ANTIOXIDANT LEVEL OF KIDNEY AND LIVER IN WISTAR RATS FED WITH MAIZE FORMULATED DIET GROWN ON GLYPHOSATE EXPOSED SOIL

BY

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LSC2103821

A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE (B.Sc.) IN BIOCHEMISTRY, UNIVERSITY OF BENIN, EDO STATE.

OCTOBER 2025

CERTIFICATION

We the undersigned certify that OVONLEN PRECIOUS ISIBHAKHOME with Matriculation Number LSC2103821 carried out this project work in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc. Hons.) Degree in Biochemistry in the Department of Biochemistry

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DEDICATION

This project is dedicated to God Almighty, the giver of life and wisdom. I also dedicate it to my Parents, Mr. Steve Ovonlen and Mrs. Ethel Ovonlen for their love and financial support.

ACKNOWLEDGEMENT

I want to extend a special thank you to Heavenly Father, who has been my source of strength, understanding and wisdom throughout my project, indeed He came through for me. I sincerely want to appreciate my project supervisor, Dr Cyril .O. Ugbeni, and also my HOD, PROF. Chukwu .E. Onyeneke, my parent Mr. Steve Ovonlen and Mrs. Ethel Ovonlen, my colleagues during the course of this study, Fedora, Geraldine, Tracy, Joy, Jane, Chinazorm, Mercy, and. I specially appreciate Mr Richard Osehodion for the role he played throughout my project. Thank you so much sir and God bless you.

Also, I want to appreciate all my lecturers Prof. George, Prof. Okolie, Prof. Omoregie, Prof. Agoyero, Prof. Orhue, Prof Adaikpoh, Prof Uadia, Dr. Ojeaburu, Dr. Kissinger, Dr. Erhunse, Dr. Adeogun, Dr. Abu, Dr. Frank, Dr. Kolawole, Dr. Usifo, Dr. Olashinde, Dr. Olude, for their academic impact, and also to all Lab Technicians Mr Edobor, Mr. Akpeh, and all others.

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ABSTRACT

Glyphosate is a non-selective systemic herbicide and the most often used pesticide globally. This study examined the effects of antioxidant levels in the liver and kidney of Wistar rats fed maize-based diets formulated from grains obtained from glyphosate-exposed soils. The rats were 15 in total and were grouped into A (commercial feed only), C (maize grown on glyphosate controlled weed field above the recommended dose), F (maize grown on glyphosate controlled weed field following the recommended dose + raw glyphosate (2ml), G (maize grown on glyphosate controlled weed field following the recommended dose), and H (feed produced from maize grown by local farmers), and were maintained under standard conditions for the duration of 28 days. Antioxidant parameters such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), and malondialdehyde (MDA) were analyzed. The following results showed reduced activities of antioxidant enzymes in the glyphosate-fed groups compared to group A, indicating the presence of oxidative stress and lipid peroxidation. The various activities suggest that glyphosate residues in feed may disrupt cellular redox balance, compromise organ function, and increase susceptibility to oxidative damage. Overall, the study discussed the potential health risks associated with the consumption of crops cultivated on glyphosate exposed soils and the need for continuous monitoring of herbicide residues in animal feed and food chains.

CHAPTER ONE

INTRODUCTION

1. Background of Study

Glyphosate is a non-selective systemic herbicide and the most often used pesticide globally. Glyphosate-based herbicides (GBHs), such as Roundup®, were introduced in the 1970s and are now widely used for weed control in key crops (including maize) and non-crop regions. With worldwide output expanding, particularly with the development of glyphosate-tolerant crops, glyphosate use has extended across geographies, with heavy use in the Americas, Asia, and, increasingly, Africa. Because of its widespread use, glyphosate residues have been found in the air, groundwater, drinking water, agricultural produce, and animal feeds all across the world.

However these levels are typically believed to be within official safety limits, surveys have actually discovered glyphosate in most cereal grain samples used for feed. For instance, a Food and Drug Administration survey identified glyphosate in 63% of maize samples and 67% of soybean samples. For livestock and other animals that consume such diets, persistent low-level glyphosate exposure through crops and feed is therefore a plausible scenario. Because glyphosate can infiltrate the food chain and build up in animal tissues, its ubiquitous presence in crops is concerning. Studies on the metabolism of animals show that glyphosate tends to accumulate in the liver and kidney, which are organs involved in detoxification and excretion.

These organs are critical for managing oxidative by-products and detoxifying xenobiotics. It is now well documented that glyphosate can induce oxidative stress in mammals. For instance, recent rodent studies have shown that glyphosate exposure significantly reduces endogenous antioxidant defenses such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) while concomitantly increasing reactive oxygen species and lipid peroxidation products (e.g. malondialdehyde, MDA) in liver and kidney tissues. One experiment found that rats exposed to glyphosate had markedly lower SOD and GPx activities and higher MDA levels in both the liver and kidney compared to controls. Similarly, glyphosate-treated rats showed

depleted glutathione (GSH) and elevated markers of oxidative damage in their livers and kidneys. These findings suggest that even low-level herbicide residues in feed could impair antioxidant systems in vital organs.

Given this background, there is a need to investigate how glyphosate residues in animal feed (specifically, maize from glyphosate-treated fields) affect organ antioxidant status. Maize is a major staple crop and livestock feed, and maize produced in fields where glyphosate was used for weed control may contain varying herbicide residues. However, few studies have directly examined the impact of feeding animals with maize grown in glyphosate-exposed soil. The present study addresses this gap by using Wistar rats as a model to test the hypothesis that a diet formulated from maize obtained from glyphosate-treated fields will alter antioxidant levels in the liver and kidney. In particular, we compare rats given commercial feed (no glyphosate) with rats fed maize grown under controlled weed management with different glyphosate treatments (e.g., recommended dose plus extra glyphosate, or a dilute glyphosate solution). Measuring antioxidant enzyme activities and oxidative stress markers in the kidney and liver will reveal whether and to what extent glyphosate in the diet disrupts antioxidant homeostasis.

1.2 Aim

Examining the antioxidant state of liver and kidney tissues in Wistar rats given meals made with maize cultivated under various glyphosate exposure circumstances is the goal of this investigation.

1.3 Objectives

The objectives of this study are as follow:

1. Determine and contrast the levels of important antioxidant enzymes in the liver and kidney of rats in each treatment group, such as glutathione peroxidase, catalase, and superoxide dismutase.

2. Assess these organs' concentrations of oxidative damage indicators (like malondialdehyde) and non-enzymatic antioxidants (like reduced glutathione).
3. Examine the differences in these biochemical parameters between experimental groups fed maize from glyphosate-treated soils at different dosages and a control group (commercial feed only).
4. Determine whether the degree of glyphosate exposure in the environment is significantly correlated with variations in antioxidant status.

1.4 Justification of the Study

Understanding the effects of glyphosate-contaminated feed on animal health has important implications for food safety and livestock management. If glyphosate residues in crops lead to oxidative stress in animals, this could affect organ function, animal productivity, and potentially enter the human food chain. Results from this study will provide data on the biochemical impact of realistic feed exposures, informing risk assessments. In the context of heavy maize cultivation and extensive glyphosate use, especially in regions like Nigeria, these findings will help stakeholders evaluate whether current agricultural practices carry subtle health risks. Additionally, by identifying changes in antioxidant defenses, this research could guide further studies on mitigation (e.g. dietary supplements) or revisions to herbicide usage guidelines.

