EFFECT OF BISPHENOL A IN THE HIPPOCAMPUS OF WISTAR RATS.

BY

IDUOROBO JERRY LUCKY

BMS2001020

SUPERVISOR: MRS UFUOMA ORHERUATA

DEPARTMENT OF ANATOMY,

SCHOOL OF BASIC MEDICAL SCIENCES,

COLLEGE OF MEDICAL SCIENCES,

UNIVERSITY OF BENIN,

BENIN CITY, NIGERIA.

JANUARY, 2025

ACKNOWLEDGEMENT

My foremost gratitude goes to God, for his immense love and guidance in this walk of life. With a grateful heart I appreciate my parents Mr & Mrs Iduorobo for their love, financial, moral and parental support throughout my stay in the university.

I am profoundly grateful to my supervisor Mrs Ufuoma Orheruata for not stressing me financially and psychologically, to my colleagues; Joy, Amelia, Elijah, Happiness, Uche and Joshua for your collective support and ideas.

I wish to express my gratitude to my siblings, in-laws and friends who have in one way or the other made a sticking impact in my life, words can't express how grateful I am to you all for their prayers and words of encouragement to me.

CERTIFICATION

This is to certify that this research work titled "**EFFECT OF BISPHENOL A IN THE HIPPOCAMPUS OF WISTAR RATS"** for the award of a degree of Bachelor of Science (B.Sc) in Anatomy was carried out by **IDUOROBO JERRY LUCKY** , under the supervision of **MRS UFUOMA ORHERUATA**. All literatures used in this study have been acknowledged and properly referenced.

MRS UFUOMA ORHERUATA **(Supervisor)**	**DATE**
DR. A.B ENOGIERU **(Head of Department)**	**DATE**
EXTERNAL EXAMINER	**DATE**

Table of Contents

ABSTRACT

Bisphenol A (BPA) is a widely used synthetic chemical found in food packaging, medical devices, and other products.BPA has been shown to cross the blood-brain barrier, alter brain structure, and impair learning and memory, even at low doses.The aim of this study investigated the effects of BPA on the hippocampus of Wistar rats. The rats were divided into three groups,group A which is the (control)was given 1ml distilled water,Group B was administered 5mg/kg BPA,Group C was administered 10mg/kg BPA for 28 days.At the end of the 28-day administration period, the rats were weighed, sacrificed via cervical dislocation, and their skulls opened to harvest the brain. The brain weights was taken and the hippocampus was carefully detached, placed in a sample bottle, fixed in 10% buffered formalin and were processed according to the method of Drury and Wallington (1980) for Hematoxylin and Eosin staining and the parameters accessed include hippocampal antioxidant enzymes (SOD, CAT, GPx and GSH), MDA concentration and the histology of the hippocampus using Haematoxylin and Eosin staining technique. Data was analyzed using SPSS/IBM statistical package version 20. Results obtained showed no significant change ($p<0.05$) in the initial body weight, final body weight and weight change of rats across experimental groups. No significant change ($p<0.05$) was observed in the cerebral and relative cerebral weight of rats across experimental groups. However, a significant decrease ($p<0.05$) was observed in hippocampus SOD, CAT, GPx and GSH activity of rats in group C (10 mg/kg bw BPA) when compared to control. A significant increase ($p<0.05$) was observed in MDA concentration of rats in group B (5 mg/kg bw BPA) and C (10 mg/kg bw BPA) when compared to control. Histological findings revealed normal archictecture of the hippocampus in group A, whereas dark shrunken neuronal cell bodies with deeply stained pyknotic nuclei and vacoulations were seen in the granular cells of rats in group B and C. In conclusion, findings from this study shows that BPA induced neurotoxic effect on the hippocampus via inducing oxidative stress and altering the architectural integrity of the hippocampus.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Bisphenol A (BPA) is a monomer that was first developed as a synthetic estrogen in the 1890s and was reported to have the efficacy of estrone in stimulating the female reproductive system in rats in the 1930s (Dodds *et al*, 1936). Subsequently, BPA has been used in many consumer products, including plastics (as a polymer, i.e. polycarbonate plastic), PVC, food packaging, dental sealants, and thermal receipts. Humans are exposed to BPA through their diet, inhalation of household dust, and dermal exposure (Vandenberg *et al.,* 2012). A total of 2.8 million metric tons of BPA was produced in 2002, and an estimated 5.5 million metric tons was produced in 2011 (kendal *et al.,* 2011). BPA is a known endocrine disruptor; it has been found to bind to estrogen receptors and have estrogenic effects in laboratory studies. Although BPA has been found to have a lower affinity for nuclear estrogen receptors relative to 17-beta estradiol (E2), its estrogenic potency is equal to E2 for responses mediated by non-nuclear estrogen receptors (Vinas *et al.,* 2012). Further, BPA can act as an antiestrogen, blocking the estrogenic response by competing with endogenous layer. BPA can also directly bind to androgen receptors, and is possibly antiandrogenic, blocking endogenous androgen action (Dudly *et al.,* 2015). BPA has been shown to bind to thyroid receptors, and have both agonistic and antagonistic effects on thyroid function (Rochester *et al.,* 2015). BPA interacts with other organs and physiological systems as well, including the developing central nervous system,pancreas and the immune system. Determining which of the different molecular mechanisms mediate the effects of BPA on different aspects of human health is the goal of a considerable number of research (Vandenberg *et al.,* 2012).

