agricultural fields. Glyphosate is the active ingredient in the Uproot brand of agricultural herbicides and a variety of other herbicide formulations. These formulations which are used widely in agricultural, forestry, and residential markets provide non-selective, post-emergent control of annual and perennial weeds (Acquavalla, *et al,* 2004).

2.1.1.2 Chemical structure and mode of action of Glyphosate

Glyphosate is a systemic herbicide with the chemical name N-(phosphonomethyl) glycine. The structure is made up of phosphonic acid (-H2PO3), methyl group (-CH2-) and a glycine amino acid moiety (-NH-CH2-COOH) with an overall chemical formula C3H8NO5P (Franz *et al*, 1997).

The mode of action of glyphosate is the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) which is essential for the biosynthesis of aromatic amino acids such phenylalanine, tyrosine and tryptophan in the shikimate pathway in plants (Duncan and Wohlhueter, 1987). The ordered mode of action is;

Uptake; Glyphosate is absorbed by plants through foliage or root.

EPSPS Inhibition; Glyphosate binds to this enzyme, preventing the conversion of phosphoenolpyruvate and shikimate -3-phosphate to 5-enolpyruvylshikimat-3-phosphate, an intermediate substrate for aromatic amino acid synthesis.

Aromatic acid depletion; the inhibition of this critical enzyme depletes aromatic amino acids. This disrupts protein synthesis essential for plant growth. This leads to the plant death due to accumulation of toxic intermediates disrupting the metabolic pathway (Duke *et al*., 2003).

The mechanisms underlying glyphosate-induced toxicity stress in catfish are multifaceted and involve complex interactions between glyphosate and cellular components. Glyphosate and its metabolites can generate ROS directly through redox cycling or indirectly by disrupting mitochondrial function, interfering with enzymatic activities, or inducing lipid peroxidation. Additionally, glyphosate can chelate essential metal ions, such as iron and manganese, leading to the formation of hydroxyl radicals via Fenton-like reactions. Furthermore, glyphosate may perturb cellular antioxidant defenses by modulating gene expression, protein synthesis, or enzyme activities involved in antioxidant pathways.

Glyphosate being an organic acid is composed of a phosphonomethyl and glycine. The chemical name is N-phosphonomethyl glycine. It is a Zwitterion with four different dissociation constants and exist as different ionic species dependent on the prevailing pH. Glyphosate inhibits 5-enoylpyruvylshikimate 3-phosphate synthase, an enzyme in the shikimic acid pathway. This inhibition prevents plants from producing the aromatic amino acids phenylalanine, tryptophan and tyrosine capable of producing proteins and lignin in plants which give plants structural support for

growth and development. However, human and other advanced animals dot not have the shikimate pathway, therefore, are unable to synthesize these amino acids (Shaner, 2014).

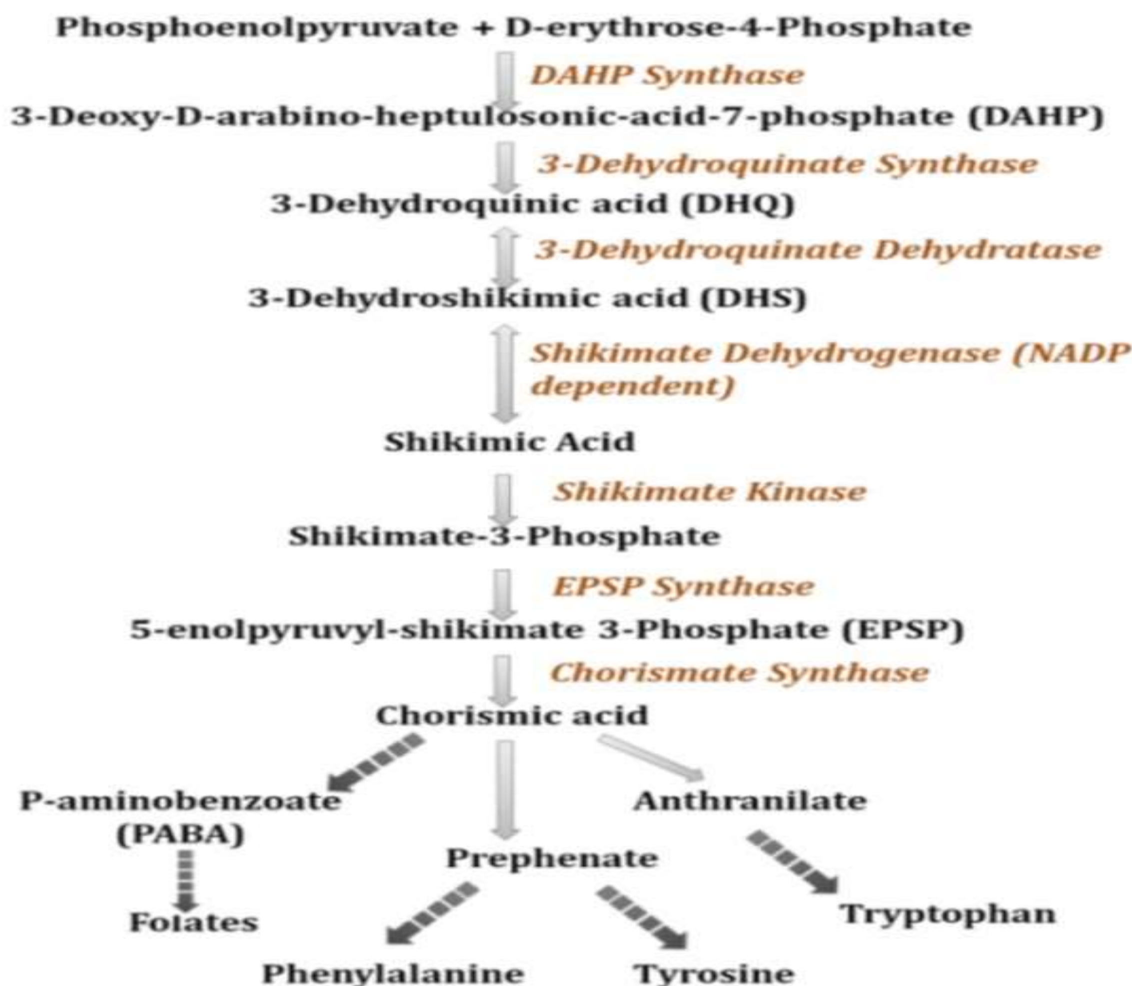


FIG3: Shikimic Acid Pathway

Source: Uploaded by Rupa Bhowmick (2020).

2.1.2 Importance of *Clarias gariepinus* as model organism

This is a special species of African catfish in tropical aquaculture. It is widely distributed and accepted by many farmers in Africa because of its fast growth, large size, low bone content, hardness and high yield, tolerance to poor water quality, omnivorous feeding pattern, crowd adaptability and appreciable market value (Osman *et al*, 2006). It serves as a model organism for studying genotoxicity and antioxidant defense systems due to:

a. Eco-relevance: This fish species is widely distributed in African fresh water ecosystem, making it an ideal species for environmental assessment impacts (Fafioye *et al*, 2017).
b. Easy to culture: It is widely cultivated for food, making it available for research (Ololade *et al*, 2020).
c. Sensitivity to pollutants: It is very sensitive to water pollutants including pesticides according to (Adeyemi *et al,* 2018).
d. Brain structure similarities: *Clarias gariepinus* brain structure shares similarities with other vertebrates (Costa, 2016).

2.1.2.1 Rationale for studying Brain tissues

Brain tissue of fish is vulnerable and sensitive to neurotoxins, leading to neurological disorders, behavioural changes and cognitive impairment. It has a very high metabolic rate and lipid content, sensitive to genotoxins and therefore, serves as a potential biomarker for environmental pollution (Fafioye, *et al*, 2017). Human health implications are of serious concern particularly on consumption of genotoxically contaminated fish (Ololade, *et al,* 2020).

Studying brain tissues of fish is critical in antioxidant defense system. The oxidative stress mitigation and neuroprotection by antioxidant defense system is crucial for maintaining fish health (Sies and Jones, 2020). Antioxidant responses in fish brain tissues portend ecological relevance because such responses can indicate environmental pollution levels (Livingstone, 2017).

2.1.3 Glyphosate toxicological effects on aquatic organisms

Glyphosate toxicity in aquatic ecosystems can be seen as acute or chronic toxicity. Some of the symptoms of herbicide toxicity in aquatic lives include respiratory abnormalities leading to laboured breathing or lethargy, equilibrium loss or exhibition of erratic swimming patterns, changes in skin and scales leading to the development of ulcers, skin colour changes and gastrointestinal effects characterized by vomiting, reduced appetite and diarrhoea (Chen, *et al,* 2020) and (Zhan, *et al,* 2018). Exposure of aquatic ecosystem to herbicides could be acute, characterize by high concentrations of the herbicide within a short exposure time leading to rapid mortality or chronic toxicity in a long-term exposure to lower concentrations with delayed mortality (Kumar, *et al,* 2022)**.** Glyphosate has been shown to elicit acute toxicity on fish, crustaceans and algae with varying LC50 values (Ma, *et al*, 2006). However, chronic exposure has been linked to various toxicological endpoints such as endocrine disruption, genotoxicity, and reproductive toxicity in different aquatic organisms (Harrman, *et al,* 2016). The toxicological effects are attributed to inhibition of 5- enolpyruvylshikimate-3-phosphate synthase in aquatic plants, compromise of membrane integrity and homeostasis, antioxidant depletion and oxidative stress (Duke, *et al*, 2003) and (Glusczak, *et al*, 2007). Consistent application of glyphosate on aquatic environment raises concern on bioaccumulation and biomagnification, impact on aquatic biodiversity and water quality degradation (Relyea, 2005) and (Giesy, *et al*. (2000).

2.1.3.1 Potential mechanism of glyphosate induced toxicity in fish

Glyphosate, the active component in Uproot herbicide can induce toxicity in fish in series of ways. Deoxyribonucleic acid damage may be one of the many ways glyphosates confer toxicity on fish. This can be seen in DNA strand breaks, micronucleus formation, chromosomal aberrations, and DNA fragmentation (Glusczak, *et al,* 2007). These changes can lead to mutation, reproductive impairment, and carcinogenesis (Bolognesi, *et al*, 2006).

Oxidative stress on glyphosate exposure can lead to increased lipid peroxidation, elevated glutathione-S-transferase and catalase activities and depletion of antioxidant defenses leading to compromised cellular homeostasis, cellular damage and mortality (Glusczak, *et al*, 2007) and (Cavas, 2011). Glyphosate induced toxicity in fish can also be seen in the compromization of membrane integrity, acetylcholinesterase inhibition and changes in kidney and liver functions (Harrman, *et al*, 2016) and (Guilherme, *et al,* 2012). Neurotoxicity is also a known damage caused by some herbicides such pendimethalin which disrupt nervous system in fish causing behavioural changes and respiratory damages (Verma, *et al,* 2019).

Apoptosis is another known means of glyphosate-induced toxicity in fish and other aquatic organisms. Glyphosate exposure induces apoptosis through increased caspase-3 activity and DNA fragmentation. The genotoxic mechanism is through direct damage or oxidative stress mediated damage, highlighting concerns for aquatic ecosystem (Cavas, 2011).

2.1.4 Genotoxicity

Genotoxicity describes the property of chemical agents that damage genetic information within a cell bringing about mutation that may lead to cancer (Nagathna, *et al*, 2013). These agents known as genotoxins have the potential to induce mutation and other genetic alterations or lesions which can lead to development of various diseases including cancer. Genotoxins are classified into carcinogens, mutagens and sometimes teratogens based on their effects according to (Mohamed, *et al,* 2017). Genotoxins have the potential to damage DNA molecules which may alter normal structure and functioning of the DNA molecule, potentially disrupting critical cellular processes, inducing errors during replication, leading to mutation in newly synthesized DNA strand. The consequences of genotoxicity vary depending on the extent of damage, the cell's ability for damage repair and the type of cells affected. Unrepaired and inaccurate repaired damage can result in several outcomes such as mutation which is a change in DNA sequence that can alter encoded instructions in DNA. It alters normal cellular processes that leads to malfunctioning proteins, abnormal cell growth and other abnormalities. Serious DNA damage can lead to carcinogenesis, where key genes that regulate cell growth and division may lead to uncontrolled cell proliferation and the eventual formation of a tumor. DNA damage can also trigger apoptosis aimed at eliminating severely damaged cells (Poetsh, *et al,* 2020).