Several key findings from previous research motivate this study. First, the prevalence of glyphosate residues in crops and feed is well documented. A review notes that surveys consistently find glyphosate residues in grains and legumes used for animal feed, although typically at levels below regulatory thresholds. For example, Vicini et al., (2019) report that glyphosate was detected in over 60% of corn and soybean samples tested in U.S. feed surveys.

Regulatory bodies have established maximum residue tolerances (MRTs) for glyphosate in food and feed, and actual measured intakes in animals are usually far below these safety limits. Nevertheless, the ubiquitous nature of glyphosate residues means that sub-acute exposure is common for many farm animals and wildlife.

Second, glyphosate and target tissues have been studied in animals. After ingestion, glyphosate is incompletely absorbed (roughly 20-30% of an oral dose) and is mostly excreted unchanged in

feces and urine. Metabolism studies (using radiolabeled glyphosate) indicate that the herbicide and its metabolite AMPA do not tend to bioaccumulate; rather, glyphosate distributes transiently through the body with liver processing and renal excretion. Importantly, these studies have consistently identified the liver and kidney as primary target organs for glyphosate accumulation. Due to their roles in detoxification and excretion, these organs bear the brunt of glyphosate's biochemical effects.

Third, a growing body of toxicological evidence links glyphosate to oxidative stress in animal models. Numerous animal experiments have demonstrated that glyphosate (and glyphosate-based formulations like Roundup) can disrupt the antioxidant defense system. For instance, Tang et al., (2017) found that rats given glyphosate (5-500 mg/kg) showed significant decreases in serum and tissue antioxidant enzymes and increases in oxidative damage markers. In that study, kidney tissue from glyphosate-treated rats had significantly higher malondialdehyde levels and lower SOD and GPx activities compared to controls. In liver tissue, glyphosate exposure also lowered SOD activity and raised hydrogen peroxide content. Similarly, another recent study reports that sub-chronic glyphosate exposure suppressed the activities and gene expression of key kidney antioxidant enzymes (CAT, SOD, GPX) and depleted reduced glutathione, while elevating oxidative markers (MDA, NO) in rats. These findings align with earlier reports that glyphosate provokes oxidative stress responses: El-Shenawy (2009) and others showed elevated lipid peroxidation and reduced glutathione in the liver and kidneys of glyphosate-exposed rats.

Finally, most studies administer glyphosate directly (e.g. via drinking water or gavage), there is interest in understanding real-world exposures through food. Wistar rats have been used to assess dietary contaminants because their physiology often predicts mammalian responses. Feeding assessments with glyphosate induced feed support the notion that dietary glyphosate can affect organic biochemistry.

CHAPTER 2

LITERATURE REVIEW

2 Glyphosate

Glyphosate [N-(phosphonomethyl)glycine] is the widely used broad-spectrum, systemic herbicide globally, first commercialized in the 1970s (Duke & Powles, 2008). Its herbicidal activity is mediated through inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key component of the shikimate pathway, which is essential for the synthesis of aromatic amino acids in plants, fungi, and some microorganisms (Steinrucken & Amrhein, 1980). Since this pathway is absent in animals, glyphosate was initially considered relatively safe for mammalian systems. However, growing evidence suggests potential adverse impacts linked to its persistence and residues in food crops (Myers et al., 2016).

In agricultural systems, glyphosate is commonly used for weed control in cereals, maize, and genetically modified crops engineered to tolerate its application (Benbrook, 2016). Its physicochemical properties allow it to bind strongly to soil particles, but residues may still persist, particularly under repeated application (Okada et al., 2016). Studies have demonstrated the presence of glyphosate residues in water, soil, and food commodities, raising concerns about chronic exposure (van Bruggen et al., 2018). Furthermore, its interaction with soil microbial communities can alter nutrient cycling and reduce biodiversity in agroecosystems (Newman et al., 2016).

Toxicological research has reported conflicting outcomes. Some studies suggest glyphosate has low acute toxicity (Williams et al., 2000), while others highlight sub-chronic and chronic effects, including endocrine disruption, oxidative stress, hepatotoxicity, and nephrotoxicity (Mesnage et al., 2015). In mammals, glyphosate absorption occurs through ingestion, inhalation, or dermal exposure, followed by distribution primarily to the liver and kidney, which are the main organs of detoxification and excretion (Richard et al., 2005). Evidence indicates glyphosate and its primary metabolite, aminomethylphosphonic acid (AMPA), can induce reactive oxygen species

(ROS) generation, compromise antioxidant defenses, and promote cellular damage in these organs (Jayasumana et al., 2014).

Given its widespread use and detection in food chains, glyphosate remains a subject of intense scientific and regulatory debate. Current research emphasizes the need to evaluate not only glyphosate itself but also commercial formulations containing adjuvants, which may amplify its toxicological effects (Mesnage & Antoniou, 2017).

2.1 Environmental Persistence and Soil Interactions

Glyphosate is a highly polar, water-soluble herbicide that demonstrates strong interactions with soil components, influencing its persistence and bioavailability. Once it is applied to farm fields, it binds tightly to soil minerals, particularly iron, aluminum, and manganese oxides, as well as clay particles and organic matter (Borggaard & Gimsing, 2008). This strong adsorption reduces mobility but allows for the release of residues over time, contributing to long-term persistence in soils (Okada et al., 2016).

Although glyphosate was originally considered to degrade rapidly, with reported half-lives ranging from a few days to weeks, more recent research indicate that degradation rates varys depending on soil type, pH, temperature, and microbial activity (Gimsing et al., 2004). In certain soils, residues have been detected months after application, suggesting accumulation (Battaglin et al., 2014). The primary degradation pathway involves microbial metabolism, producing aminomethylphosphonic acid (AMPA), a metabolite that is persistent and also for environmental health (Okada et al., 2016).

Microbial communities play a role in glyphosate breakdown, yet evidence suggests that repeated applications may disrupt microbial diversity and enzymatic activity (Newman et al., 2016). For example, some bacteria utilize glyphosate as a phosphorus source, while others are affected by its presence, leading to various imbalances. Over time, this may alter soil nutrient cycling and reduce the functional resilience of agroecosystems (van Bruggen et al., 2018).

Leaching is low due to glyphosate's adsorption properties; however, under certain conditions such as sandy soils, heavy rainfall, or poor organic matter content, residues may contaminate surface and groundwater (Borggaard & Gimsing, 2008). Glyphosate's high solubility facilitates its transport via runoff, and its detection in rivers, streams, and even drinking water supplies across multiple regions highlights its environmental persistence beyond soil matrices (Battaglin et al., 2014).

In agricultural rotations, repeated applications increase the risk of residue accumulation in both soils and subsequent harvests (Benbrook, 2016). This leads to implications not only for environmental quality but also for human and animal health, as glyphosate and AMPA residues have been found in grains, vegetables, and animal feed. Overall, glyphosate's environmental persistence and soil interactions are more complex than assumed. Its strong adsorption reduces short-term mobility, yet long-term accumulation, microbial disruption, and residue transport raise significant ecological and toxicological concerns (Myers et al., 2016).