One of the human body's most intricate systems, the nervous system interacts with other bodily systems to preserve physiologic equilibrium. The postnatal function of the human brain is contingent upon appropriate embryologic and fetal development, making it a delicate and intricate organ(Rutten et al,20012). Changes in the development of the embryo can result in structural or functional disorders that have long-term effects on

neurologic function. These changes might be caused by environmental factor, genetic factors, or maternal health impacts (Carlson,2014). The nervous system has an impact on all facets of bodily health, such as: the neurological system's ability to function affects thoughts, memory, learning, and emotions (Squire *et al.,* 2020), regulation and coordination of movement, balance (Ito, 2013) as well as sensory functions (Purves *et al.,* 2018), It regulates sleeping and aging processes (Nir *et al.*, 2010). It is activated in response to stressful situations (McEwen *et al.,* 2011). The nervous system is made up of two major structures: the brain and spinal cord. Together, these structures combine information from the sensory organs and peripheral nervous system to produce output signals that are sent to the autonomic nervous system, which controls the body's internal organs, and the skeletal muscles, enabling both voluntary and involuntary movement (Waxman, 2017). Because the central nervous system (CNS) is the master control center of the body, aging's effects on the CNS are widespread and profoundly affect almost every physical function, including daily functioning. Spinal cord and brain have specific subfunctions within the central nervous system (CNS) (Springer,2019).

1.2 AIM

The aim of this study is to evaluate the effects of bisphenol A in the hippocampus of Wistars rats.

1.3 SPECIFIC OBJECTIVES

The following are the precise goals of this study:

i. The body and brain weight in rats treated with or without Bisphenol A.

ii. BPA exposure was found to alter the activity of antioxidants enzyme(CAT,GPx and SOD) in the hippocampus of rats.

iii.BPA exposure was found to affect lipid approximation as measured by MDA concentration in the hippocampus of rats.

iv.Histology of the hippocampus of rats treated with or without Bisphenol A.

1.4 SIGNIFICANCE OF THE STUDY

The work is significant because it focuses on neurotoxicity caused by bisphenol A, a known environmental toxin. In recent years in the scientific community, the harmful effect of a phenolic type of environmental toxicant, known as bisphenol A (BPA), has achieved great relevance (Richter *et al*,2009). The interest in this compound is increasing owing to its possible adverse effects on several organs, which has led several organisations to recommend the prohibition or reduced usage. BPA has also aroused interest in the nephrological community, as it has been linked to kidney and endocrine disorders. Since BPA is cleared by the kidneys, plasma and tissue levels of BPA are markedly increased in patients with impaired renal function.