2.1.4.1 DNA Fragmentation

DNA fragmentation is the separation or breaking of DNA strands into smaller pieces. This can be induced or occur spontaneously due to exposure to genotoxins or radiations. DNA double strand breaks are harmful lesions which can lead to genomic instability and eventual cell death if repair mechanisms are altered (Gonzalez-Marin, *et al,* 2012). DNA fragmentation can be classified to

apoptotic, necrotic or enzyme mediated. During programmed cell death, caspase activates DNase which cleaves the DNA molecule into smaller units or fragments of about 180-200 base pairs (Nagata, *et al,* 2003). DNA fragmentation due to necrosis is made possible due to cell injuries and infections (Soldani and Scovassi, 2004). DNase 1, DNase 11 or restriction endonucleases activities can lead to fragmentation of the DNA molecule (Zhang, *et al,* 2015). DNA fragmentation is caused by oxidative stress, genotoxic stress, inflammatory cells activation and ageing (Cooke, *et al*, 2003). Some of the resultant effects of DNA fragmentation include genomic instability, epigenetic changes and cell death (Zhang, *et al*, 2015).

2.1.4.2 Potential Genotoxic effects of Glyphosate in Brain tissue of *C. gariepinus*

Studies on the genotoxic effects of glyphosate in the brain of Clarias gariepinus is limited. However, studies on other fish species and organisms provide insights on glyphosate genotoxic mechanisms. Potential genotoxic effects are DNA damages which include DNA fragmentation and strand breaks, micronucleus formation and chromosomal aberrations (Bolognesi *et al,* 2006). Oxidative stress is another potent potential mechanism that triggers DNA damage and neurotoxic effects. Different genetic effects in fish include micronuclei formation characterized by the cytosolic creation of small membrane bound nuclei, housing chromosomal materials, indicating chromosomal damage. Chromosomal aberration is another genotoxic effect characterized by changes in chromosomal structures and numbers through duplications, deletions and translocation (Verma *et al,* 2019). Genomic instability which is the elevated frequency of genetic changes can ultimately lead to cancer and other associated diseases. DNA strand breaks, DNA fragmentation and mutations are genetic lesions that can lead to genetic instability and changes in protein functions and expressions (Rao, *et al*, 2017).

Aquatic organisms such as fish has different genotoxic responses, and these include;

Mutagenicity. This is changes in the genetic information resulting from the induction of mutation. Clastogenicity involves the induction of chromosomal breaks, leading to chromosomal abnormalities (Kumar *et al,* 2022). Aneugenicity is a change leading to aneuploidy, described as the numerical chromosomal changes induced by genotoxins in the aquatic environment, while oxidative genotoxicity is damage induced by reactive oxygen species (ROS) (Chen, *et al*. (2022). Epigenotoxicity is changes in gene expression without a corresponding change in the DNA (Zhang *et al,* 2022).

2.1.5 Oxidative Stress and Antioxidant Defense System

Oxidative stress is a condition where the balance between free radical production and antioxidant defense is disrupted. Free radicals including reactive oxygen species (ROS) and reactive nitrogen species (RNO) are highly reactive molecules that can effectively damage cell organelles, leading to cell death (Sies, 2015). Some of the sources of oxidative stress include ultraviolet radiations, metabolic processes, inflammation and environmental pollutants such as heavy metals, and pesticides (Glusczak *et al*, 2007).

Antioxidant defense system encompasses the neutralization of free radicals by antioxidants, protecting cells from oxidative damage. This defenses are mediated by enzymes and non-

enzymatic molecules. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione –S-transferase (GST) (Hayes *et al*, 2005). The non- enzymatic antioxidant defense system includes reduced glutathione, vitamin C and E, polyphenols (flavonoids, phenolic acids), and carotenoids such as lycopenes, beta- carotenes (Krinsky *et al,* 2003).

2.1.5.1 Aquatic Organisms and Antioxidant Responses to Glyphosate

Antioxidants system in aquatic organisms respond differently to different pollutants in different tissues other than the brain tissue. However, glyphosate specific responses of superoxide dismutase (SOD) in fish and shrimp has increased activity. Catalase (CAT) has increased activity in fish but decreased activity in crustaceans. Glutathione peroxidase (GPx) has increased activity in fish but decreased activity in shrimp. Glutathione –s- transferase has increased activity in both fish and crustaceans (Glusczak *et al*, 2007) and (Moraes, *et al,* 2017). However, reduced glutathione (GSH), vitamins C and E deplete levels in fish and shrimp. Oxidative stress markers such as lipid peroxidation (LPO), malondialdehyde and protein carbonylation increases in fish (Guilherme, *et al,* 2012) and (Cavas, 2011).

2.1.5.2 Antioxidant Defense Mechanisms in *C. gariepinus* Brain Tissue

Clarias gariepinus brain tissue antioxidant defense mechanism is an interplay of enzymatic and non-enzymatic antioxidants, antioxidant related genes and signalling pathways. The enzymatic antioxidant superoxide dismutase converts superoxide radicals to hydrogen peroxide. Catalase degrade or decomposes hydrogen peroxide to water and oxygen. Glutathione peroxidase reduces lipid peroxide to the corresponding alcohols while glutathione-s-transferase conjugate glutathione to electrophilic compounds. The non- enzymatic antioxidants such as reduced glutathione scavenges free radicals for maintenance of redox balance. Vitamin C scavenges against oxidative stress while vitamin E protects against lipid peroxidation (Halliwell, *et al,* 2007), Ogbeibu, *et al,* (2019). Nitric oxide has been seen to be a potent antioxidant in recent years (Radi, 2018). It reacts with superoxide radicals to form peroxinitrite (ONOO). This intermediate does pro-oxidant response and scavenge lipid peroxyl radicals (LOO). This role is seen as an antioxidant response (Goss, *et al,* 1997).

The antioxidant related genes in brain tissue of *C. gariepinus* are the nuclear factor erythroid 2-related factor 2 (Nrf2) which regulates antioxidant gene expression (Sies, 2015). Kelch-like ECH-associated protein 1 (Keap1) regulates Nrf2 gene activity (Halliwell, *et al*, 2007). The glutathione-s-transferase pi (GST π) is associated with detoxification and antioxidant defenses (Akintonwa, *et al,* 2018).

The signalling pathways in this species of fish brain are the nitrogen- activated protein kinase (MAPK) which regulate antioxidant responses according to (Ogbeibu, *et al*, 2019). PI3K/Akt signalling pathway regulates antioxidant responses and cell survival (Akintonwa, *et al*, 2018).

2.1.5.3 Some Brain-specific Antioxidant Proteins in *C. gariepinus*

There are certain brain-specific proteins other than the conventional antioxidant system with active antioxidant roles that protect the brain from oxidative stress. These include Thioredoxin (Trx), Peroxiredoxin (Prx) and Neuroglobins. Thioredoxin regulates protein redox state and inflammation. Peroxiredoxin down-regulates lipid peroxidation and hydrogen peroxide. Neuroglobins binds oxygen and regulate oxidative stress (Adeyemi, *et al*, 2018) and (Matinez-Alvarez *et al*, 2015).

2.1.6 Oxidative Stress and Lipid Peroxidation

Lipid peroxidation occur when lipids particularly poly unsaturated fatty acids (PUFAs) react with reactive oxygen species (ROS) to form lipid radicals, damaging cell membranes that lead to various pathological conditions. Lipid peroxidation can be enzyme mediated such cyclooxygenase and lipooxygenase or non-enzyme mediated (Halliwell, *et al,* 2017). Lipid peroxidation have some deadly consequences, some of which are cell membrane damage, alteration of cellular signaling, inflammation pathways activation, protein modification and DNA damage (Kehrer and Klotz, 2015). Lipid peroxidation has assayable markers which include malondialdehyde (MDA), lipid hydroperoxide, thiobabituric acid reactive substance (TBARS) (Ohkawa, *et al,* 1979). Diseases linking to lipid peroxidation include inflammatory conditions (asthma,arthritis), neurodegenerative diseases, cancer, aging and astheroclerosis (Khrer and Klotz, 2015).

2.1.6.1 Oxidative stress and Lipid Peroxidation in *C. gariepinus* Brain

Oxidative stress and lipid peroxidation in the brain of *C. gariepinus* can be caused by water pollutants such as heavy metals (Hg, Cd, Pb) and industrial chemicals. Others causes include hypoxia, ultraviolet radiations and temperature changes. These can trigger oxidative stress leading to neuronal damages, cognitive impairment, neurodegeneration and neuroinflammation (Khrer and Klotz, 2015) and (Bols, *et al,* 2001).

2.1.7 Histopathology of Fish Brain Tissue Exposed to Water Pollutants

Histopathology is the study of tissue changes caused by disease or injury involving examination of tissue samples under a microscope (Kumar, *et al*, 2017). Histopathological lesions induced by pollutants can lead to neurological dysfunctions, behavioural alterations and increased mortality (Bols, *et al*, 2011). Histopathological changes are characterized by;

Neuronal Degeneration: This is characterized by shrinkage and reduction of neuronal density. Neuronal cells become condensed; a term referred as Pyknosis. Sometime neuronal cells become fragmented, a condition termed (Karyorrhesis Cattaneo, *et al,* 2017).

Reactive Gliosis: This is the activation and proliferation of glial cells, forming the glial fibrilary acidic protein (GFAP). Hyperplasia and hypertrophy of glial cells are common features for gliosis (Ajala, *et al*, 2020).

Inflammation: Inflammation is a common histopathological change as immune cells such as macrophages and lymphocytes are activated and pro-inflammatory molecules such as TNF-alpha and IL-1B are up regulated (Cattaneo, *et al,* 2017).

Cerebral Edema and vascular changes: This is characterized by increased fluid accumulation and expression of water channel protein aquaporin-4 (AQP4). Blood vessel diameter become increased and congestion become evident. Hemorrhage and thrombosis characterize vascular changes (Ajala, *et al*, 2020) and (Cattaneo, *et al,* 2017).

2.1.8. Myloperoxidase (MPO)

This is a heme containing enzyme that performs immune responses and oxidative stress regulations in the brain of *C. gariepinus*. It is found in microglial and neuronal cells in the hypothalamus, cerebellum and optic tectum. It functions in oxidative stress regulation, neuroinflammation modulation, antimicrobial defense and neurodegeneration prevention (Li, *et al,* 2018) and (Nwani, *et al*, 2020). It works by converting hydrogen peroxide and chloride ions into hypochlorous acid (HOCl), a potent antimicrobial agent. The process involves;

a. Binding of hydrogen peroxide (H2O2) to MPO's binding site
b. Oxidation of chloride ions (Cl') to hypochlorite (OCl')
c. Hypochlorous acid formation (HOCl) through protonation of hypochlorite
d. HOCl is released which reacts with microbial targets.

Myloperoxidase is regulated by calcium ions which activates its activity. Reduced glutathione inhibit its activity and hydrogen peroxide levels induces its expression Ola-Fadahunsi et al (2019), Adeyemi et al (2018).

2.1.9 Total Protein and Protein Thio in the Brain of *C. gariepinus*

Total protein in the brain tissue of C. gariepinus is the overall content of proteins present in brain cells, tissue and fluids and functions in structural support, enzyme activity, neurotransmission and antioxidant defenses (Dasgupta, *et al,* 2016). Proteins are essential in maintaining structures and functions of neurons and other associated cells, are vary across different regions of the brain (Li, *et al*, 2018).

Protein thiols (PT) refers to the thiol group (-SH) called sulfhydryl groups present in proteins, particularly cysteine residues. Protein thiol group functions in antioxidant defenses, redox regulation, protein folding, enzyme activity and signal transduction. The type present in brain tissue of C. gariepinus are cysteine residue (-SH), glutathione (GSH) and protein disulfide isomerases (PDI) (Ola-Fadahunsi, *et al,* 2020).

There are several factors affecting total protein and protein thiol in the brain of *C. gariepinus.* These factors ranges from biological, environmental, chemical, physiological and sometime analytical. Biological factors that affect total protein and protein thiol ranges from species, sex, age, dietary protein quality as well as health status of the fish. Salinity, water pollution, pH, oxygen level and temperature are key environmental factors that affect total protein and protein thiol level).