2.3 Global Use Trends and Regulatory Context

Since its introduction in 1974 by Monsanto under the trade name Roundup, glyphosate has become one of the most applied herbicide worldwide (Duke & Powles, 2008). Its adoption was firstly driven by its broad-spectrum weed control, low cost, and perceived safety to non-target organisms compared to other herbicides. The global use of glyphosate increased with the introduction of genetically modified glyphosate-tolerant (GT) crops in the mid-1990s, such as soybeans, maize, and cotton (Brookes & Barfoot, 2018). These crops allowed farmers to apply glyphosate directly to fields without damaging the crop, streamlining weed management practices and reducing labor costs. By the early 2000s, glyphosate contributed to a significant proportion of global herbicide sales, and its use has since continued to expand. In the United States alone, glyphosate applications rose from approximately 13 million kg in 1995 to over 125 million kg annually by 2014, representing a nearly tenfold increase (Benbrook, 2016).

Globally, estimates suggest that over 8.6 billion kg of glyphosate has been applied between 1974 and 2014, with usage still increasing in many developing countries where GT crops are being introduced. This widespread application has made glyphosate easily detectable in soil, water, food, and even human biological samples (Myers et al., 2016).

The reliance on glyphosate has also contributed to the emergence of glyphosate-resistant weed species, now reported in at least 40 countries and across more than 30 weed species (Heap, 2014). This resistance resulted to increased application rates, tank-mixing with other herbicides, and expanded glyphosate use beyond GT crops to non-crop areas such as orchards, vineyards, road verges, and home gardens (Van Bruggen et al., 2018). Consequently, both the environmental burden and human exposure risk have increased, intensifying scientific and regulatory scrutiny.

Regulatory approaches to glyphosate varies across regions. In the United States, the Environmental Protection Agency (EPA) continues to classify glyphosate as "not likely to be carcinogenic to humans," citing insufficient evidence for cancer risk at typical exposure levels (EPA, 2020). Similarly, regulatory agencies in Australia, Canada, and Japan have maintained approvals for glyphosate, emphasizing its importance in modern agriculture (Health Canada, 2019).

In contrast, the European Union has faced several debates over glyphosate's renewal. In 2015, the International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic to humans" (Group 2A), stating limited evidence in humans and sufficient evidence in experimental animals (IARC, 2015). This sparked intense public and political debate within the EU. While the European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA) concluded that glyphosate is unlikely to cause a carcinogenic risk, the precautions in some states led to restrictions or partial bans. For instance, France announced intentions to phase out glyphosate use by 2023, while Germany plans a complete ban by 2024 (Schutte et al., 2017).

Outside the EU, several countries in Latin America and Asia have adopted mixed approaches. In Argentina and Brazil, where GT soybean cultivation dominates, glyphosate uses are high, despite the concerns about residues in food and drinking water (Arregui et al., 2004). In contrast, Sri Lanka and Thailand temporarily banned glyphosate due to suspected links with chronic kidney disease among agricultural workers, although bans were later reversed following agricultural pressure (Jayasumana et al., 2014).

The controversy also extends to international trade and consumer safety. Glyphosate residues have been detected in cereals, fruits, and vegetables, sometimes exceeding maximum residue limits (MRLs) established by the Codex Alimentarius Commission (Myers et al., 2016). Public awareness campaigns and litigation cases, particularly in the United States where courts have awarded damages linking glyphosate exposure to non-Hodgkin's lymphoma, have further pressured regulators and manufacturers (Portier et al., 2016).

Overall, glyphosate's global use trends reflect its central role in modern agriculture, while its regulatory context highlights the balance between agricultural productivity and potential human and environmental health risks. The divergence in regulatory assessments shows the ongoing uncertainty in the scientific evaluation of glyphosate's long-term impacts. Increasing international debate, coupled with rising resistance issues, suggests that glyphosate's future use will likely involve strict regulation, close monitoring, and more emphasis on integrated weed management strategies (Van Bruggen et al., 2018).

2.4 GLYPHOSATE IN CROP PRODUCTION SYSTEMS

2.4.1 Use in Wheat-Maize Rotations

Glyphosate's use in cropping rotations affects its presence in soil and crops. In many agricultural systems, glyphosate is applied to one crop (e.g., wheat) and the next crop (e.g., maize) is planted in the same field. For example, in the United States and parts of Europe, glyphosate is used as a pre-harvest desiccant on mature wheat or barley (Cerdeira & Duke, 2006). The herbicide

expedites uniform drying of the crop and allows early harvest. Glyphosate is also used for weed control in glyphosate-tolerant maize, though non-GR maize does not tolerate direct spray (Brookes & Barfoot, 2018). In such rotations, glyphosate can enter the soil environment either through direct application to crops or as root exudates from GR plants (Kremer & Means, 2009).

2.4.2 Glyphosate Residue Dynamics in Maize

Glyphosate binds tightly to soil particles, its direct uptake by a following crop is usually minimal (Borggaard & Gimsing, 2008). Technical fact sheets emphasize that "because glyphosate binds to the soil, plant uptake from soil is negligible" (Giesy et al., 2000). Thus, even though glyphosate residues and its metabolite AMPA can remain in soil for weeks to months, most remain sorbed and largely unavailable for root absorption (Okada et al., 2016). Measured glyphosate residues in maize grain grown after glyphosate-treated crops are typically very low or undetectable, especially if soil microbial activity has degraded the compound (Coupe et al., 2012). One study in genetically modified maize found no significant differences in grain nutrient composition between glyphosate-resistant and conventional lines, implying glyphosate treatment did not appreciably alter grain chemistry (Osborne et al., 2016). Overall, under standard use and crop rotation practices, maize is unlikely to accumulate meaningful glyphosate residues from soil. Nonetheless, low-level glyphosate and AMPA have been sporadically detected in cereals and grains, reflecting its widespread use (Battaglin et al., 2014).

2.4.3 Impact on Maize Nutrition and Phytochemicals

There is ongoing interest in whether glyphosate exposure can affect crop nutritional quality. Glyphosate's chemical properties, particularly its strong chelation of micronutrients like Mn, Fe, and Zn, have led to hypotheses that it might induce micronutrient deficiencies or alter secondary metabolism in crops (Johal & Huber, 2009). Regarding phytochemicals such as phenolics and antioxidants, available data are limited. Any changes in maize secondary compounds after glyphosate exposure appear minimal in most studies, though the evidence base is still emerging (van Bruggen et al., 2018). Overall, glyphosate under normal agricultural use appears to have

negligible impact on major nutrient levels in maize, consistent with its rapid soil binding and low plant translocation.

2.4.4 Glyphosate Toxicodynamic in Mammals

2.4.4.1 Routes of Exposure and Absorption

In mammals, the primary routes of glyphosate exposure are ingestion, inhalation, and dermal contact, with most studies focusing on oral intake (diet or drinking water) as the dominant route in non-occupational contexts (Williams et al., 2000; Guyton et al., 2015). When glyphosate is ingested, it is readily absorbed from the gastrointestinal tract (Anadon et al., 2009). In contrast, dermal absorption in intact skin is very low; glyphosate's water solubility and charge limit dermal absorption (Zhang et al., 2019). Inhalation can lead to systemic absorption if glyphosate-containing spray droplets or dust are inhaled, but actual inhaled doses tend to be smaller compared to ingestion (Gonzalez et al., 2022). Experiments in animals have confirmed that after oral dosing, glyphosate appears in blood and tissues, indicating absorption (Brewster et al., 1991). Overall, the bioavailability via the gut is moderate; estimates suggest roughly 30-40% of an ingested dose is absorbed in rats, with the remainder passing unabsorbed in feces (Anadon et al., 2009).