1.5 EXPECTED CONTRIBUTION TO KNOWLEDGE

The study will provide additional information on the possible toxic effects of bisphenol A in the hippocampus.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION TO BISPHENOL A

Bisphenol A (BPA) is an organic compound that has garnered significant attention due to its widespread use and potential health effects (Vandenberg *et al*., 2019). BPA was first synthesized in photonic materials by the Russian chemist Aleksandr Dianin (Rubin, 2021). However, it wasn't until the 1950s that it began to be used commercially in the production of polycarbonate plastics and epoxy resins (Staples *et al*., 2011). Its widespread application in consumer products emerged during this time, leading to its significant presence in various industries today,BPA consists of two phenolic groups connected by a carbon bridge. Its structure allows it to mimic natural hormones, particularly estrogen, which is a critical factor in its biological effects (Richter *et al*., 2017). The chemical properties of Bisphenol A include the chemical formula $C_{15}H_{16}O_2$, a molecular weight of 228.24 g/mol, and an IUPAC name of 4,4'-(propane-2,2-diyl) diphenol (Rochester, 2023). The presence of hydroxyl groups (-OH) contributes to its reactivity and ability to form polymers.

Regarding its physical properties, BPA usually appears as a white crystalline powder, has a melting point of 158-159 °C, a boiling point of 300 °C, and is soluble in organic solvents like ethanol and acetone but poorly soluble in water (Rochester and Bolden, 2017). However, concerns regarding its potential health effects have surged in recent decades, leading to increased regulatory scrutiny and consumer awareness (Gould, 2016).

BPA is primarily used in the production of:

- **Polycarbonate Plastics**: Found in items like water bottles, food containers, and safety

equipment (Staples *et al*., 2019).

- **Epoxy Resins**: Used in coatings for food cans, dental sealants, and adhesives (Vandenberg *et al*., 2017).
- **Thermal Paper**: Commonly used in receipts and labels (Biedermann *et al*., 2020).

2.1.1 DESCRIPTION OF BISPHENOL A

Bisphenol A (BPA) is a colorless, crystalline solid at room temperature, typically appearing as white to light yellow granules or powder (Rochester, 2013). It has a relatively high melting point of approximately 160°C (320°F) and is soluble in organic solvents such as ethanol and acetone but has limited solubility in water (Gencha and Seth, 2019). The distinct chemical structure of BPA features two phenolic groups connected by a propane bridge, which contributes to its stability and ability to interact with biological systems, particularly as an endocrine disruptor (Rubin, 2021).

The representation of Bisphenol A (BPA) can be depicted in various forms, including its molecular formula ($C_{15}H_{16}O_2$), structural formula, and 2D representation, which visually illustrates the arrangement of atoms within the molecule (Vandenberg *et al*., 2017). The structural formula highlights the two hydroxyl (-OH) functional groups, which are crucial for BPA's reactivity and biological interactions (Rochester and Bolden, 2015).

2.1.3 CHEMICAL PROPERTIES OF BISPHINOL A

Bisphenol A (BPA) is an industrial chemical that has been widely used since the 1950s in the production of polycarbonate plastics and epoxy resins (Vandenberg *et al*., 2017). It is a synthetic compound characterized by its phenolic and methylene structural features. Chemically, BPA consists of two hydroxyphenyl groups (phenol moieties) connected by a propane bridge. Its IUPAC name, 4,4'-isopropylidenediphenol, reflects the central

isopropylidene (methane) bridge linking the two phenolic rings, which contributes to its chemical stability and widespread industrial application (Rochester, 2013; Staples *et al*., 2018). These structural properties make BPA highly versatile in manufacturing but also enable it to interact with biological systems, raising concerns about its potential endocrine-disrupting effects (Rubin, 2021).

2.1.4 CHEMICAL REACTIVITY

BPA's chemical reactivity is largely influenced by its two phenolic hydroxyl groups, which can undergo various reactions typical of phenols. Esterification occurs when BPA forms esters with organic acids, such as acetic acid, through reactions involving its hydroxyl groups (Staples *et al*., 2019). Etherification involves the conversion of the phenolic groups into ethers in the presence of alkylating agents, making BPA a versatile intermediate in organic synthesis (Rochester, 2023). Polymerization is one of BPA's most important industrial applications, as it serves as a key monomer in the production of polycarbonate plastics and epoxy resins. In polycarbonate production, BPA reacts with phosgene or diphenyl carbonate to form long polymer chains, while epoxy resins are synthesized through its reaction with epichlorohydrin (Vandenberg *et al*., 2017). Although BPA is relatively stable under standard conditions, it can degrade under exposure to light and high temperatures, leading to the release of breakdown products with potential biological activity (Rubin, 2021). Additionally, BPA reacts with strong oxidizing agents and bases, which can alter its chemical structure and influence its environmental persistence and toxicity (Flint *et al*., 2012).