The chemical factors include heavy metals, industrial contaminants, pesticides and pharmaceuticals such as antidepressants. The physiological factors lined from hormonal changes, stress and physical activities, the analytical factors ranges from sample processing and storage and the assay method deployed for the analysis (Quintana, *et al,* 2013).

2.2 Theoretical Review

2.2.1 Oxidative Stress Theory

According to Harman, in 1956, the oxidative stress theory posits that an imbalance between the production of reactive oxygen species (ROS) and the capacity of an organism's antioxidant defense system leads to oxidative damage in cells. ROS are highly reactive molecules, including free radicals such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH·). These species are by-products of normal cellular metabolism but can accumulate to toxic levels due to environmental stressors like pollutants, toxins, or xenobiotics, including glyphosate. When exposed to glyphosate, organisms like *Clarias gariepinus* (African catfish) may experience heightened ROS levels, overwhelming their antioxidant defense systems. This results in oxidative damage to cellular components such as lipids (lipid peroxidation), proteins, and DNA. Biomarkers like malondialdehyde (MDA) measure lipid peroxidation, while antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) reflect the defense response. The theory has become instrumental in explaining oxidative damage in organisms exposed to environmental pollutants, with specific studies applying it to *C. gariepinus* and other aquatic species exposed to glyphosate and other herbicides. However, some argue the role of oxidative stress in glyphosate-induced damage is overstated due to insufficient dose-response consistency.

2.2.2 Genotoxicity Assessment Paradigm

This concept was first conceptualized by Bruce Ames in 1975 which evaluates the potential of substances, such as environmental pollutants, to cause damage to an organism's genetic material (DNA). Genotoxic substances can induce mutations, chromosomal aberrations, and DNA strand breaks, which may lead to cancer, heritable genetic changes, or compromised cellular functions. This framework employs a suite of biomarkers and assays to detect and quantify genotoxic effects in exposed organisms and provides direct evidence of genetic damage as well as offering insights into sub-lethal effects, critical for understanding long-term impacts. And it is applicable across diverse species and ecosystems. However, it does not always differentiate between primary genotoxic effects and secondary damage due to oxidative stress. Sensitivity to environmental factors (e.g., pH, temperature) complicates result interpretation. Some critics question the paradigm's ability to link laboratory findings to real-world scenarios, particularly for complex mixtures like glyphosate formulations.

2.2.3 Hormesis Theory

Hugo Schulz in 1888, first observed hormesis while studying yeast's response to low and high concentrations of toxins, noting stimulatory effects at low doses. The hormesis theory posits that a substance or stressor can have a biphasic dose-response effect on biological systems. At low

doses, the stressor can stimulate beneficial or adaptive responses, while at high doses, it induces toxic effects. Hormesis is characterized by a "J-shaped" or "inverted U-shaped" curve, where the response to a stressor varies significantly depending on the dose. In the context of glyphosate exposure in *Clarias gariepinus*, the hormesis theory suggests that low concentrations of glyphosate may activate adaptive mechanisms, enhancing cellular defense systems like antioxidant enzymes. However, as concentrations increase, these systems are overwhelmed, leading to toxicity, oxidative stress, and genotoxicity. The concept provides a nuanced understanding of dose-response relationships and highlights potential adaptive responses that conventional toxicity models overlook. It improves ecological risk assessments by considering non-linear effects. However, unpredictable hormetic responses are not uniform across species or stressors. Biphasic responses complicate setting safe exposure limits and potential misuse is cautioned as critics caution against using hormesis as a justification for low-dose exposures of harmful chemicals, including glyphosate.

2.2.4 Ecotoxicological Perspective

René Truhaut in 1969 was the first to introduce the concept of ecotoxicology. The Ecotoxicological perspective focuses on understanding how chemicals or environmental stressors, such as pollutants, affect living organisms within ecosystems. It combines elements of toxicology, ecology, and environmental science to evaluate both direct effects on individual species and broader implications for ecological communities and processes. This approach is particularly useful for assessing the impacts of pollutants like glyphosate on aquatic species such as *Clarias gariepinus*. Ecotoxicology investigates the pathways through which pollutants enter ecosystems, their bioavailability, bioaccumulation, and potential to disrupt physiological, biochemical, and genetic functions in organisms. It also examines indirect effects, such as changes in biodiversity, habitat integrity, and food web dynamics. Mechanisms in the context of glyphosate exposure includes direct toxicity to aquatic organisms can harm non-target species like *C. gariepinus* by inducing oxidative stress, genotoxicity, and physiological dysfunctions, bioaccumulation and trophic transfer which may bio- accumulate in tissues, potentially moving through food webs and affecting predators or higher trophic levels, habitat alteration through leaching and runoff of glyphosate into water bodies may disrupt aquatic vegetation, altering habitats and reducing food sources for fish and invertebrates, and community-level impacts which may cause changes in species diversity and abundance due to glyphosate exposure can destabilize ecological interactions and nutrient cycling.

2.3 Empirical Literature

Osioma and Ejoh, (2021) in their study exposed *Clarias gariepinus* to different concentrations of 60mg/L, 80mg/L, and 100mg/L of Uproot, a glyphosate-based herbicide and the activities of acetylcholinesterase, oxidative stress and nitrosative stress markers were determined in serum liver, gills and brain were determined using standard assays. Acetylcholinesterase activities was not significantly inhibited, but superoxide dismutase activity was elevated ($P < 0.05$) at 60mg/L exposure, but decreased at 80mgL and 100mg/L concentrations. Increased concentration was observed in nitric oxide levels in brain tissue. Malondialdehyde concentration was low

significantly and GST activity was elevated. The impaired activities of antioxidant enzymes assayed and the elevation of nitric oxide suggest disruption of antioxidant response in Clarias gariepinus exposed to glyphosate.

Toxicity of sub lethal concentrations of glyphosate and paraquat herbicide in Clarias gariepinus was investigated. The effects of exposing juveniles of the fish to two commonly applied herbicides over eight weeks and some biochemical parameters were assessed. Fractionated concentrations of glyphosate (0.026, 0.053 and 1.06mg/L) and paraquat (0.0035, 0.007 and 0.014mg/L) were administered for eight weeks. Antioxidant enzyme activities of superoxide dismutase, catalase, lipid peroxidation and glutathione peroxidase were observed. The result showed that the two herbicide formulations caused changes in antioxidant enzymes, showing aquatic Ecotoxicological effects (Isaac, *et al*, 2018).

Ani, *et al,* (2017) studied glyphosate toxicity on juvenile African catfish, Clarias gariepinus. The acute toxicity bioassay was conducted to consider the 96-hour LC50 following the probit analysis method. There were significant differences (P <0.05) in the LC50 values obtained at different exposure times. Mortality increased with increased concentrations of glyphosate and time of exposure. The study indicated that glyphosate has toxic effects on Clarias gariepinus. The herbicide should be professionally and prudently used in both terrestrial and aquatic ecosystems to avoid Ecotoxicological hazards.

Integrative assessment of biomarker responses in teleostean fishes exposed to glyphosate-based herbicide Excel Mera 71 was evaluated. Anabas testudineus, Heteropnestes fosilis and Oreochromis nilotecus in field conditions based on anti-oxidative, metabolic and digestive responses were studied. AChE, LPO, CAT, GST, ALP, AST and ALT were investigated in different tissue including the brain. Result showed significant alteration of enzyme activities. These results could be used for assessment of ecological risks of glyphosate on fish (Sukhendu, *et al*, 2016).

Effects of glyphosate-based herbicide on the biochemical parameters of Rhamdia quelen (silver catfish) and Leporinus obtusidens were investigated after exposing them to a commercial formulation Roundup with 0.2mg/L and 0.4mg/L concentrations for 96 hours. Effects of the herbicide were assessed on ALT, AST, and protein in mucus layer, nucleotide hydrolysis in brain and protein carbonyl in liver. AST, ALT activities increased after exposure. Protein and glucose levels in both fish increased. Result showed that glyphosate caused liver damage as evidenced by increased plasma transaminases and liver protein carbonyl in both fish species investigated. Brain nucleotide hydrolysis showed a different response for each fish species studied. These parameters indicated some important and potential indicators of glyphosate contamination in aquatic ecosystems (Vania, *et al*, 2015)

The histopathological changes of Mugil cephalus exposed to glyphosate was determined by (Cavas, *et al*, 2015). Fish were exposed to 0, 1, 5 and 10mg/L of glyphosate for 96 hours. Result showed glyphosate exposure caused significant histopathological alterations in liver, kidney and gills. The observed changes were hepatocyte degeneration, renal tubular degeneration and gill damage. The severity of damage was dose-dependent. This shows that glyphosate exposure can

cause significant histopathological changes which can be used as biomarkers for monitoring glyphosate pollution.

Valeria, *et al*, (2014) investigated the effect of acute glyphosate exposure on the oxidative stress biomarkers and the antioxidant defenses of h ybrid siribum. The fish were exposed to different herbicide concentrations for 96 hours. The thiobarbituric acid reactive substance (TBARS), protein and antioxidant defenses were analyzed. 15mg/L of herbicide resulted in the death of 50% of fish after 96 hours. Antioxidant activities decreased in brain, but protein content increased with all changes showed concentration dependence. Result showed that glyphosate exposure at tested concentrations affected the health of the fish by promoting changes that can affect their survival in natural environment.

The biochemical effects of glyphosate on acetylcholinesterase, lipid peroxidation, catalase, glutathione-s-transferase and protein content on teleost fish was studied. 17.20mg/L dose was administered into two species for 30 days under laboratory condition. LPO in all tissues such as liver, muscle and gills changed. GST and total protein content decreased. AChE, CAT activity was all altered the result showed that Excel Mera 71 caused serious alterations in the enzyme activities, resulting into severe deterioration of fish health Palas, et al (2014).

Acute toxicity of glyphosate on Clarias gariepinus fingerlings was assessed. Mortality rate and behavioural responses were investigated under laboratory ambient conditions for 96 hours exposure. Lethal concentration LC50 value of glyphosate on fingerlings of Clarias gariepinus was 0.0018ml/l for 96 hours of exposure. Findings shows that fingerlings exhibited several abnormal behaviours including uncoordinated movement, loss of equilibrium and motionless at bottom of tank. Oxygen consumption decreased with increased glyphosate concentration (Okayi, *et al*, 2013).

Seyed, *et al*, (2013) evaluated optimization of recovery patterns in common carp exposed to roundup using response surface methodology. Genotoxicity and neurotoxicity effects and biochemical parameters were evaluated. The sub-lethal toxicity bioassay of roundup of 2mg/L LC50 96 hours in common carp 1,4,9,16,25,35, and40 day was investigated. After 16 days of exposure, some of the fishes were introduced to herbicide free water. The effect of four recovery parameters, time, temperature, water exchange and salinity on the levels of biomarkers of genotoxicity (DNA damage), neurotoxicity (AChE) activity showed promising improvement for the recovery trend of fishes exposed to roundup

Diego, *et al*, (2013) studied fish toxicity of commercial herbicides formulated with glyphosate. The acute and chronic toxicity of two commercial formulations of glyphosate-based herbicides were reported. The acute toxicity was tested towards two fish species of Danio rerio and Poeciliia reticulate by evaluating the mortality. The chronic toxicity was assessed I Danio rerio by measuring the biochemical parameters of glutamic pyruvic transaminase (AST) and glutamic oxaloacetic transaminase. The result showed both herbicides produced acute toxicity towards the two tested species. The biochemical parameters showed high values as a mark of chronic toxicity, thereby, showing both herbicides may produce environmental damage.

Tamsyn, *et al*, (2013) investigated effects of glyphosate and its formulation, Roundup on reproduction in Zebrafish (Danio rerio). The fish were exposed to glyphosate for 21 days at 0.1, 0.5, and 10mg/L concentrations. Result showed that 10mg/L increased early-stage embryo mortalities and premature hatching, but exposure during embryogenesis alone did not increase embryo mortality, suggesting effect was cursed by exposure during gametogenesis. Result showed that these chemicals caused reproductive toxicity in zebrafish.