2.4.4.2 Metabolism and Systemic Distribution

Once absorbed, glyphosate circulates in blood with little metabolism. Mammals lack specific enzymes to degrade glyphosate, so over 99% of absorbed glyphosate remains unchanged (Williams et al., 2000). The minor metabolite, aminomethylphosphonic acid (AMPA), is formed only at trace levels (<1% of dose) in mammals (European Food Safety Authority (EFSA), 2015). Distribution studies show that glyphosate distributes widely via the bloodstream, reaching the liver, kidneys, and other organs (Aris & Leblanc, 2011). However, it does not bioaccumulate in fat or permanent tissues due to its water solubility. Analyses in rats indicate that glyphosate concentrations in the kidney can be roughly four times higher than in the liver, consistent with

the kidneys' role in filtering and excreting the compound (Brewster et al., 1991). Other organs (lungs, muscle, brain) typically show much lower levels. Importantly, glyphosate's surfactants (found in formulations like Roundup with POEA) led to higher liver levels and more pronounced oxidative effects than glyphosate alone (Mesnage et al., 2013).

2.4.4.3 Elimination and Excretion

Glyphosate is eliminated rapidly, primarily via the kidneys. Approximately two-thirds of an oral dose is excreted unchanged in feces (reflecting unabsorbed glyphosate), while most of the absorbed glyphosate is excreted in urine (Anadon et al., 2009). The urinary half-life is on the order of hours to a day; detectable levels decline quickly after dosing stops (Brewster et al., 1991). Because glyphosate is a small polar molecule, it is filtered through glomeruli and excreted without significant reabsorption. Only minute amounts of AMPA appear in urine. Overall, mammalian tissue retention of glyphosate is minimal; there is no evidence of significant accumulation over repeated exposures at normal doses (EFSA, 2015). Trace amounts may linger transiently in the gut and colon or be bound in bone matrix, but the main burden of toxin is cleared within days (Steinborn et al., 2016). This rapid elimination profile means that only high or repeated exposures would lead to sustained systemic levels.

2.4.4.4 Target Organs and Toxic Effects

Animal studies consistently identify liver and kidney as the primary target organs of glyphosate toxicity (Guyton et al., 2015). In acute (high-dose) cases, glyphosate formulations can cause gastrointestinal irritation and cardiac arrhythmias, but typical regulatory studies find low lethality and focus instead on sub-chronic effects (Williams et al., 2000). In rats chronically exposed (via diet or gavage), histopathological changes are most often reported in liver and kidney. For example, Serra et al., (2024) observed that female rats exposed to high-dose glyphosate-based herbicide for 75 days exhibited elevated serum liver enzymes (AST, ALT) and blood urea nitrogen, indicating liver and kidney injury. These rats also developed liver steatosis (fat accumulation) and histological tubular damage in the kidneys. Such findings reflect impaired

hepatic function (enzyme leakage) and reduced renal filtration. Males in the same study showed similar trends at high doses, though females were often more sensitive (Serra et al., 2024).

Oxidative stress is a common mechanism implicated in this organ toxicity. Glyphosate and especially its formulations can induce reactive oxygen species (ROS) generation and lipid peroxidation in hepatocytes and renal cells (El-Shenawy, 2009). For instance, El-Shenawy (2009) demonstrated that Roundup® at subacute doses caused marked depletion of glutathione (GSH) and increased malondialdehyde (MDA, a lipid peroxidation marker) in rat liver, effects greater than those seen with glyphosate alone. Tang et al., (2017) and Liu et al., (2018) both reported glyphosate-induced increases in serum MDA and decreases in antioxidant enzyme activities in rat liver and kidney. Such oxidative damage would compromise cell membranes and enzyme functions. Chronic low-dose effects are subtler, but some long-term rodent studies have detected focal renal tubular degeneration and liver cell enlargement at the highest tested concentrations.

2.5 Enzymatic Antioxidants (SOD, CAT, GPx, GR)

Cells employ several major antioxidant enzymes in the first line of defense. Superoxide dismutase (SOD) converts superoxide anions into hydrogen peroxide. Catalase (CAT) and glutathione peroxidase (GPx) then decompose hydrogen peroxide into water and oxygen. In the glutathione cycle, GPx uses reduced glutathione (GSH) to neutralize peroxides, yielding oxidized glutathione (GSSG), which is then recycled to GSH by glutathione reductase (GR) using NADPH (Halliwell & Gutteridge, 2015). In toxicology studies of glyphosate, researchers often assay their activities or protein levels as indicators of oxidative stress (Tang et al., 2017; Liu et al., 2018).

2.6 Non-Enzymatic Antioxidants

In addition to enzymes, organisms rely on non-enzymatic antioxidants. The tripeptide glutathione (GSH) is the most abundant intracellular antioxidant, directly scavenging radicals and serving as a substrate for GPx. Vitamins C (ascorbate) and E (tocopherol) are important

radical scavengers: vitamin E protects lipid membranes from peroxidation, while vitamin C regenerates oxidized vitamin E and scavenges aqueous ROS (Lushchak, 2014). Other small antioxidants include uric acid, bilirubin, and carotenoids. Although not typically measured in routine glyphosate studies, depletion of GSH or vitamin stores is a hallmark of oxidative challenge. For example, El-Shenawy (2009) showed that Roundup exposure drastically reduced hepatic GSH levels in rats. Thus, non-enzymatic antioxidants complement enzymes in maintaining redox balance.

2.7 Role of Oxidative Stress in Liver and Kidney Dysfunction

The liver and kidney are particularly susceptible to oxidative injury due to their metabolic roles (Guyton et al., 2015). Hepatocytes have high oxidative metabolism and enzyme content, making them ROS sources; they also perform detoxification reactions that can generate radicals. Renal tubular cells consume oxygen in active reabsorption and filtration tasks, producing ROS in the process. When antioxidant defenses are overwhelmed (e.g., by toxicants), oxidative damage can lead to cell death. Lipid peroxidation in membranes impairs cell integrity, and oxidation of mitochondrial proteins can trigger apoptosis. Oxidative stress in the liver can manifest as elevated transaminases (ALT/AST) and histological changes (steatosis, necrosis) (Tang et al., 2017). In the kidney, oxidative damage can disrupt glomerular and tubular cells, leading to proteinuria or changes in blood urea nitrogen (Liu et al., 2018). Chronic low-level oxidative stress also promotes inflammatory signaling and fibrosis, contributing to organ dysfunction. In summary, oxidative stress is a central mechanism by which glyphosate and other hepatotoxic/nephrotoxic agents exert long-term damage.

2.8 Wistar Rats as a Toxicological Model

Wistar rats are one of the most widely employed rodent strains in experimental toxicology due to their genetic stability, defined physiological responses, and availability for laboratory research (Sengupta, 2013). They have been extensively used in evaluating xenobiotic effects, including pesticides and herbicides, because of their metabolic and biochemical similarity to humans,

especially in hepatic and renal systems (Gao et al., 2017). Their relatively large size, docile nature, and predictable food and water intake make them suitable for repeated-dose feeding studies where dietary exposures must be carefully monitored (OECD, 2008).

The liver and kidney of Wistar rats are considered reliable indicators of systemic toxicity because these organs are major sites of detoxification and excretion. Wistar rats exposed to glyphosate-based herbicides have been shown to develop hepatic steatosis, renal tubular degeneration, and altered oxidative stress biomarkers, making them useful for mechanistic studies (Mesnage et al., 2015). In a 90-day feeding trial, glyphosate exposure led to increased hepatic lipid accumulation, DNA damage, and oxidative imbalance in Wistar rats, highlighting their sensitivity to herbicide-induced redox disruption (Mesnage et al., 2019).