2.1.5 HISTORY/DISCOVERY AND EARLY DEVELOPMENT (1905-1950)

One of the most precious and ancient compounds is bisphenol A. Bisphenol A (BPA) is

an organic compound first synthesized in 1905 by the Russian chemist Aleksandr Dianin (Vandenberg *et al*., 2019). Initially, it was studied for potential use in pharmaceuticals, but its properties as a chemical building block led to its application in plastics (Rubin, 2021). In the 1930s, BPA was recognized for its ability to be polymerized with epichlorohydrin, leading to the development of polycarbonate plastics and epoxy resins (Geens *et al.*, 2021). By the 1950s, these materials gained widespread use in consumer products, including containers, coatings, and dental materials (Michałowicz, 2014).

Concerns about BPA emerged in the late 20th century, particularly regarding its endocrine-disrupting properties (Richter *et al*., 2017). Research began to link BPA exposure to health issues, prompting regulatory scrutiny (Vandenberg *et al*., 2022). Over the years, many countries have implemented restrictions on BPA in certain products, especially those intended for food and beverages (European Food Safety Authority, 2015).

The discovery of bisphenol A (BPA) in the 1930s faced several challenges:

- **Limited Analytical Techniques:** Early chemical analysis methods were not advanced enough to accurately identify and quantify BPA in various materials (Flint *et al*., 2022).
- **Industrial Applications:** Initially, BPA was developed for its industrial applications in plastics and resins, which overshadowed concerns about its safety (Rubin, 2021).
- **Lack of Awareness:** There was limited understanding of endocrine disruptors and their potential health effects, leading to insufficient regulatory scrutiny (Vandenberg *et al*., 2013).

- **Research Funding:** Early studies on BPA's biological effects struggled with funding and interest compared to other chemicals, delaying comprehensive research (Gould, 2019).
- **Public Perception:** The general public was initially unaware of BPA's presence in everyday products, which delayed consumer advocacy for safer alternatives (Vogel, 2019).
- **Global Disparities:** Different countries had varying regulations and levels of concern regarding BPA, leading to inconsistent safety standards and practices worldwide (Geens *et al.*, 2021).

2.1.6 EXPOSURE TO BISPHENOL A

BPA is an endocrine disruptor that structurally mimics estrogen, allowing it to bind to estrogen receptors in the body and interfere with natural hormone functions. It's primarily introduced to the human body through ingestion, as it can leach from food containers or cans lined with BPA-based resins(Peretz,2022). However, BPA can also be absorbed through the skin when handling thermal paper receipts or inhaled in dust particles , Once inside the body, BPA is metabolized in the liver and excreted in urine. However, studies have detected BPA in human biological samples (blood, urine, and even breast milk), indicating chronic exposure and bioaccumulation, particularly in individuals exposed to BPA regularly. BPA leaches from plastic containers and packaging materials, especially when they are heated, such as during microwaving or with hot foods (Lehmler,2022). Additionally, BPA is prevalent in certain epoxy resins, used in the lining of food and beverage cans to prevent corrosion. This widespread use means people can be exposed to BPA through food, drinks, air, and even dust (Vandenberg *et al*., 2019).

2.1.7 TOXICITY OF BISPHENOL A

Due to its widespread presence, BPA can leach into food and beverages, exposing humans to its potential health risks (Frontiers in public health 2021). Studies indicate that BPA acts as an endocrine disruptor, mimicking estrogen and interfering with hormonal processes (Springer,2019). This disruptor can negatively impact reproductive health, immune function, and neurodevelopment, with links to conditions such as infertility, hormonal imbalances, and certain cancers. Animal studies have shown that BPA exposure can affect growth, development, and immune responses, while human studies have associated BPA with increased risks of metabolic diseases and neurobehavioral issues in children. The potential dangers of BPA have led to global scrutiny, driving some countries to limit its use, particularly in food packaging and baby products. recent years, research has shifted towards exploring BPA alternatives, like bisphenol S (BPS) and bisphenol F (BPF), although these analogs may have similar toxic profiles. The National Institute of Environmental Health Sciences and other organizations are focused on understanding the long-term effects of both BPA and its alternatives to better inform public health guidelines (Richter *et al*., 2017).