DNA damage in fish (Anguilla Anguilla) exposed to glyphosate was investigated by (Guilherme, *et al*, 2012). Comet assay was carried out on the liver and gill of the fish after being exposed to varying concentrations of glyphosate. Antioxidants were assessed in both organs as indicators of pro-oxidant state. Result showed that both organs displayed increased DNA damage. It was recorded that the type of DNA damage was concentration dependent and was also a function of exposure duration.

Daiane, *et al, (*2010) studied oxidative stress in Rhamdia quelen exposed to agrichemicals. The sample fish were exposed to varying sub-lethal concentrations of methyl parathion (MP), a glyphosate herbicide and tabuconazole (Teb). MP and Teb exposed to R. quelen showed enhanced level of TBARS higher than control. In contrast, glyphosate did not alter TBARS generation. Protein carbonyl content increased only in fish exposed to Teb. Catalase activity decreased and GST activity increased on exposure to the chemicals. Result showed that MP and Teb cause changes in oxidative parameters.

The effects of a glyphosate-based herbicide on activity of acetylecholinesterase and antioxidant defense system on neotropical fish Prochilodus lineatus was studied. The juvenile fish were treated (6, 24, and 96 hours) to 1mg/L of RTD, 5mg/L of RTD and a control. Brain acetylcholinesterase activity and liver antioxidant activity were analyzed. After 6 hours of exposure, fish showed transient reduction in superoxide dismutase activity. GST activity was inhibited after 6- and 24-hours exposure. The reduction in activities of this may be related to lipid peroxidation. Glutathione peroxidase activity increased as lipid peroxidase activity return to normal after 24 and 96 hours. Reduced glutathione increased after 96 hours. The result showed that after 24- and 96-hours exposure, antioxidant defenses were apparently enough to combat reactive oxygen species. Acetylcholinesterase inhibition not considered a life-threatening situation (Kathya, *et al*, 2010).

2.4 Summary of Literature Review

The surge in the uncontrolled use of herbicide for weed control in the Niger Delta region of Nigeria may throw a serious environmental effect especially to the aquatic ecosystem as unintended potential effects on fish and other aquatic organisms remained dimly researched. The African catfish of *Clarias gariepinus* species is a staple protein source found in the fresh water bodies and remained largely cultured in most aqua culturing in the region. The evaluation of genotoxicity and antioxidant defense system in the brain of *Clarias gariepinus* is critical as exposure may lead to oxidative stress, growth retardation, immunosuppression and chromosomal abnormalities. This may result to mutation and other genotoxic aberrations such as DNA fragmentation. Glyphosate exposure may affect brain health of *Clarias gariepinus,* oxidative stress and inflammation which can lead to neurodegeneration and behavioral changes.

The evaluation of genotoxicity and antioxidant defense system in *Clarias gariepinus* exposed to glyphosate highlights the importance of monitoring and mitigating the effects of pesticides on aquatic lives.

2.5 Research Gaps

The research gaps in this study are numerous which include:

- There is lack of comprehensive studies on glyphosate mediated DNA damage in the brain cells of *Clarias gariepinus.*
- Limited research on the assessment of antioxidant enzyme activities such as SOD, CAT, GPx in *Clarias gariepinus* brain exposed to Uproot.
- Insufficient knowledge on glyphosate effects on brain cell cycle and oxidative stress.
- There is need for study on glyphosate effects on brain glutathione (GSH) levels and its related enzymes.
- Need for assessment of glyphosate mediated oxidative stress and lipid peroxidation in the brain of *Clarias gariepinus*
- Need in for standardized protocols for genotoxicity and antioxidant defense system in fish brain cells.
- Limited knowledge of effects of glyphosate on the integrity of blood brain barrier.

CHAPTER THREE

3.0: MATERIALS AND METHODS

3.1: **Chemicals and Reagents**

5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylcholine iodide, thiobarbituric acid, reduced glutathione, 1-chloro-2, 4-dinitrobenzene (CDNB), epinephrine and hydrochloric acid were products of Sigma-Aldrich (Steinheim, Germany). Ethylenediamine tetraacetic acid (EDTA), trichloroacetic acid (TCA), sodium citrate, sodium azide, formaldehyde, sodium chloride and metaphosphoric acid were products of BDH Chemicals Limited, Poole, England. All other reagents were of analytical grade and were prepared in all-glass distilled water

3.2: Collection of Fish Samples

Twenty (20) specimens of African catfish (*Clarias gariepinus*) were procured from a commercial fish pond at Oria, Abraka, Delta State, Nigeria. They were transported on the same day to the laboratory in a well-ventilated container with the pond water to avoid injury. Fish were left to acclimatize in the congenial laboratory condition for 7 days in aquaria of 200 L capacity. They were fed once a day with commercial fish pellets during both acclimation and exposure periods.

3.3: Experimental Design

Uproot (commercial brand name) of herbicide containing isopropyl - ammonium salt of glyphosate as active ingredient (equivalent to 360 g/L) was purchased from a local trader at Otuoke, Bayelsa State, Nigeria. In preparing the required doses used for the experiment, 10 mL of the stock was taken and made up to 500 mL with distilled water. From this dilution, the required doses (30 mg/L, 50 mg/L and 70 mg/L) of glyphosate were calculated and multiplied by capacity of the aquaria, 20L. Treatment with herbicide commenced on the 2nd week.

Fish were divided into four groups (A, B, C and D) comprising five fish per group (n = 5) and separated into 20 L capacity aquaria. Group A served as control without glyphosate exposure. Groups B, C and D were exposed to 30 mg/L, 50 mg/L and 70 mg/L glyphosate, respectively. The water was changed every 24 h and exposure was repeated for 14 days. Experiments were conducted at natural photoperiod.

3.3.1: Preparation of Brain Tissue Supernatant for Biochemical Assay

The fish were dissected and the brain tissue was quickly removed. 0.5 g of wet tissues (brain) was homogenized in 4.5 ml of the physiological solution (phosphate buffer, pH 7.4). The resulting homogenates were centrifuged at x5000g for 20 minutes. The supernatants were decanted and used for further biochemical analysis.

3.3.2: Preparation of Brain Tissue for Histopathology Examinations

The brain tissues obtained from fish were fixed immediately in 10 % formyl saline solution

3.4: Biochemical Analyses

3.4.1: Determination of Total Protein Concentration

The total protein concentration in supernatant of brain was determined by the method of (Doumas *et al.* 1981).

Principle:

Protein forms a purple complex when it reacts with cupric ions in an alkaline solution. The intensity of the violet colour is proportional to the amount of protein present in the sample.

Procedure:

The assay mixture was prepared by adding 0.05 ml of sample (supernatant) to 3.0 ml of total protein reagent containing 600 mM NaOH, 12 mM $CuSO_4$, 32 mM potassium tartrate, 30 mM potassium iodide and non – reactive ingredients. The content was mixed by inversion and left undisturbed at room temperature (29°C) for 10 minutes. The standard and blank were constituted by replacing the sample with 0.05 ml of bovine albumin (total protein standard) and distilled water respectively. The absorbance of all tubes was read against blank at 540 nm.

Calculation:

Total protein (g/dl) = $\frac{Abs.of\ Samples}{Abs.of\ Standard} \times Conc.of\ Standard\ (5.0 g/dl)$

3.4.2: Estimation of Tissue Reduced Glutathione

The reduced glutathione levels in the supernatant of brain was determined using the procedure of (Ellman, 1959).

Principle:

The sulfhydryl group of GSH reacts with DTNB (5,5-dithio-bis-2-nitrobenzoic acid) and produces a yellow coloured 5-thio-2-nitrobenzoic acid (TNB). The mixed disulfide GSTNB (between GSH and TNB) that is concomitantly produced is reduced by glutathione reductase to recycle the GSH and produce more TNB. The rate of TNB production is directly proportional to the recycling reaction which in turn is directly proportional to the concentration of GSH in the sample. The absorbance of TNB at 412 nm provides an estimation of GSH in the sample.

Procedure:

Tissue supernatant (0.5 ml) was added to 2ml of 10% (w/v) trichloroacetic acid, mixed thoroughly and centrifuged at x5000g. 1 ml of supernatant was mixed with 0.5 ml Ellman's reagent and 3 ml of 0.2 M phosphate buffer (pH 8.0). The absorbance was read against the blank at 412 nm. A series of standards were prepared along with a blank containing 3.5 ml of buffer.

Calculation:

The concentration of reduced GSH in 1mole of GSH/g of wet tissue was extrapolated from a standard calibration plot (Appendix i).

3.4.3: Determination of Protein Thiols

The protein thiols in supernatant of brain was determined using the method of (Sedlack and Lindsey, 1968).

Principle:

The sulphydryl groups in tissues was estimated using Ellman's reagent. DTNB is reduced by SH group to form one mole of 2 – nitro -5- mercapto benzoic acid per mole of SH.

Procedure:

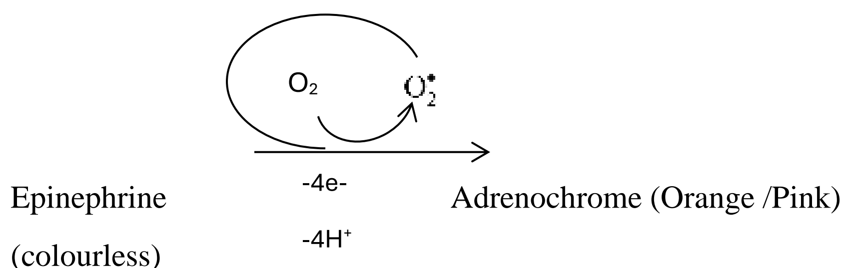
The reaction mixture contained 0.2 mL of the supernatant, 1.5 mL of buffer (0.2 M tris, pH 8.0, containing 0.2M EDTA) and 1.0 mL of DTNB and made up to 10.0 mL with absolute methanol. Two tubes containing a reagent blank without sample and another without DTNB were stoppered and allowed to stand for 15 minutes with occasional shaking at room temperature. The reaction mixture was then centrifuged at 3000g for 15 minutes and absorbance measured at 420 nm. The level of total thiols minus glutathione levels gives the amount of protein thiols in the tissue. Protein thiols are expressed as mg/g wet tissue.

3.4.4: Assay of Superoxide Dismutase Activity

The method of Misra and Fridovich (1972) was used to determine the activity of superoxide dismutase in brain tissue.

Principle:

The superoxide anion ($O_2^{\bullet-}$) substrate for superoxide dismutase (SOD) is generated indirectly in the oxidation of epinephrine at alkaline pH by the action of oxygen on epinephrine.



As $O_2^{\bullet-}$ builds in the solution, the formation of adrenochrome accelerates because $O_2^{\bullet-}$ also reacts with epinephrine to form adrenochrome. SOD dismutases the $O_2^{\bullet-}$ formed during the epinephrine oxidation and therefore slows down the rate of formation of the adrenochrome as well as the amount that is formed. Because of this slowing process, SOD is said to inhibit the oxidation of epinephrine. The percent inhibition (%I) is hyperbolic with respect to the SOD activity.

Procedure:

To 0.2ml of supernatant, 2.5 ml of 0.05 M carbonate buffer (pH 10.2) was added. The reaction was initiated by adding 0.3 ml of freshly prepared 0.3 mM of epinephrine. This was mixed by inversion. The reference cuvette contained 2.5 ml of the buffer, 0.2 ml of distilled water and 0.3 ml of the substrate (epinephrine). The increase in absorbance was monitored at 480 nm at interval of 30 seconds for 150 seconds.

Calculation:

The percentage inhibition of SOD activity was calculated as follows:

$$\% \text{ inhibition} = 100\% - \frac{Abs.of\ blank}{Abs.of\ Sample} \times \frac{100}{1}$$

Unit required of SOD in one minute was calculated as:

$$\text{SOD in Unit/ml} = \% \text{ inhibition X } \frac{1}{50\%} \times \frac{V}{Vo}$$

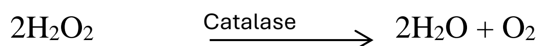
Where,

V = Total volume,   VO = Volume of sample

3.4.5: Assay of Catalase Activity

The catalase activity in brain was determined using the method of Kaplan and Groves (1972).

Principle:



$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$

Catalase activity was estimated by measuring the breakdown of hydrogen peroxide in the reaction mixture by monitoring changes in absorbance at a wavelength of 360 nm.