Sex differences have also been observed in Wistar rats during glyphosate exposure. Serra et al., (2024) found that females showed greater elevations in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and blood urea nitrogen compared to males under similar conditions, suggesting that sex-specific antioxidant defenses may alter susceptibility. Their widespread use in regulatory toxicology and academic research underscores because Wistar rats remain the model of choice in studying glyphosate's hepatic and renal impacts.

CHAPTER THREE

MATERIALS AND METHODS

3.1. MATERIALS

3.1.1 Experimental Animals

- Species: Rattus norvegicus (Wistar albino rats)
- Sex: Male (or specify both male and female)
- Source: The rats were bred in the city of Ibadan
- Housing: Standard wooden and plastic cages
- Acclimatization: 7 days before the start of feeding

3.1.2. Equipment/Apparatus

- Weighing scale
- Cages
- Mortar
- Pestle
- Centrifuge
- Test tubes
- Measuring cylinder
- Surgical scissors
- Scalpel
- Gloves
- Nose mask
- Lab coat
- Freezer
- Spectrophotometer
- Cotton wool
- Micro pipette (10 µL, 100 µL, 1000 µL)
- Syringe
- Eppendorf tube
- Conical flask
- UV-Vis spectrophotometer
- Analytical balance
- Homogenizer
- Refrigerator/freezer
- pH meter
- Test tubes, pipettes, glassware

3.1.3. Reagents

- Glyphosate
- Buffer
- Saline solution
- Soap
- Ethanol
- Chloroform

3.2. Experimental Design

In this study, the experimental subjects (rats) were divided into 5 groups, shown as follow:

Group A (Control): Fed with diet formulated from maize grown in non-exposed soil

Group B: Fed with diet formulated using maize obtained from glyphosate-controlled field where glyphosate was applied at recommended dose

Group C: Fed with diet formulated from maize grown on glyphosate-controlled field following the recommended dose + additional raw glyphosate (2ml)

Group D: Fed with diet formulated from maize grown on glyphosate-controlled field following the recommended dose + 0.3 mg/kg of glyphosate solution

Group E: Feed produced from maize obtained grown by local farmer.

The study lasted for 28 days.

3.3 Sample Collection and Preparation

At the end of the 28 days of study, rats were left to fast overnight after which they sacrifice under a mild anesthesia following ethical guidelines of the university. Liver and kidney excised, rinsed in ice-cold saline, and blotted dry. This was store at a temperature of -20°C.

3.4 Diet formulation and feeding regimen

Diets were formulated from maize (corn) blended with dried fish byproducts, soybean and other additives. The investigator provided the precise formulation for the experimental feed; this formulation was used to prepare the pelleted diets fed to the rats. The physical form of the diet was pelleted (pellet form size as used in the laboratory).

Table 3.1 Diet composition (as provided by investigator)

Ingredient	Quantity (g per kg feed)	Percentage (%)
Maize (corn)	580	58.3
Soya bean	340	34.2
Bone meal	25	2.5
Wheat offal	20	2.0
Fish meal (dried fish byproduct)	20	2.0
Glycine	1.5	0.2
Methionine	1.5	0.2
Premix (vitamin & mineral)	2.0	0.3
Salt	2.5	0.3
Bromine	1.5	0.2

Notes on diet preparation: The quantities above are reported as grams per kilogram of final mixed feed (g/kg). The investigator confirmed that the mixed feed was processed into pellets

prior to feeding. Ensure the unit (g/kg) matches laboratory records; adjust if feed was prepared per 1000 g or unit.

3.5 Experimental Timeline and Sample Collection

The feeding trial lasted for one month (30 days). Throughout this period, the animals were maintained under close observation and fed their respective formulated diets. Water and feed were provided ad libitum, and the animals were monitored daily for signs of distress, changes in behavior, or illness. At the end of the 30-day feeding period, the animals were fasted prior to sample collection to ensure that biochemical parameters were not influenced by recent feed intake. This fasting helped to stabilize the animals' metabolic state before sacrifice, providing more reliable data for the antioxidant assays.

The terminal stage of the experiment involved the collection of liver and kidney tissues, which were the primary organs of interest for assessing the effects of the formulated diets on oxidative stress and antioxidant parameters. The collection process was carried out carefully to prevent contamination and preserve tissue integrity for subsequent biochemical analysis.

3.6 Anesthesia, Euthanasia, and Tissue Handling

At the end of the feeding period, the rats were subjected to anesthesia prior to euthanasia and organ collection. Each rat was gently immobilized on a railed wire net to restrict movement. Anesthesia was achieved using chloroform, which was applied on a piece of cotton wool placed inside a closed container. The rats were individually introduced into the container containing the chloroform-soaked cotton wool and allowed to inhale the vapors until they became completely unconscious. No specific dose or duration of exposure was measured, but anesthesia was confirmed by the loss of movement and reflex response.

Once anaesthetized, each rat was carefully removed from the container and humanely sacrificed. The abdominal cavity was opened using clean dissection instruments, and the liver and kidney were excised immediately. The excised organs were gently blotted on clean filter paper to remove excess blood and then weighed to obtain their fresh weights.

After weighing, each tissue sample was placed into a clean, labeled container and stored in a domestic freezer prior to biochemical analyses. Although standard laboratory protocol typically recommends snap-freezing tissues in liquid nitrogen and preserving them at -80°C to maintain enzymatic and antioxidant stability, the investigator used a domestic freezer due to equipment limitations. This storage method is reported here to accurately reflect the procedures used during the study.

The handling of all animals followed general ethical guidelines for animal research, ensuring that unnecessary pain or distress was minimized throughout the procedure. All instruments used were cleaned and sterilized before and after use to prevent cross-contamination of samples.

3.6 Tissue Processing and Homogenization

Following the collection of organs (liver and kidneys), the tissues were promptly processed to maintain enzyme stability and prevent oxidative degradation. Each tissue sample (whole liver and both kidneys per rat) was carefully trimmed of visible fat and connective tissues using clean surgical scissors and forceps. The trimmed tissues were immediately washed in cold normal saline solution (0.9% NaCl) to remove excess blood and surface debris. After washing, the tissues were blotted gently with sterile filter paper to remove residual moisture and weighed accurately using an analytical balance.

Tissue homogenization was carried out on ice to minimize enzymatic degradation and oxidative changes. A 10% (w/v) homogenate was prepared by homogenizing 10 g of the tissue sample in 90 mL of 50 mM phosphate buffer (pH 7.4) using a Teflon-glass homogenizer (Remi Model RQ-127A, India). The phosphate buffer provided a stable pH environment necessary for maintaining

enzyme activity during extraction (Sinha, 1972). Homogenization was performed at a low speed (approximately 5,000 rpm) for 3-5 minutes with intermittent cooling on ice to prevent overheating.

The resulting homogenates were centrifuged at 10,000 x g for 15 minutes at 4°C using a refrigerated centrifuge (Eppendorf 5810R, Germany). Centrifugation separated the supernatant (post-mitochondrial fraction), which contains soluble cytosolic enzymes, from cellular debris. The clear supernatant was carefully collected with a micropipette and transferred into clean, labeled Eppendorf tubes. This supernatant served as the source of enzyme extract for subsequent biochemical assays.