2.1.8 NEUROTOXICITY OF BISPHENOL A

Neurotoxicity is any unfavorable consequence that exposure to biological, chemical, or physical agents causes on the structure or function of the nervous Bisphenol A is widely used industrial chemical, has gained attention for its potential neurotoxic effects. Research over the past two decades has indicated that BPA exposure may lead to various neurodevelopmental and neurobehavioral disorders. BPA's neurotoxicity is attributed to its endocrine-disrupting properties. BPA can mimic estrogen and bind to estrogen

receptors in the brain, potentially leading to altered neurodevelopment and behavior. Research has demonstrated that BPA can disrupt hormonal signaling pathways crucial for brain development (Yin, 2020). The hormone-like action of BPA can interfere with neurogenesis, synaptogenesis, and the organization of neural circuits. BPA exposure has been linked to increased oxidative stress and neuroinflammation, both of which can contribute to neuronal damage and cognitive deficits (Mocarelli et al. (2021). BPA may also alter or influence neurotransmitter systems, particularly those involving dopamine and serotonin, leading to behavioral changes. BPA can lead to cognitive impairments, anxiety, and hyperactivity (Kern,2017). BPA exposure during critical developmental windows has been linked to increased anxiety-like behaviors and hyperactivity in animal Patisaul and Adewale (2019). BPA has been shown to promote neuro-inflammation, a condition characterized by the activation of glial cells and the release of inflammatory cytokines. Chronic neuro-inflammation can have detrimental effects on brain health and function. The neurotoxicity of bisphenol A is a pressing public health concern, with potential implications for neurodevelopment and behavior (Patisaul,2017). Ongoing research is critical to further elucidate the mechanisms of BPA-induced neurotoxicity and to inform regulatory policies aimed at reducing exposure, particularly among vulnerable populations such as pregnant women and children (kern,2017).

It has been discovered that the compound bisphinol A causes hippocampus toxicity in a variety of experimental animals, including adult wistar rats (Aschner, 2011). The hippocampus is essential for cognitive functions such as memory formation and learning. BPA, a common component in plastics, acts as an endocrine disruptor, mimicking

estrogenic activity in the brain. This interference particularly affects the hippocampus, where BPA has been shown to impair neurogenesis (the growth of new neurons), alter dendritic spine density, and disturb key signaling pathways that support cognitive development and function (Ding *et al.,* 2015) Studies on animal models, particularly rodents, demonstrate that BPA exposure results in physical and functional alterations within hippocampal neurons. Dendritic spine density—a critical aspect of neuron connectivity and synaptic strength—has been found to decrease with BPA exposure, particularly in the hippocampal CA1 region,This reduction can impair synaptic plasticity, a process vital for learning and memory. Long-term BPA exposure leads to diminished synaptic efficacy, impacting behaviors that rely on hippocampal functions, such as spatial memory and contextual learning. (Xu *et al.,* 2010) Another significant mechanism through which BPA affects the hippocampus is through the induction of oxidative stress(Yoshihara,2020).

BPA has been shown to increase oxidative biomarkers in hippocampal cells, leading to an imbalance in redox states and contributing to neuronal damage. This oxidative stress triggers inflammatory pathways, which further damages neuronal structures and contributes to cognitive decline (Johnson *et al.,* 2018).

2.1.9 CARDIOVASCULAR TOXICTY OF BISPHENOL A

Research indicates possible connections to heart disease .In response to rising concerns, various countries and regions have taken steps to regulate or ban BPA e.g European Union restricted BPA in baby bottles and is evaluating its safety in food contact materials,united States the FDA has banned BPA in baby bottles and sippy cups and canada Recognized BPA as a toxic substance and has implemented regulations to limit its

use as awareness of BPA's potential risks has grown, several alternatives have been developed. These include: BPS (Bisphenol S) a common substitute, although concerns about its safety are also emerging,BPF (Bisphenol F) another alternative, but similar studies are needed to evaluate its safety.Non-bisphenol Alternatives Materials like polyethylene and polypropylene are being used in place of BPA-containing products(Kumar,2022).