Procedure:

To 2 ml of the tissue supernatant, 1 ml of H_2O_2 substrate was added to the reaction cuvette. The absorbance was read at 360 nm for 70 sec. The reference cuvette contained 1ml of H_2O_2 and 2ml of water.

Calculation:

The disappearance of hydrogen peroxide may be described by the equation for the first order kinetics.

$Log_{10}A = Log_{10}Ao - kt/2.3$

Therefore,

$$K = \frac{log_{10}A}{Log_{10}Ao} \times \frac{2.3}{t}$$

A = Absorbance of H_2O_2 at time t sec.

Ao = Absorbance of H_2O_2 at time 0 sec.

K = rate constant

T = time in min. ---70s (1.167min).

3.4.6: Assay of Glutathione -s-transferase Activity

The activity of glutathione-s- transferase in the supernatant of brain was assayed by the method of (Habig, *et al*, 1974).

Principle:

Glutathione-S-transferase catalyzes the conjugation of L-glutathione to 1-chloro-2,4-dinitrobenzene (CDNB) through the thiol group of the glutathione. The formation of the GS-DNB conjugate is proportional to the enzyme activity and can be used for photometric GST activity determination. The rate of increase in the absorption of GS-DNB conjugate at 340nm is directly proportional to the GST activity in the sample.

$$GSH + CDNB \xrightarrow{GST} GS\text{-}DNB \text{ conjugate} + HCl$$

Procedure:

The reaction mixture was prepared by mixing 1.5 ml sodium phosphate buffer (0.1 M pH 6.5), 0.2 ml GSH (9.2 mM), 0.02 ml CDNB (0.1 M) and 0.1 ml of the sample (supernatant). The reaction solution without the supernatant was used as the blank. The increase in absorbance was monitored at 340 nm at interval of 60 seconds for 3 minutes.

Calculation:

GST activity expressed in nmol/mg protein/min in brain tissue was calculated as follows:

$$A = \frac{1000 \times (Eexp - Econt) \times 1.82}{9.6 \times V \times t \times C}$$

Where,

A = Enzyme activity conjugate (nmol/mg protein/ minute.

E_{exp} = Increase in the absorbance of the sample at 340 nm.

E_{cont} = Increase in the absorbance of the blank at 340 nm.

1000 = Factor for enzyme activity to be expressed in nmol/min/ml.

1.82 = Total volume of the mixture.

9.6 = Molar coefficient of the conjugate formation

V = Volume of the sample (homogenate) in ml

t = Time (minute)

c = Total protein concentration

3.4.7: Assay for Glutathione Peroxidase (GPx) Activity

The activity of Glutathione Peroxidase (GPx) in brain tissue supernatant was determined using the method of Flohe and Gunzler. (1984).

Principle:

$2GSH + H_2O_2 \xrightarrow{\text{Glutathione Peroxidase}} GSSG + 2H_2O$

$GSSG + \beta\text{-NADPH} \xrightarrow{\text{Glutathione Peroxidase}} \beta\text{-NADP} + GSH$

Glutathione peroxidase activity was measured by monitoring the disappearance of β-NADPH.

Procedure:

The reagents, 1.49ml phosphate buffer (0.1M; pH 7.4), 0.1ml EDTA (1mM), 0.1ml sodium azide (1mM), 0.05ml glutathione reductase (1 IU/ml), 0.05ml GSH (1mM), 0.1ml NADPH (0.2mM), 0.01ml H_2O_2 (0.25mM) were mixed with 0.1ml of brain homogenate in a total volume of 2ml. Using a spectrophotometer at 340nm the disappearance of NADPH was recorded at 25^oC.

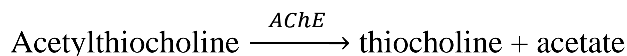
The activity of the enzyme was calculated as nM NADPH oxidized per minutes per mg protein using molar extinction of 6.22×10^3 $M^{-1}cm^{-1}$.

3.4.8: Assay of Acetylcholinesterase Activity

The activities acetylcholinesterase in the supernatant of brain was assayed according to the method of (Ellman, et al, 1961).

Principle:

The principle of this method is based on the measurement of the rate of production of thiocholine as acetylthiocholine is hydrolysed. This is accomplished by the continuous reaction of the thiol with 5', 5-dithiobis -2-nitrobenzoate ion to produce the yellow anion of 5- thio-2-nitro-benzoic acid (II). The rate of colour production read at 412 nm is directly proportional to acetylcholinesterase activity.


Acetylthiocholine $\xrightarrow{AChE}$ thiocholine + acetate

Thiocholine + dithiobisnitrobenzoate → yellow colour

Procedure:

25 µl of supernatant was added to a cuvette containing 2.925 µl of 0.1M phosphate buffer (pH 8.0), 25 µl of 8 mM of DTNB and 25 µl of 45 mM acetylcholine iodide at room temperature (25^oC). The contents in the cuvette were mixed, and the absorbance was read continuously at intervals of 30 seconds for 2 minutes at 412 nm.

Calculation:

The enzyme (Acetylcholinesterase) activity was calculated as follows:

$$A = \frac{1000 \times (Eexp - Econt) \times 3}{13600 \times V \times t \times C}$$

Where,

A = Acetylcholinesterase activity in nmol/mg protein/minute

E_{exp} = Increase in the absorbance of the sample at 412 nm

E_{cont} = Increase in the absorbance of the blank at 412 nm

1000 = Coefficient (This factor is introduced so that the enzyme activity can be expressed in nMol/min/ml

3 = Total volume of the mixture (ml)

13600 = Molar extinction coefficient in Mol^{-1} cm^{-1} DTNB

V = Volume of sample (ml

t = Time in minute

c = Total protein concentration

3.4.9: Determination of Nitric Oxide

The colorimetric determination of nitric oxide was carried out in brain tissue supernatant by the method of (Green *et al*. 1982).

Principle:

In acid medium and in the presence of nitrite, the formed nitrous acid diazotize sulphanilamide and the product is coupled with N – (1 –naphthyl) ethylenediamine. The resulting azo – dye has a bright reddish – purple colour which absorbs strongly at 540nm.

Procedure:

The assay mixture was prepared by mixing 0.5 mL of the sample supernatant with an equal volume of Griess reagent, [(1.0 mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 mL of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 mins and a reddish – purple colour change was observed. The absorbance was measured at 540 nm.

Calculations:

The amount of nitric oxide radical was calculated by this equation:

$$\%NO = \frac{Ao - A1}{Ao} x\,100$$

Where; A_o = Blank absorbance

A_1 = Sample absorbance

3.4.10: Determination of myeloperoxidase activity

The myeloperoxidase activity in tissue supernatants were assayed for as described by (Bradley *et al,* 1982).

Principle:

The rate at which a coloured product formed during the MPO – dependent reaction of O – dianisidine and hydrogen peroxide was measured at 460 nm in a UV spectrophotometer. One unit of MPO activity is defined as that which degrades 1 µmol of peroxide/min at 25°C.

Procedure:

Pre- weighed tissue was homogenized (1: 10 w/v) in 0.5% hexadecyltrimethyl ammonium bromide in 50 mM potassium phosphate buffer (pH 6.0) before sonication in an ice bath for 20 s. Three freeze/thaw cycles were performed followed by sonication (20 s in ice bath). The samples were centrifuged at 17000g (5 min, 4^0C) and myeloperoxidase in the supernatant was assayed by mixing 0.1 ml of supernatant and 2.9 ml of 50 mM potassium phosphate buffer (pH 6.0) containing 0.167 g/L o-dianisidine dihydrochloride and 0.0005% hydrogen peroxide (H_2O_2). The change in absorbance at 460 nm was measured for 4 min using an UV visible spectrophotometer.

3.4.11: Assay of Peroxidase

The method proposed by (Kochba *et al*. 1977) was adopted for assaying the activity of peroxidase in brain.

Principle:

In the presence of the hydrogen donor pyrogallol, peroxidase converts H_2O_2 to H_2O and O_2. The oxidation of pyrogallol to a coloured product called purpurogalli can be followed spectrophotometrically at 430nm.

Procedure:

To 3.0 mL of pyrogallol solution (0.05 M in 0.1 M phosphate buffer (pH 6.5), 0.1 mL of supernatant was added and the spectrophotometer was adjusted to zero at 430 nm. To the test cuvette, 0.5 mL of H_2O_2 (1% in 0.1 M phosphate buffer, pH 6.5) was added and mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer. One unit of peroxidase is defined as the change in absorbance/minute at 430 nm.

3.4.12: Evaluation of Malondialdehyde Concentration

The concentrations of malondialdehyde in serum and the supernatant of brain was evaluated using the method of (Buege and Aust, 1978).

Principle:

The thiobarbituric acid reactive substances (TBARS) assay measures lipid peroxides and aldehydes, such as malondialdehyde (MDA) in the cell, culture media and cell lysate. MDA combines with thiobarbituric acid (TBA) in a 1:2 ratio to form fluorescent adduct that is read at 530 nm.

Procedure:

To 1.0 ml of the sample (serum/supernatant) was added 2.0 ml of TCA –TBA – HCl reagent (15 % (w/v), TCA, 0.375 % (w/v) TBA and 0.25 N HCl. The contents were boiled for 15 minutes, cooled and centrifuged at 10,000g for 10 minutes to remove the precipitate. The absorbance was read at 535 nm using the reagent blank.

Calculation:

Malondialdehyde (units/ml) $= \frac{OD \times V}{E \times Vsample}$

OD = Absorbance,

E = Molar extinction coefficient ($1.56 \times 10^5 Mol^{-1} cm^{-1}$)

V = Total volume of mixture,

V_{sample} = Volume of sample (serum/supernatant)

2.4.13: DNA Fragmentation Assay

DNA fragmentation assay was conducted using the procedure of Wu *et al*. (2005) with some modifications.

Principle:

DNA fragmentation lysis buffer separate the intact chromatin in the cell. The pellet and supernatant fractions react with diphenylamine solution, which absorbs at 620 nm

Procedure:

The tissue (50 mg) was homogenized in 10 volumes of a TE solution pH 8.0 (5 mmol Tris–HCl, 20 mmol EDTA) and 0.2% triton X-100. 1.0 ml aliquot of each sample was centrifuged at 27,000

× g for 20 min to separate the intact chromatin (pellet, B) from the fragmented DNA (supernatant, T). The pellet and supernatant fractions were assayed for DNA content using a freshly prepared DPA (Diphenylamine) solution for reaction. Optical density was read at 620 nm with spectrophotometer.

Calculation:

The results were expressed as amount of % fragmented

DNA by the following formula;

% Fragmented DNA = T x100/(T+B)

3.4.14: Histopathology Examination

The histopathological examination was carried out as previously described by (Onyesom, *et al*, 2015)

Procedure:

Samples from the brain was fixed in 10% neutral buffered formalin for 24 h and processed via routine tissue processing technique. The samples were dehydrated in a graded series of ethanol, cleared in xylene, embedded in paraffin wax, sectioned at 4–7 µm and stained with hematoxylin and eosin (H&E). The stained sections were carefully examined under binocular compound light microscope. After which, photomicrograph of sections from selected samples were taken under a magnification x400 using automated built-in digital photo camera.

2.7 Statistical Analysis

The data obtained for the various biochemical parameters were expressed as Mean ± SD. Data were analyzed using analysis of variance (ANOVA) and group means compared by the Tukey's post hoc test. Values were considered statistically different at $p < 0.05$. All statistical analysis was performed using SPSS version 21 (SPSS, Inc – Chicago, Illinois, USA)

CHAPTER FOUR

4.0: RESULTS AND DISCUSSION

4.1: Biochemical Parameters

The results of the biochemical parameters obtained in the brain of *Clarias gariepinus* induced with different concentrations of glyphosate herbicide are shown below.