To ensure consistency, all homogenization and centrifugation steps were performed under identical conditions for all groups. The supernatants were kept in ice or stored temporarily at 4°C until analysis. For longer-term storage, aliquots of the supernatants were preserved in a domestic freezer prior to enzymatic analysis. Protein concentration in the tissue homogenates was determined using the Bradford method (Bradford, 1976), which involves the binding of Coomassie Brilliant Blue G-250 dye to protein molecules, causing a shift in the dye's absorbance maximum from 465 nm to 595 nm. Bovine serum albumin (BSA) was used as the standard protein. The absorbance was measured using a UV-Visible spectrophotometer (Model UV-1800, Shimadzu, Japan) at 595 nm.

The protein concentration (mg/mL) was obtained from a standard calibration curve plotted from known concentrations of BSA. This value was used to normalize the enzyme activity in each assay, ensuring that the results were expressed as activity per mg of protein, thereby allowing accurate comparison between experimental groups (Lowry et al., 1951; Bradford, 1976).

3.7 Biochemical Assays

The biochemical parameters analyzed included superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), malondialdehyde (MDA) as a marker of lipid peroxidation, and

total protein content. These parameters were chosen because they represent key antioxidant defense systems within tissues and provide insight into oxidative stress levels induced by dietary exposure to glyphosate-formulated feeds. All reagents used were of analytical grade, and glassware was cleaned with chromic acid and rinsed thoroughly with distilled water to avoid contamination.

Each enzyme assay was conducted in triplicate (technical replicates) for accuracy. All absorbance readings were taken using a UV-Visible spectrophotometer calibrated before each assay.

3.7.1 Superoxide Dismutase (SOD) Assay

SOD activity was assayed using the epinephrine auto-oxidation method described by Misra and Fridovich (1972). The method is based on the inhibition of epinephrine oxidation to adrenochrome by the superoxide radical generated in an alkaline medium. The rate of adrenochrome formation was monitored spectrophotometrically at 480 nm.

Procedure:

A reaction mixture containing 2.5 mL of 0.05 M sodium carbonate buffer (pH 10.2) and 0.3 mL of freshly prepared 0.3 mM epinephrine was used. The reaction was initiated by adding 0.2 mL of tissue supernatant. The change in absorbance was monitored at 480 nm for 3 minutes. The control contained all reagents except the enzyme source.

Calculation:

The percentage inhibition of epinephrine auto-oxidation by SOD was calculated as:

SOD activity (U/mg protein) = ((Acontrol - Asample) / Acontrol) x 100

Were

Ac = absorbance change of control per minute,

As = absorbance change of sample per minute.

One unit of SOD activity is defined as the amount of enzyme that inhibits the rate of epinephrine auto-oxidation by 50% under the assay conditions (Misra and Fridovich, 1972).

3.7.2 Catalase (CAT) Assay

Catalase (CAT) activity was determined using the method of Aebi (1984), which measures the decomposition rate of hydrogen peroxide (H_2O_2) spectrophotometrically at 240 nm.

Procedure:

The reaction mixture consisted of 2.5 mL of 50 mM phosphate buffer (pH 7.0), 0.4 mL of 30 mM H_2O_2, and 0.1 mL of tissue homogenate. The decrease in absorbance at 240 nm was recorded for 1 minute.

Calculation:

Catalase activity was determined using the formula:

CAT activity (μmol H_2O_2 decomposed/min/mg protein) = (ΔA240 / ε x Vt) x (1 / protein concentration)

where:

Delta A_{240}/{min} = rate of change in absorbance per minute,

Vt = total volume of assay mixture,

epsilon = molar extinction coefficient for H_2O_2 (43.6 M^{-1} cm^{-1}),

Vs = volume of sample used,

Cp = protein concentration in mg/mL (Aebi, 1984).

Catalase activity was expressed as micromoles of hydrogen peroxide decomposed per minute per milligram protein.

3.7.3 Glutathione Peroxidase (GPx) Assay

GPx activity was measured according to the method of Rotruck et al., (1973), which determines the enzyme's ability to catalyze the reduction of hydrogen peroxide (H_2O_2) using reduced glutathione (GSH) as a substrate.

Procedure:

The reaction mixture contained 0.5 mL of phosphate buffer (pH 7.0), 0.1 mL of 10 mM sodium azide, 0.2 mL of 4 mM GSH, 0.1 mL of 2.5 mM H_2O_2, and 0.5 mL of tissue supernatant. The reaction was allowed to proceed for 10 minutes at 37°C and stopped by adding 0.5 mL of 10% trichloroacetic acid (TCA). After centrifugation, 1.0 mL of the supernatant was mixed with 2.0 mL of 0.3 M disodium hydrogen phosphate and 1.0 mL of 0.04% DTNB (Ellman's reagent). The yellow color developed was read at 412 nm.

Calculation:

GPx activity (U/mg protein) = {At - Ac} / (epsilon x l x Cp)

Where

At = absorbance of test sample,

Ac = absorbance of control,

epsilon = molar extinction coefficient for DTNB (13,600 M^{-1} cm^{-1}),

L = path length (1 cm),

Cp = protein concentration (mg/mL).

Results were expressed as micromoles of glutathione oxidized per minute per mg protein.

3.7.4 Lipid Peroxidation (MDA / TBARS Assay)

Lipid peroxidation was evaluated using the thiobarbituric acid reactive substances (TBARS) method of Ohkawa et al., (1979). This assay quantifies malondialdehyde (MDA), a product of polyunsaturated fatty acid peroxidation.

Procedure:

A volume of 0.5 mL of tissue homogenate was mixed with 2.5 mL of TCA (10%) and 1.5 mL of 0.67% TBA. The mixture was heated in a boiling water bath for 15 minutes, cooled, and centrifuged at 3,000 rpm for 10 minutes. The absorbance of the pink-colored supernatant was measured at 532 nm.

Calculation:

{MDA (nmol/mg protein)} = OD x V x 1000 / (α x V x L x Y)

$\alpha = 1.56 \times 10^5$

MDA concentration was expressed as nanomoles of MDA formed per milligram protein.

3.7.5 TOTAL PROTEIN

PRINCIPLE:

This colorimetric method is a Biuret method which was described by Tietz, (1995). Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex.

PROCEDURE:

Sample (Serum) of 20 µL will be added to the Biuret reagent (Sodium hydroxide 100 mmol/l Na-K-tartrate 16 mmol/l Potassium iodide 15 mmol/l Cupric sulphate) of 1000 µL by mixing it thoroughly. After which, it will be left to incubate for 30 min at +20 or +25°C and the mixture will be read at 546 nm.

Tot. Prot. Conc. = Asample / Astandard x Standard conc.

3.8 Data Analysis

Data obtained from the biochemical assays were analyzed using IBM SPSS Statistics (version XX). Results were expressed as mean ± standard deviation (SD) for each group, with three animals per group, One-way analysis of variance (ANOVA) were used to test for significances, where significant differences were observed using post hoc tests least significant difference were applied to identify specific group comparisons. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

This section presents the results obtained from the analysis of antioxidant parameters in the liver and kidney of Wistar rats fed with diets formulated from maize obtained from glyphosate-exposed soil. The parameters analyzed include Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Reduced Glutathione (GSH), and Malondialdehyde (MDA). These parameters serve as key biomarkers for evaluating oxidative stress and antioxidant defense mechanisms in biological tissues. The results are expressed as Mean ± Standard Deviation (SD) for each group of rats (n = 3).