BPA's prevalence in consumer products poses significant health and environmental concerns. Ongoing research is critical to understanding its effects, leading to informed regulatory decisions and consumer choices. As science progresses, finding safe alternatives will be essential for public health and safety (Rochester *et al,*2016).

BPA's structural similarity to estrogen allows it to bind to estrogen receptors in the body, which can interfere with normal hormonal functions (Rubin, 2021). Research has demonstrated that BPA exposure can lead to adverse health effects by disrupting the endocrine system, which regulates processes like growth, metabolism, and reproduction. Rubin (2021) reviewed studies on BPA as an endocrine disruptor, highlighting the hormone-like effects of BPA on the reproductive and developmental systems. Rubin's work laid the groundwork for further studies examining low-dose effects of BPA on the endocrine system. (Vandenberg *et al*.2019) expanded upon Rubin's work by examining over 300 studies that investigated low-dose BPA effects on various biological systems. They concluded that BPA can cause significant health effects even at levels lower than those deemed safe by regulatory agencies,Some research has found associations between BPA exposure and neurodevelopmental issues, particularly in children. BPA may impact

brain development, potentially leading to behavioral issues, cognitive impairments, and increased risk of conditions like attention deficient hyperactivity disorder (Rochester, 2013).

2.1.11 METABOLIC DISORDERS

BPA exposure is also associated with an increased risk of metabolic disorders, including obesity, diabetes, and cardiovascular disease. Research suggests that BPA may affect insulin function, lipid metabolism, and other processes critical to metabolic health. (Mustieles and Fernandez, 2020). Due to its estrogen-mimicking properties, BPA has been studied in connection with hormone-dependent cancers, such as breast and prostate cancer. Animal studies and some human epidemiological data have shown correlations, although more research is needed to establish a definitive causal link (Gencha *et al,*2018).

2.2 OVERVIEW OF THE BRAIN

The brain is an extraordinarily intricate and sophisticated organ that is in charge of almost every element of the human experience, including perception, emotion, and thought. It is made up of billions of neurons and glial cells that serve as support structures. These neurons are arranged into an intricate web of circuits and pathways that enable perception, thought, and behaviour in the environment (Sousa, 2016). The nerve systems' central command center is located in the human brain. It transmits data to the muscles after absorbing it from the body's sensory organs. It is made up of billions of glia, which are supporting cells, and more than 100 billion neurons, which are nerve cells (Purves *et al.*, 2018). Neuroglial cells are classified into multiple categories. The CNS receives support from astrocytes, which establish synapses, store glycogen, preserve the

blood brain barrier, and provide an anionic homeostatic environment for neuronal communication. Oligodendrocytes produce the myelin wrapping of nerve fibers in the CNS (Bear *et al.,* 2015). Generally speaking, there are three main, age-related alterations that are associated with the impacts of aging on the central nervous system, ranging from peripheral neurons through the brain: Pro-inflammatory aging processes are fueled by aged astrocytes (Matias *et al.,* 2019). In spinal neural circuits, there are fewer motor neurons and slower reflexes (Hunter *et al.* 2016) and neuronal loss (Singh *et al.*2017).The brain is separated into the cerebrum, cerebellum, and brainstem and is located within the skull. The brain's main region, the cerebrum, is in charge of conscious thought, voluntary movement, and sensation. The cerebellum, which sits beneath the cerebrum, is in charge of balance and coordination. According to (Kande*l et al.,* 2012), the brainstem regulates vital bodily processes like blood pressure, heart rate, and respiration in addition to serving as a link between the brain and spinal cord. The nervous system, which consists of the spinal cord and a network of nerves that carry information from the brain to various body parts, allows the brain to connect with the rest of the body. Additionally, the brain regulates behavior, emotion, and mental processes like perception, memory and learning(Kolb,2022).According to Kandel *et al.* (2012), the brain is fundamental to our capacity for thought, emotion, and interaction with the outside world. Brains have centralized physiological control over the body's organs (Carlson *et al.,* 2013). Through the production of patterns in muscle activity and the stimulation of chemical release known as hormones, they ex(ert an influence on the rest of the body (Squire *et al.,* 2012). Quick and well-coordinated reactions to environmental changes are

made possible by this centralized control.