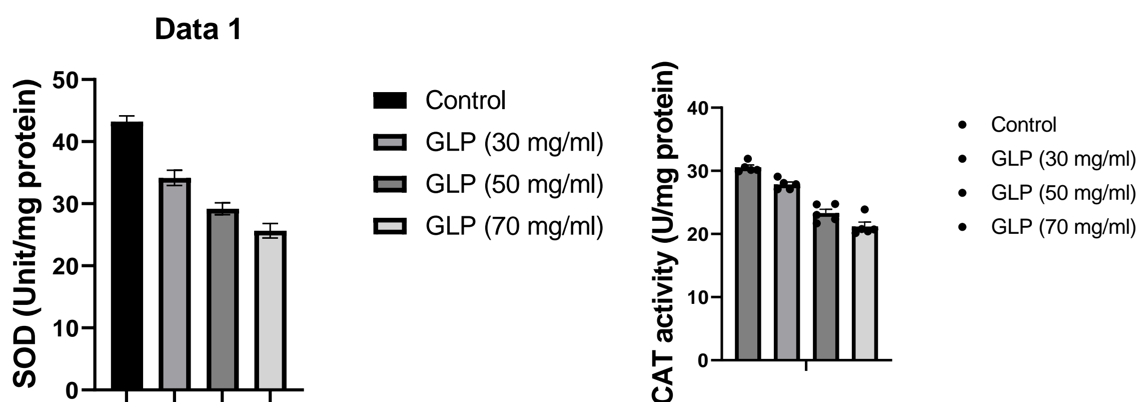


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Figure 4.1: Activities of Superoxide dismutase and Catalase in brain of *Clarias gariepinus* induced with varying concentration of glyphosate.

Bars represent the Mean ± SEM (n=5). # P < 0.05 vs Control, *P < 0.05 vs GLP using one-way ANOVA followed by Tukey's post hoc test.

Figure 4.1 demonstrates the impact of Uproot a glyphosate herbicide on the changes in the activities of superoxide dismutase and catalase. Induction of glyphosate resulted in a noteworthy reduction of SOD activity in the brain of treated catfish compared to the control. However, administration of glyphosate at doses ranging from 30 mg/ml to 70mg/ml resulted in a considerable inhibition of the activity of SOD and CAT whish also appears to be dose dependent.

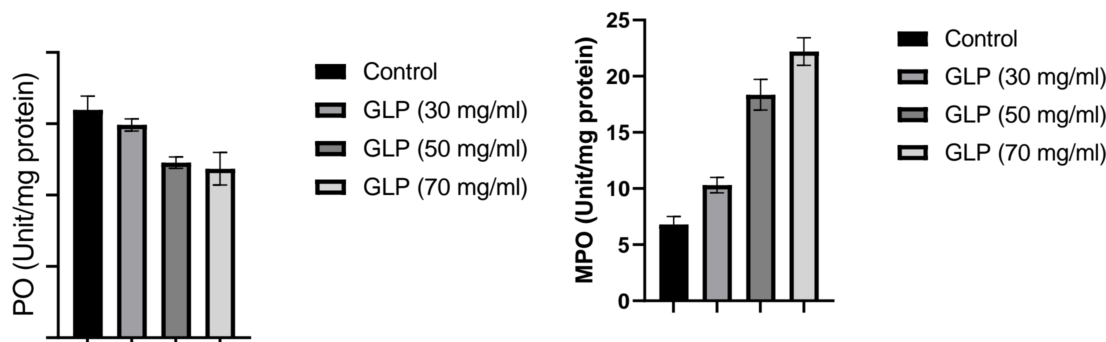

Figure 4.2: Activities of Peroxidase and Myeloperoxidase Enzymes in brain of *Clarias gariepinus* treated with various concentrations of Glyphosate

Bars represent the Mean ± SEM (n=5). # P < 0.05 vs Control, *P < 0.05 vs GLP using one-way ANOVA followed by Tukey's post hoc test.

Figure 4.2, (A) glyphosate induced catfish (30 mg/ml) showed a mild decrease but not significant (P > 0.05) in peroxidase (PO) activity in the brain of catfish, while a significant reduction (p < 0 .05) in the PO activity was observe at (50 and 70 mg/ml) when compared to the control group. (B) The contamination with glyphosate (30, 50 and 70 mg/ml) significantly increased (P < 0.05) the myeloperoxidase (MPO) levels compared to the control group at graded dose level.

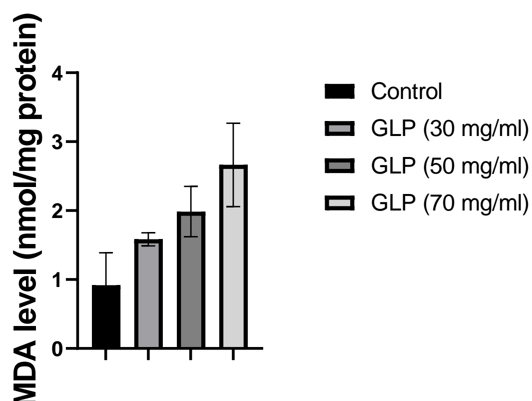

Figure 4.3: Levels of malondialdehyde (MDA) in brain of *Clarias gariepinus* exposed to different concentrations of Uproot, a glyphosate herbicide

Bars represent the Mean ± SEM (n=5). # P < 0.05 vs Control, *P < 0.05 vs GLP using one-way ANOVA followed by Tukey's post hoc test.

The results in Figure 4.3 indicated that the exposure of *Clarias gariepinus* to different concentrations of glyphosate (i.e., 30, 50 and 70 mg/ml) significantly increased ($p < 0.05$) the MDA levels compared to the control group at graded dose level.

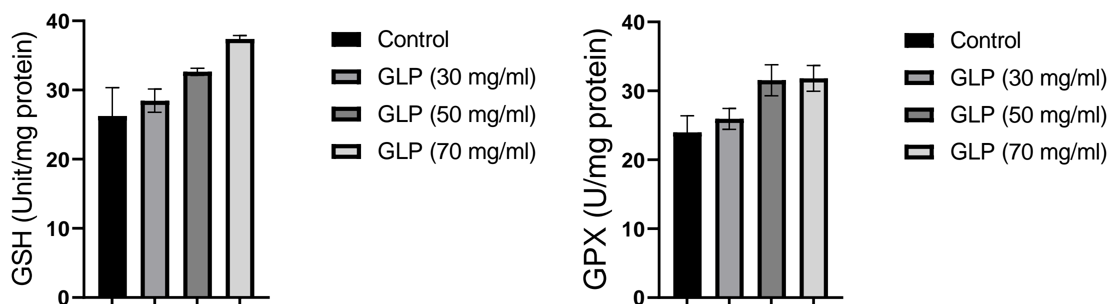

Figure 4.4: Concentration of Reduced Glutathione and Glutathione Peroxidase activities in brain of *Clarias gariepinus* exposed to different concentrations of glyphosate herbicide.

Bars represent the Mean ± SEM (n=5). # $P < 0.05$ vs Control, *$P < 0.05$ vs GLP using one-way ANOVA followed by Tukey's post hoc test.

Results in Figure 4.4, (A) indicated that glyphosate induction at 30 mg/ml shows a mild increase but not significant ($p > 0.05$) in the reduced glutathione levels in the brain of catfish, while a significant elevation ($p < 0.05$) in the GSH activity was observe at (50 and 70 mg/ml) when compared to the control group at graded dose level.

The results in Figure 4.4 (B) also showed that contamination with glyphosate (30 mg/ml) lead to a mild increase but not significant ($p > 0.05$) in the brain glutathione peroxidase activity of catfish when compared with control group. Exposure of *Clarias gariepinus* to glyphosate at 50 mg/ml and 70 mg/ml) significantly increases ($p < 0.05$) the activity of glutathione peroxidase enzyme compared to the control group at graded dose level. However, catfish exposed to 50mg/ml and 70 mg/ml had comparable ($p > 0.05$) glutathione peroxidase activities, although, higher ($p < 0.05$) when compared with catfish exposed to 30mg/ml of glyphosate.

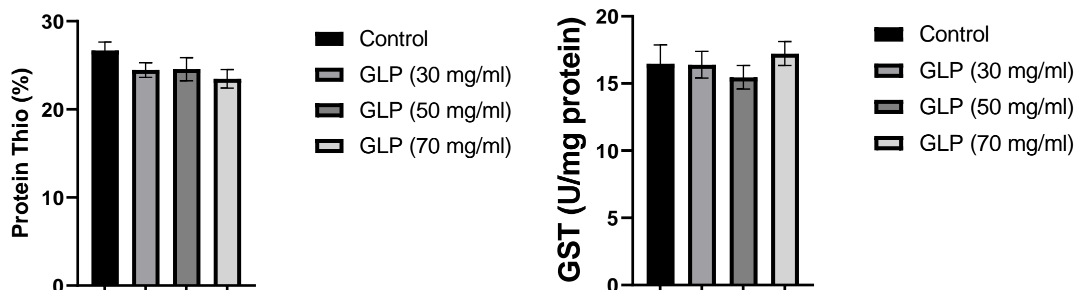

Figure 4.5: Percentage Protein Thio and Glutathione s- transferase activity in brain of *Clarias gariepinus* exposed to different concentrations of Uproot, a glyphosate herbicide

Bars represent the Mean ± SEM (n=5). # $p < 0.05$ vs Control, *$p < 0.05$ vs GLP using one-way ANOVA followed by Tukey's post hoc test.

The percentage protein thio as shown in Figure 4.5 A, indicated that catfish exposed to 30mg/ml, 50mg/ml and 70 mg/ml of glyphosate had comparable brain protein thio content with the control fish. Also, glyphosate induced catfish (30, 50 and 70 mg/ml) showed no significantly difference ($p > 0.05$) in brain GST activity when compared with control *Clarias gariepinus*.

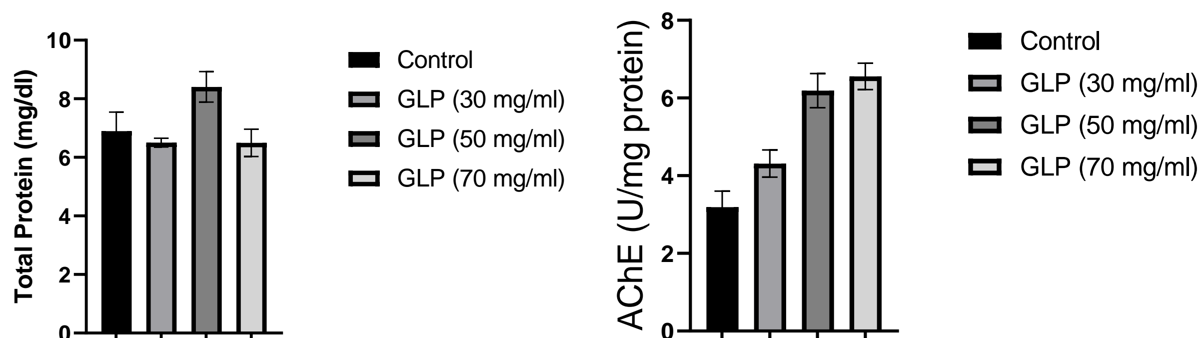

Figure 4.6: Concentration of Total Protein and Activities of Acetylcholinesterase in the Brain of *Clarias gariepinus* exposed to different Concentrations of Uproot, a glyphosate herbicide

Bars represent the Mean ± SEM (n=5). # $P < 0.05$ vs Control, *$P < 0.05$ vs GLP using one-way ANOVA followed by Tukey's post hoc test.

Results in Figure 4.6 (A), showed mild reduction in total protein concentration in catfish exposed to 30mg/ml and 70 mg/ml glyphosate but not significant ($p > 0.05$) in the total protein level in the

brain of catfish and 50mg/ml of glyphosate exposure significantly increases protein level when compared with control group.

The activities of acetylcholinesterase as shown in Figure4.6 (B), indicated that catfish exposed to 30 mg/ml of glyphosate expresses a mild increase but not significant ($p > 0.05$) in the AChE activity in the brain of catfish, while a significant elevation in the AChE activity was observe at (50 and 70 mg/ml) when compared to the control group at graded dose level. However, catfish exposed to 50 mg/ml and 70 mg/ml had comparable brain AChE activities.