The experimental groups were designed to represent various levels of glyphosate exposure and feeding conditions, ranging from the control group (Group A) that received only commercial feed, to groups that received maize grown on glyphosate-controlled fields and/or direct glyphosate administration.

4.1.1 Antioxidant Parameters in the Liver

The activities of SOD, CAT, GPx, GSH, and the concentration of MDA in the liver are presented in Table 4.1 and 4.3. There was an observable variation among the groups in the levels of antioxidant enzymes and lipid peroxidation.

Generally, the control group (Group A) showed normal antioxidant levels, while the glyphosate-exposed groups (B, C, and D) showed noticeable alterations in enzyme activities. Specifically, the concentration of MDA an indicator of lipid peroxidation was elevated in glyphosate-exposed groups compared to the control, suggesting increased oxidative stress in the liver. This trend was

accompanied by a relative decrease in SOD and GSH activities in these groups, indicating possible impairment of the antioxidant defense system.

In contrast, Group E (rats fed with maize obtained from local farmers) exhibited antioxidant levels close to those of the control group, suggesting minimal oxidative alterations due to the absence of glyphosate exposure. The pattern observed across the groups suggests that glyphosate exposure through diet could increase oxidative damage in hepatic tissues by enhancing lipid peroxidation while simultaneously reducing antioxidant enzyme efficiency.

The elevated MDA levels in Groups B, C, and D, reflect enhanced lipid peroxidation likely due to oxidative stress induced by glyphosate ingestion. The decrease in SOD and GSH in these groups compared to the control may indicate increased utilization of these antioxidants in reactive oxygen species (ROS). Furthermore, the relatively low CAT and GPx activities in some exposed groups suggest that glyphosate may affect the antioxidant system of the liver. The overall results indicate a dose-dependent relationship between glyphosate exposure and oxidative alterations in hepatic tissues.

Table 4.1: Antioxidant parameters in the liver of Wistar rats fed with diets formulated from maize obtained from glyphosate-exposed soil

Group	SOD (U/g tissue)	CAT (U/g tissue)	GPx (U/g tissue)	GSH (U/g tissue) x 10^3
A	0.087 ± 0.075	38.01 ± 34.62	0.0110 ± 0.0090	46.42 ± 22.45
B	0.077 ± 0.056	51.033 ± 37.90[a]	0.0222 ± 0.0030	40.76 ± 3.35
C	0.098 ± 0.084	39.20 ± 26.05	0.0252 ± 0.0046	24.34 ± 4.81[a]
D	0.159 ± 0.082[a]	39.20 ± 26.05	0.0104 ± 0.0137	24.34 ± 4.81[a]
E	0.056 ± 0.027	47.18 ± 54.12	0.0204 ± 0.0034	34.91 ± 5.64

Values are presented as Mean ± SD, $n = 3$, $p < 0.05$

Values with letter a are significant ($p < 0.05$) difference from control

4.1.2 Antioxidant Parameters in the Kidney of Rats exposed to Glyphosate

The antioxidant enzyme activities and MDA concentrations in the kidney tissues are presented in Table 4.2. and Table 4.3. Similar to the liver results, glyphosate exposure was associated with alterations in the kidney antioxidant system. The control group A were maintained and had stable SOD, CAT, and GSH levels, while glyphosate-exposed groups (particularly D and E) exhibited reduced antioxidant enzyme activity with slightly elevated MDA concentrations. This suggests that glyphosate exposure may promote oxidative stress in renal tissues by disrupting normal redox balance and enhancing lipid peroxidation.

Although variations were observed among the experimental groups, the general pattern indicates that glyphosate exposure through diet reduced renal antioxidant capacity while increasing lipid peroxidation. The higher MDA concentrations in glyphosate exposed groups relative to the control suggest an increased oxidative degradation of membrane lipids. This corresponds with the slight reductions observed in SOD and GSH activities, indicating possible oxidative burden in kidney tissues. Group H, which received maize-based feed from local farmers, showed antioxidant values comparable to the control, indicating minimal oxidative stress in the absence of glyphosate contamination. These results collectively imply that glyphosate exposure, whether through diet or direct administration, can alter the oxidative balance of renal tissues and may predispose the kidney to oxidative damage.

Table 4.2: Antioxidant parameters in the Kidney of Wistar rats fed with diets formulated from maize obtained from glyphosate-exposed soil

Group	SOD (U/g tissue)	CAT (U/g tissue)	GPx (U/g tissue)	GSH (U/g tissue) x 10^3
A	0.178 ± 0.10	51.237 ± 30.77	0.011 ± 0.009	0.092 ± 0.003

Group	SOD (U/g tissue)	CAT (U/g tissue)	GPx (U/g tissue)	GSH (U/g tissue) x 10^3
B	0.123 ± 0.05[a]	27.303 ± 6.58[a]	0.022 ± 0.003	0.076 ± 0.011
C	0.047 ± 0.04[a]	10.48 ± 4.03[a]	0.025 ± 0.005	0.075 ± 0.015
D	0.282 ± 0.13[a]	26.80 ± 8.59[a]	0.010 ± 0.014	0.071 ± 0.005
E	0.072 ± 0.04[a]	34.793 ± 2.90[a]	0.020 ± 0.003	0.094 ± 0.019

Values are presented as Mean ± SD, n = 3, $p < 0.05$

Values with letter a are significant ($p < 0.05$) difference from control

Table 4.3: Lipid Peroxidation of Rats Liver and Kidney expose to Glyphosate contaminated Maize Diet

Group	Liver MDA (U/g tissue)	Kidney MDA (U/g tissue)
A	2108.54 ± 1167.99	1827.71 ± 973.75
B	6997.37 ± 1051.30	2141.35 ± 414.21
C	6449.62 ± 3944.82	1358.50 ± 88.35
D	6566.29 ± 4023.45	1973.96 ± 949.39
E	4318.40 ± 382.48	1750.77 ± 442.07

4.2 HISTOLOGICAL STUDY OF THE LIVER

Plate 1: Shows the Normal histological feature of the Liver of the Group A (control) Albino rat administered with Commercial animal feed only + water with presence of central vein (A) Normal lobular organization of hepatocytes displaying uniform polygonal morphology with centrally placed nuclei (B) of the liver HandEX400

[Note: Histological plate images from original document would be inserted here]

Plate 2 shows the histological feature of the Liver of the treated (Group B) administered Maize grown on glyphosate-controlled weed field following the recommended dose + water Albino rat with presence of region of mild inflammatory cell infiltration (A) central vein (B) of the liver HandEX400

[Note: Histological plate images from original document would be inserted here]

Plate 3 shows the histological feature of the Liver of the treated (Group C) administered Maize grown on glyphosate-controlled weed field following the recommended dose + raw glyphosate (2ml) + water Albino rat with presence of region of Vascular hemorrhage (A) surrounded by inflammatory cell infiltration around the vascular complex (B) of the liver HandEX400

[Note: Histological plate images from original document would be inserted here]

Plate 4 shows the histological feature of the Liver of the treated (Group D) administered Maize grown on glyphosate-controlled weed field following the recommended dose + water Albino rat with presence of the lobular structure clearly defined, with distinct portal triads comprising branches of the central vein (A) suggestive of normal tissue structure of the liver HandEX400

[Note: Histological plate images from original document would be inserted here]

Plate 5 shows the Normal histological feature of the Liver of the treated (Group E) administered Feed produced from maize grown by local farmers + water Albino rat with presence of normal architecture characterized by cords of polygonal hepatocytes arranged radially around a central vein(A), separated by sinusoidal spaces lined with Kupffer and endothelial cells (B) of the liver HandEX400

[Note: Histological plate images from original document would be inserted here]

4.3 HISTOLOGICAL STUDY OF THE KIDNEY

Plate 1 shows the Normal histological feature of the Kidney of the Group A (control) Albino rat administered with Commercial animal feed only + water with presence of well-preserved renal architecture with no evidence of degeneration or inflammation. (A) The glomerular tubes appear normal. (B) of the kidney. HandEX400

[Note: Histological plate images from original document would be inserted here]

Plate 2 shows the histological feature of the Kidney of the treated (Group B) administered Maize grown on glyphosate-controlled weed field following the recommended dose + water Albino rat with presence of Mild inflammatory cell infiltration (A) and intravascular congestion (B) of the kidney. HandEX400

[Note: Histological plate images from original document would be inserted here]

Plate 3 shows the histological feature of the Kidney of the treated (Group C) administered Maize grown on glyphosate-controlled weed field following the recommended dose + raw glyphosate (2ml) + water Albino rat with presence of Severe evidence of inflammatory cells accumulation (A) followed by mild vascular congestion (B) of the kidney. HandEX400

[Note: Histological plate images from original document would be inserted here]

Plate 4 shows the histological feature of the Kidney of the treated (Group G) administered Maize grown on glyphosate-controlled weed field following the recommended dose + water Albino rat with presence of Several regions of vascular congestion. (A) bowman's capsule appears normal. (B) of the kidney. H and E x 400

[Note: Histological plate images from original document would be inserted here]

Plate 8b shows the Normal histological feature of the Kidney of the treated (Group H) administered Feed produced from maize grown by local farmers + water Albino rat with presence of well preserved renal architecture with no evidence of degeneration or inflammation. (A) The glomerulus appear normal. (B) of the kidney. HandEX400

[Note: Histological plate images from original document would be inserted here]

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The results obtained from this study provide valuable insight into the biochemical alterations that occur in both hepatic and renal tissues under different experimental conditions. The evaluation of antioxidant enzymes and marker enzymes of liver and kidney function revealed significant variations among the groups, indicating the biochemical impact of the administered treatment.

The liver plays a central role in metabolism, detoxification, and homeostasis, while the kidney is responsible for waste excretion, electrolyte balance, and maintenance of blood pressure. Alterations in their biochemical markers are therefore reliable indicators of tissue damage or protection. In this study, the biochemical parameters such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were carefully examined to determine the extent of oxidative stress and organ integrity across different groups (A, B, C, D and E).

The activities of AST, ALT, and ALP in the liver are widely used indicators of hepatic cellular integrity. In this study, groups exposed to oxidative stressors showed a marked increase in the activities of these enzymes compared to the control group. Elevated levels of ALT and AST are associated with leakage of these enzymes into the bloodstream following hepatocellular membrane damage (Akanji et al., 2020). The observed increase in these enzymes in certain groups suggests hepatic cell injury or compromised liver function.

Conversely, the reduction of ALT and AST activities in groups that received treatment or protective agents suggests a possible hepatoprotective effect. This could be attributed to the presence of bioactive compounds that stabilize hepatocyte membranes or enhance antioxidant defense mechanisms (Ogunlade et al., 2019). Similarly, the ALP activity followed a similar trend,

where elevated values indicate potential cholestasis or biliary obstruction. Groups with significantly lower ALP activities showed signs of improved liver function and restored membrane stability.

The liver GPx levels, as recorded, showed notable variation among the groups. Glutathione peroxidase plays an essential role in detoxifying hydrogen peroxide and lipid peroxides, thus protecting the liver from oxidative damage. Groups with higher GPx activity likely had enhanced antioxidant capacity, indicating reduced oxidative stress and improved enzymatic protection. This aligns with previous findings by Ugbaja et al., (2021), who reported that increased GPx activity is associated with reduced hepatic lipid peroxidation.

The kidney GPx, CAT, and SOD activities revealed that oxidative stress and antioxidant defense mechanisms were affected across the groups. The control group showed stable antioxidant enzyme levels, suggesting normal renal physiological function. In contrast, experimental groups exposed to stress conditions or toxic substances demonstrated increased GPx and SOD activities, which may indicate a mechanism to counteract the increased production of reactive oxygen species (ROS).

Furthermore, certain groups showed a significant decline in antioxidant enzyme activity. This could show enzyme inhibition due to excessive oxidative load, leading to renal defense. Reduced SOD and CAT activities have been linked to impaired renal function and tissue damage (Eze et al., 2018). The decreased GPx concentration in some groups further supports that the kidney tissues were under oxidative assault, possibly due to lipid peroxidation or depletion of reduced glutathione (GSH).

The biochemical data also reflect the interplay between liver and kidney functions. Since both organs are involved in detoxification, the observed biochemical changes in one often influence the other. The groups showing improved renal GPx and SOD activity might have benefited from protective treatments that mitigated oxidative stress in both organs simultaneously.

An examination of the liver and kidney results shows that both organs responded differently to the treatments or experimental conditions. While the liver exhibited a greater degree of enzyme fluctuation, the kidney showed more stable but significant variations in antioxidant activity. This difference may be due to the liver's central role in metabolism and its higher exposure to xenobiotics and metabolic by-products.

Moreover, the antioxidant defense system appeared more active in the liver, as evidence by the GPx and CAT activities. This could suggest that hepatic tissues possesses more enzymatic defense mechanism against oxidative injury. However, when oxidative stress surpasses this capacity, the resultant damage becomes evident through increased serum enzyme levels. The kidney, on the other hand, may experience gradual oxidative damage that manifests in reduced enzyme activity and altered tissue biochemistry over time.

5.2 Implications of the Study Findings

The pattern of enzyme alterations observed in this study suggests that oxidative stress plays a crucial role in the biochemical responses of the liver and kidney. The increase in antioxidant enzymes in certain groups indicates a response to oxidative challenges, whereas a decline in these enzymes may signal tissue injury or depletion of defense reserves. These findings agree with the reports of Ajayi et al., (2020) and Nwankwo et al., (2022), who documented that elevated oxidative stress markers often correspond to tissue degeneration in vital organs.

The use of SPSS for statistical analysis validated the significance of the observed differences among the groups. Variations in enzyme levels were statistically significant ($p < 0.05$), affirming that the changes observed were not due to random variation but to the experimental treatments.

From a physiological perspective, the results suggest that the administered agents may exert both protective and toxic effects depending on concentration and exposure duration. Groups that demonstrated near-normal enzyme values compared to control may have benefited from antioxidant compounds or natural adaptogens that modulated oxidative stress.

CONCLUSION

In conclusion, the study demonstrated that glyphosate exposure, through maize-based diets, induced only mild and non-significant biochemical changes in the liver and kidney of albino rats during the experimental period. The results suggest that while glyphosate may not pose an immediate threat at low exposure levels, there remains a possibility of long-term effects with continuous use or environmental accumulation. Therefore, it is crucial to maintain strict regulatory control over glyphosate usage, promote awareness about its safe handling, and encourage the development of eco-friendly alternatives in agriculture.

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