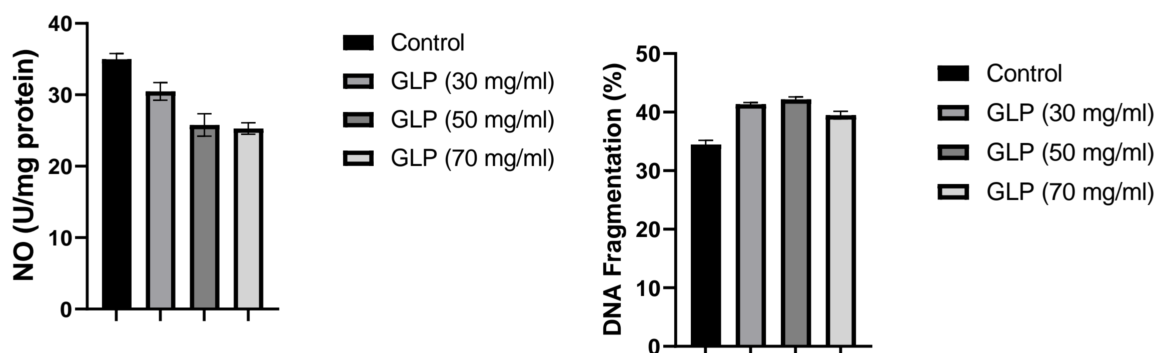

Figure 4.7: Levels of Nitric Oxide and Percentage DNA Fragmentation in the Brain of *Clarias gariepinus* exposed to varying concentration of Uproot, a glyphosate herbicide

Bars represent the Mean ± SEM (n=5). # $P < 0.05$ vs Control, *$P < 0.05$ vs GLP using one-way ANOVA followed by Tukey's post hoc test.

The brain nitric oxide concentration in *Clarias gariepinus* as shown in Figure 4.7 (A), indicated that there was a significant reduction ($p < 0.05$) in the concentration of nitric oxide in catfish exposed to glyphosate (30, 50 and 70 mg/ml) as compared with the control catfish.

Results in Figure 4.7 (B) showed significant elevation in percentage DNA fragmentation in the brain of glyphosate induced catfish as compared with the control fish.

4.2: Histological Analysis

A

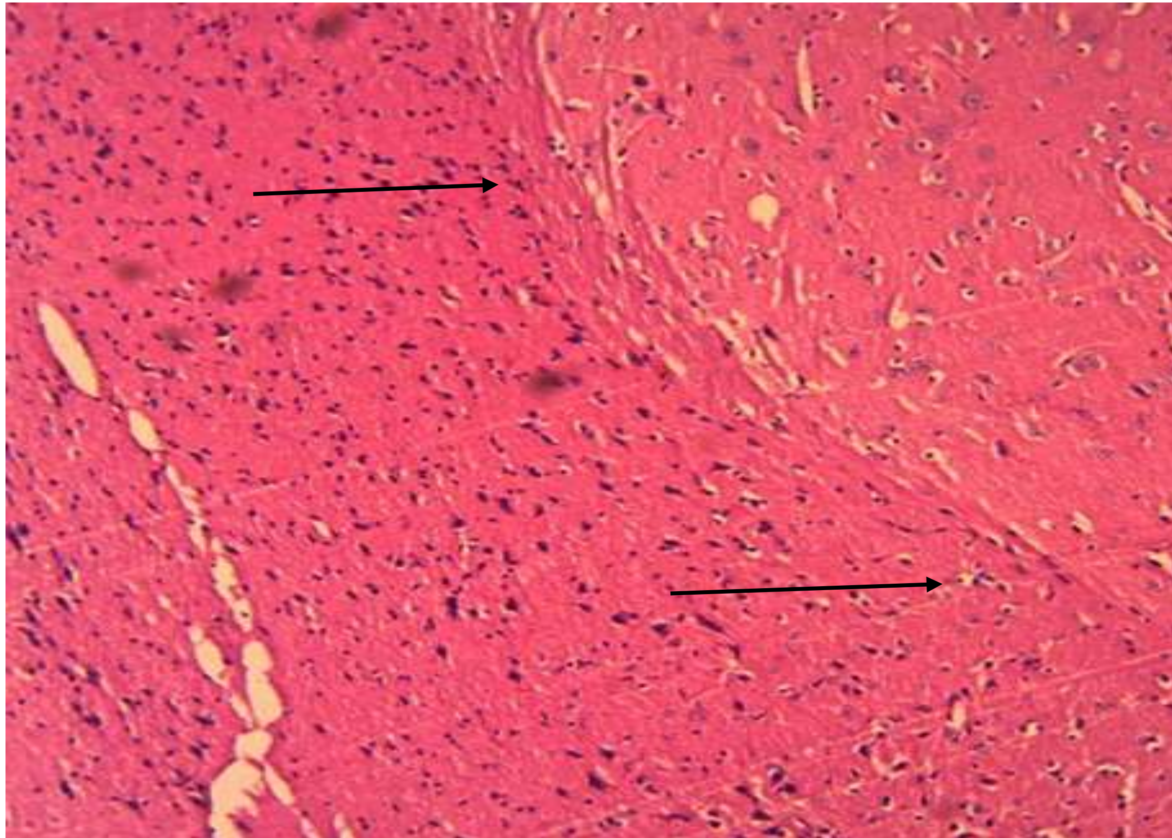

Plate 1: Photomicrograph of fish tissue brain without treatment with GLP with H&E stain at magnification of x100.

A. Control group showing normal stratum album centrale, Purkinje cell nucleus and Purkinje cell dendrites, inner granular layer and well arrange stratum periventricular.

… Histological Analysis

B

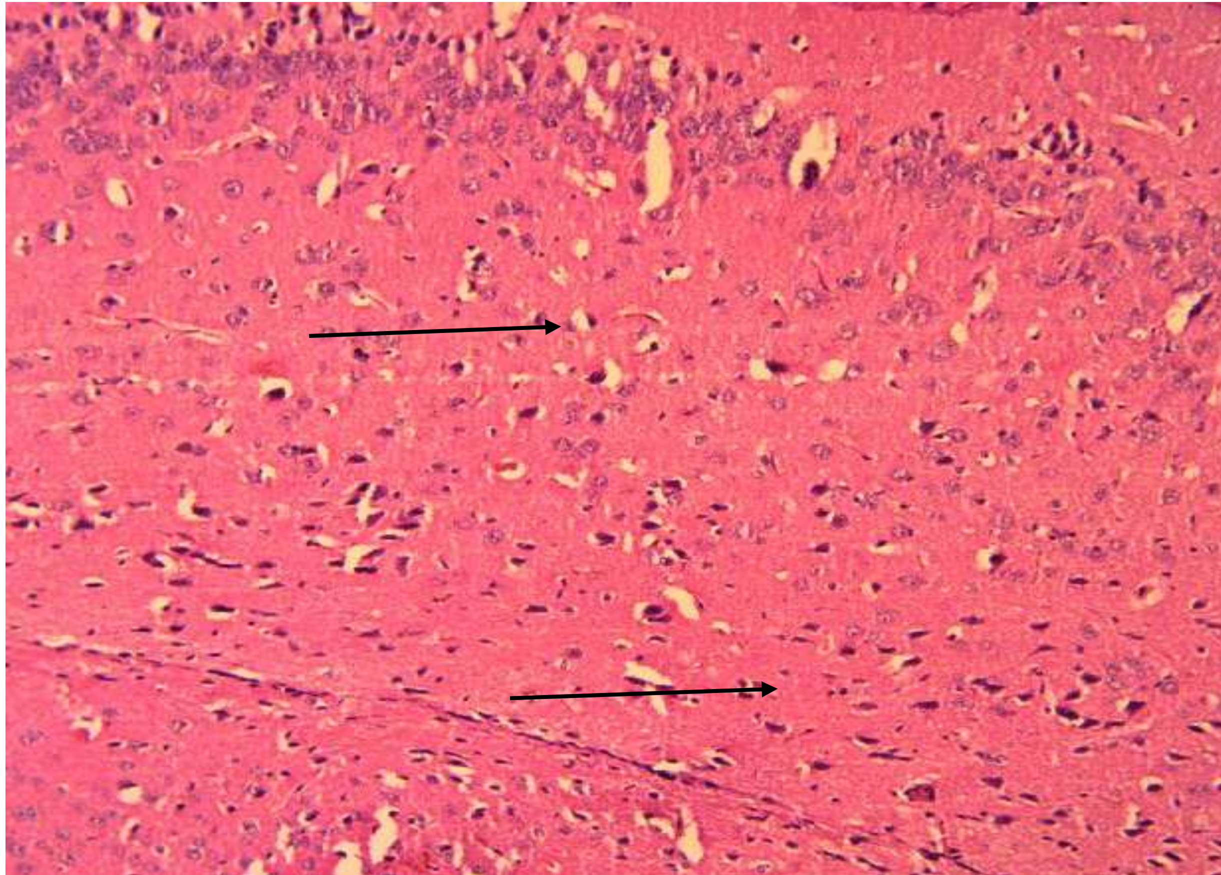

Plate 2: Photomicrograph of fish brain tissue with 30mg/ml treatment with GLP with H&E stain at magnification of x100.

B Showing normal stratum album centrale, Purkinje cell nucleus and Purkinje cell dendrites and congested inner granular layer and stratum periventricular (green arrow).

… Histological Analysis

C

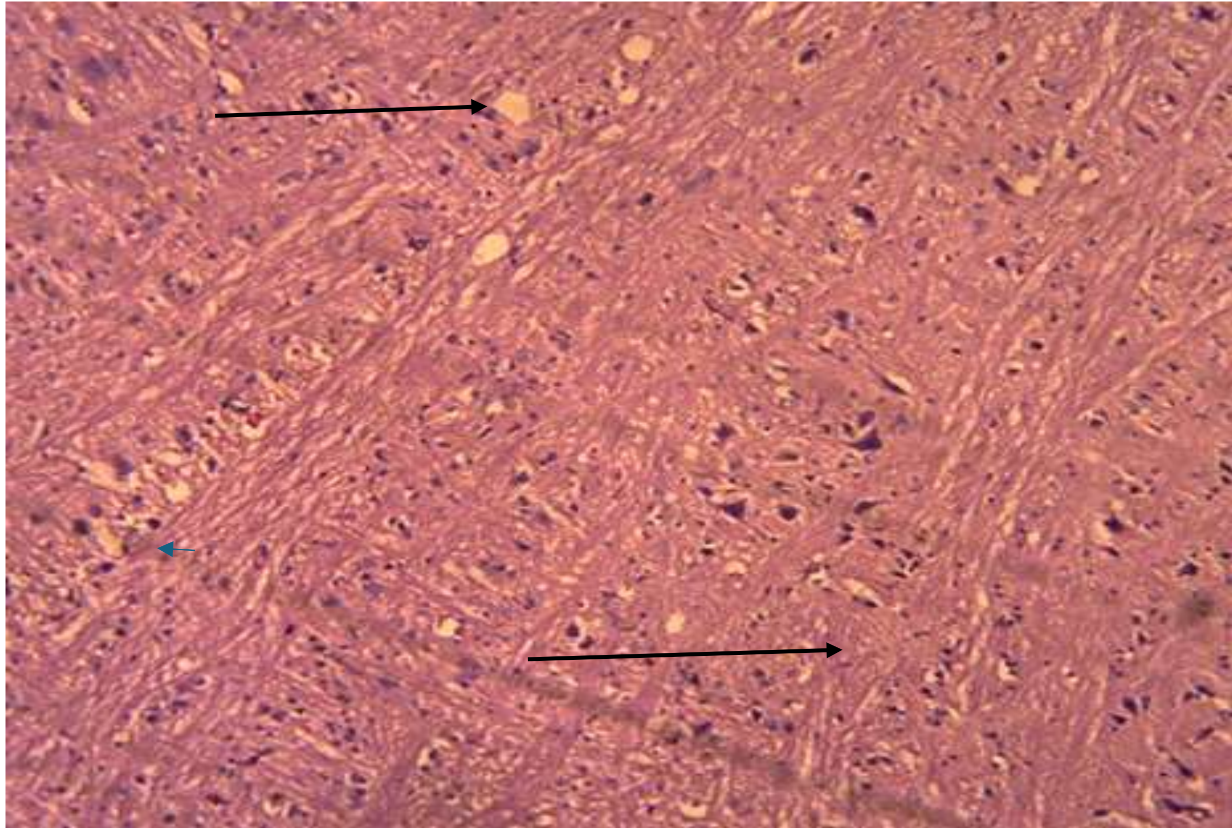

Plate 3: Photomicrograph of fish tissue brain treated with 50mg/ml dose of GLP with H&E stain at magnification of x100.

C slide shows degenerated neurons, altered stratum album centrale, with no visible Purkinje cell nucleus and Purkinje cell dendrites, inner granular layer and moderate vacuolar changes with empty space.

… Histological Analysis

D

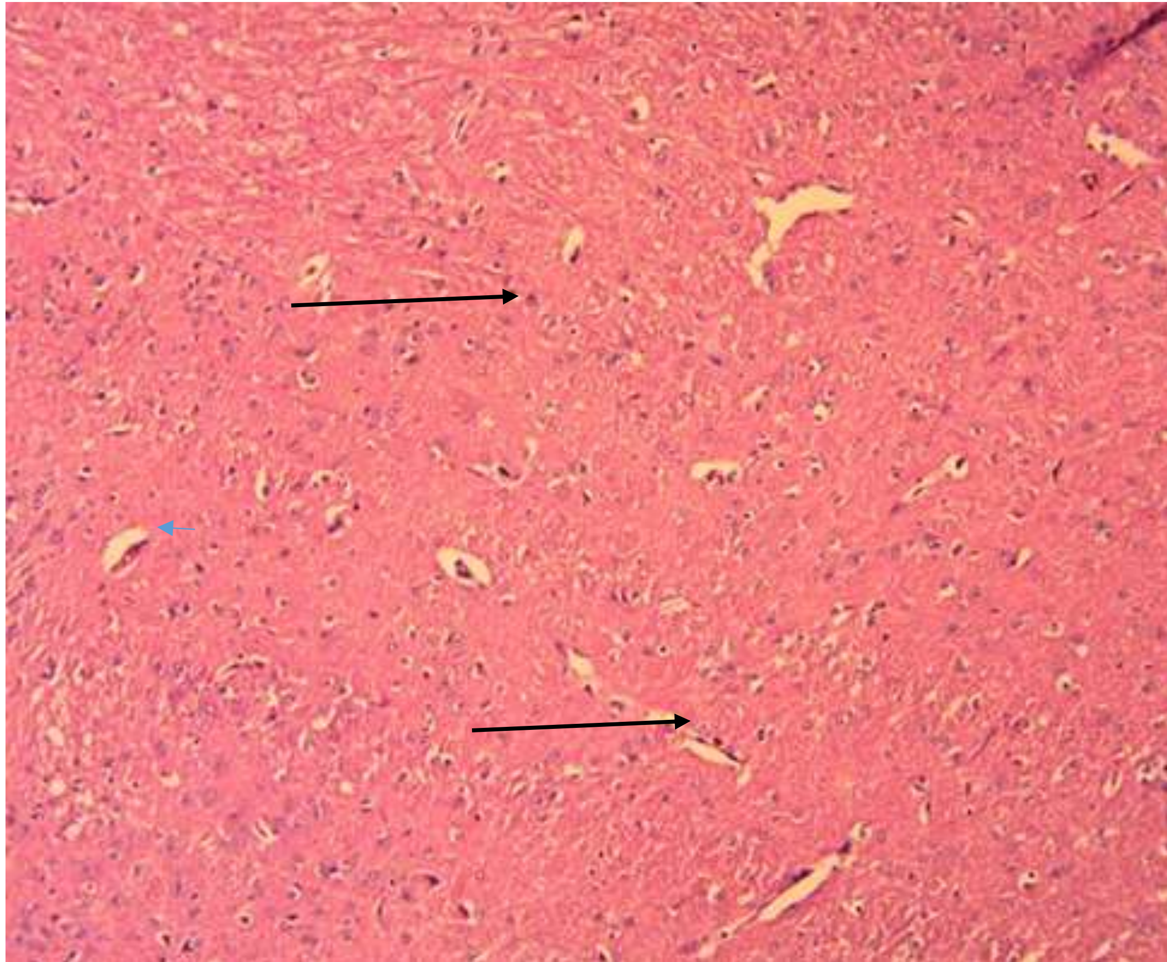

Plate 4: Photomicrograph of fish tissue brain treated with 70mg/ml dose of GLP with H&E stain at magnification of x100.

D slide indicating degenerated neurons, altered stratum album centrale, with no visible Purkinje cell nucleus and Purkinje cell dendrites, inner granular layer and moderate vacuolar changes with empty space.

4.3 Discussion of Result

It is an established fact that herbicide contamination in aquatic habitat is drawing a global attention Kelly et al (2018). Fishes could be exposed to contamination concentrations which is used in different times on different crops in the region. The contamination may alter cellular and biochemical parameters in fish, and evaluation of changes in biochemical enzymes activities and other useful biomarkers could link glyphosate exposure and risk assessment (Dey, *et al,* 2016).

The current research exposed *Clarias gariepinus* to 30mg/L, 50mg/L, and 70mg/L of glyphosate based herbicide (Uproot) for 14 days in the ambient laboratory environment and some biochemical parameters in the brain tissue such as superoxide dismutase (SOD), catalase (CAT), lipid peroxidase (LPO), myloperoxidase (MPO), maoldialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GPx), protein thio, glutathione-S- transferase (GST), total protein (TP), nitric oxide (NO), DNA fragmentation and histopathological parameters were evaluated.

The activities of superoxide dismutase (SOD) were seen decreased significantly ($P < 0.05$) in brain tissue of *Clarias gariepinus* exposed to all treatment doses of 30mg/L, 50mg/L and 70mg/L compared to control. The decrease in activity may indicate overwhelmed antioxidant defenses leading to oxidative stress. Catalase (CAT) activity showed similar pattern of effect in this study. This data is consistent with previous findings by (Osioma, and Ejoh, 2021).

Lipid peroxidase (LPO) activity showed slight decrease ($P > 0.05$) at lowest concentration but decrease become significant ($P < 0.05$) at intermediate and highest concentrations. This shows contamination dose dependent decreases. This aligns with studies that pesticides can impair antioxidant defense mechanisms Livingstone (2001). This may exacerbate oxidative damage to cell membranes, potentially leading to cellular dysfunction (Halliwell and Chirico, 1993).

Myloperoxidase (MPO) activity in the brain tissue showed concentration dependent significant increase ($P < 0.05$). This suggests possible glyphosate induction of inflammatory response leading to increased production of reactive oxygen species (ROS). Elevated MPO activity may contribute to tissue damage and alteration of cellular homeostasis according to (Klebano, 2005).

Malondialdehyde (MDA) level increased significantly ($P < 0.05$) which was concentration dependent. This increase is indicative of enhanced lipid peroxidation and oxidative stress. This result shows consistency with previous study that exposure to contamination induces oxidative stress Livingstone (2001). Implication of this is cell dysfunction and alteration of cell homeostasis.

Reduced glutathione (GSH) level increase was not significant ($P > 0.05$) at the lowest dose in comparison with the control. However, at intermediate and highest doses GSH level increased appreciably ($P < 0.05$). This indicates adaptive response, and it aligns with the concept of threshold effects in antioxidant regulation (Sies, *et al*, 2017).

Glutathione peroxidase (GPx) activity had mild increase ($P > 0.05$) at lowest concentration (30mg/L) compared to control but increased significantly ($P < 0.05$) at intermediate and highest concentration indicate adaptive response. This confirms the dose-dependent regulation of GPx activity as put by (Flohe, *et al,* 1973).

Protein Thio concentrations show comparable levels between control and test groups indicating insignificant oxidative damage or cellular redox status. This indicates unaltered protein function and structure (Kempe, *et al*, 2008).

Glutathione-S-transferase (GST) showed insignificant increase ($P > 0.05$) in activity across all test concentrations compared to control implies that GST was not significantly induced and that the detoxification capacity of the enzyme may not be overtly affected. This means that cellular detoxification mechanism due to GST not substantially activated or induced.

Total Protein concentration in the brain tissue was low in lowest and highest doses ($P > 0.05$) of exposure compared to control but significant increase ($P < 0.05$) at intermediate dose. This shows non-monotonic response, and it is consistent with the biphasic response where intermediate triggers significant increase in protein synthesis or alters its degradation. Findings in this study aligns with the hormesis response concept of cellular adaptation as canvassed by Calabrese and (Baldwin, 2001).

Acetylcholinesterase (AChE) activity showed concentration dependent inhibition. The lowest dose not significantly increased ($P > 0.05$) AChE activity but at intermediate and highest doses significantly increased ($P < 0.05$) activity. This significant increase in activity suggests glyphosate exhibition of neurotoxic effects. This is consistent with previous studies of contamination-based inhibition of AChE activity as advanced by (Costa, *et al*, 2016).

Nitric oxide (NO) result showed a significant reduction ($P < 0.05$) in brain tissue exposed to glyphosate. This decrease suggest neurotoxic effect of glyphosate, potentially disrupting neurotransmission as nitric oxide plays prominently in synaptic plasticity. The reduction of nitric oxide may be attributed to oxidative stress and inflammation which is consistent with previous study of (Sutleoglu, *et al*, 2015).

DNA fragmentation significantly increased ($P < 0.05$) in all test doses compared to control. The response was dose-dependent. Result suggests induction of neurotoxicity with implication of apoptosis mediated neurodegeneration leading to behavioural and cognitive impairment. The findings correlate with previous work done by (Cavas, *et al*, 2015).

Histopathological examination of brain tissue of *Clarias gariepinus* showed insignificant changes in the lowest exposure concentration compared to control. However, intermediate and highest test concentrations displayed significant neurotoxic effects. These effects are characterized by degenerated neurons, granular layer alterations, purkinje cells and purkinje dendritic damages and changes in stratum album central. These changes are implicated on disrupted brain architecture as they compromise neuronal connectivity, neuronal degeneration which confers loss in cognitive and motor functions. These changes however are dose-dependent. This finding aligns with previous findings by (Cavas, *et al*, 2015).

CHAPTER FIVE

5.1 Summary of findings

The activities of superoxide dismutase (SOD) and catalase (CAT) were significantly inhibited which showed a concentration dependent pattern in the brain tissue of *Clarias gariepinus.*

Lipid peroxidase (LPO) activity decreased significantly at intermediate and highest doses of glyphosate, indicating dose-dependent inhibition. Myloperoxidase (MPO) activity showed marked increase showing its dose-dependent induction by the contaminant glyphosate. Malondialdehyde (MDA) level increased linearly with concentration suggesting lipid peroxidation. Reduced glutathione (GSH) level increased across test concentration which was dose-dependent, implicating adaptive response to oxidative stress. Glutathione peroxidase (GPx) activity increased compared to control showing effect of the herbicide on its activity. Protein Thio concentration showed comparable level with control, and therefore, no noticeable cellular redox balance alterations, protein functions and structure. Glutathione-S-transferase (GST) activity was not affected compared with control, suggesting that GST may not be sensitive to glyphosate. Total protein in the brain tissue of *Clarias gariepinus* showed biphasic response compared to control, indicating induction of cellular adaptation, promoting protein anabolism. Acetylcholinesterase (AChE) activity showed significant dose-dependent increase, suggesting glyphosate mediated neurotoxicity. Nitric oxide level showed significant reduction compared to control, implicating neurotoxicity and DNA damage, potential behavioural and cognitive impairment. DNA fragmentation result showed marked increase in percentage DNA fragmentation, suggestive of dose-dependent genotoxic effect. Histopathological examination of the brain tissue of *Clarias gariepinus* showed neurotoxic effects such as degenerated neurons, damage to Purkinje cells and dendrites, changes in granular layers, vacuolation and empty spaces as these changes compromise brain architecture.

5.2 Conclusion

Findings in this study indicate that exposure to glyphosate induces significant neurotoxic effects in the brain tissue of test organism. The glyphosate showed substantial effects on various antioxidants analyzed in the brain tissue. The herbicide showed genotoxic effects as neuronal DNA fragmentation showed a concentration dependent damage. The study also showed concentration dependent histopathological changes such as degenerated neurons and alteration in brain architecture. These results suggest harm to aquatic lives and raise concern about environmental pollution. The dose-dependent effects observed lay more emphases on the need for further studies on underlying mechanisms and long-term consequences.

5.3 Recommendations

i. Launch further investigation into oxidative stress, inflammation and apoptosis.
ii. Investigate cognitive impairment and behavioural changes in fish exposed to glyphosate
iii. Assess potential human health concerns associated with glyphosate pollution in the environment.

5.4 Contribution to knowledge

This study contributes to knowledge to the understanding of neurotoxic effects in aquatic organisms, informing strategies for mitigating ecosystem pollution.

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