2.2.2 ORGAN OF STUDY -- HIPPOCAMPUS

2.2.2.1 Gross Anatomy of Hippocampus

The hippocampus consists of two main parts: the dentate gyrus and the cornu ammonis (or hippocampus proper). The hippocampal sulcus divides and shapes these two sections into one structure, with the subiculum located below the sulcus. Since the hippocampus is part of the allocortex (also known as the archicortex), there is a transitional zone separating it from the neocortex. Anatomists often divide the hippocampal region into the entorhinal cortex (EC), subiculum, dentate gyrus (DG), and the hippocampus proper (cornu ammonis, CA), collectively known as the hippocampal formation (Anand and Dhikav, 2012).

The hippocampus is further divided into three main sections: the head, body, and tail. The head is the enlarged portion of the hippocampus. Histologically, the hippocampus proper is divided into regions CA1, CA2, and CA3. The subiculum, which connects the entorhinal cortex with the hippocampal area, is positioned directly across from CA1 in the ventricle. Blood supply to the hippocampal region comes from the posterior cerebral artery, which branches into anterior, middle (Fumagalli and Prior,2012).

2.2.2.2 HISTOLOGY OF THE HIPPOCAMPUS

The hippocampus is a complex structure with distinct cell layers and specialized cell types essential to its function. The dentate gyrus is made up of two primary layers: the granule cell layer and the molecular layer. The granule cell layer contains densely packed granule cells, which are primarily excitatory neurons in the dentate gyrus (Keshvari *et al.,* 2020). The molecular layer contains dendritic processes, axons, and various types of

interneurons (Mandour *et al.,* 2021). The CA1 region is composed of layered pyramidal neurons, distinguished by their large, triangular shape and prominent apical dendrites (de Flores et al., 2020; Piancino *et al.,* 2020). In contrast, the CA2 region has less distinct cellular characteristics compared to other CA regions (Piancino *et al.,* 2020).The CA3 region contains densely packed pyramidal neurons with a similar triangular shape and is notable for its recurrent collateral connections, forming an "auto-associative network" (Lamtai *et al.,* 2021). The subiculum consists of pyramidal neurons and interneurons, with pyramidal neurons functioning as the primary excitatory cells in the hippocampus, essential for information flow and connectivity with other brain regions (de Flores *et al.,* 2020; Mandour *et al.,* 2021). Additionally, the hippocampus contains various inhibitory interneurons that use gamma-aminobutyric acid (GABA) as their main neurotransmitter (Lamtai *et al.,* 2021; Rahmati *et al.,* 2022). These interneurons regulate the excitability and synchronization of hippocampal circuits, contributing to the generation of specific activity patterns (Rahmati *et al.,* 2022).

2.2.2.3 ARTERIAL SUPPLY AND VENOUS DRAINAGE OF THE HIPPOCAMPUS

The hippocampus receives its arterial supply from branches of the posterior cerebral artery (PCA) and the anterior choroidal artery (AChA) (Genha *et al*., 2015; Liu *et al.,* 2015). The PCA arises from the basilar artery and divides into several branches, including the posterior hippocampal artery (PHA) and the anterior hippocampal artery (AHA) (Anyachor *et al.,* 2020).

Posterior Hippocampal Artery (PHA)

The PHA is the primary arterial supply to the posterior hippocampus, providing oxygenated blood to the dentate gyrus, CA1, and subiculum regions (Liu *et al.,* 2015). Studies have shown that PHA occlusion can lead to hippocampal damage and cognitive impairment (Heiss *et al.,* 2011).

Anterior Hippocampal Artery (AHA)

The AChA contributes to the arterial supply of the hippocampus, particularly the lateral and posterior segments (Keshvari *et al.,* 2020). AChA occlusion has been linked to hippocampal atrophy and cognitive decline (Klijn *et al.,* 2010).

The hippocampus drains venous blood through several pathways, including:

1. Internal Cerebral Veins (ICVs): The ICVs receive venous blood from the hippocampus and merge to form the Great Cerebral Vein of Galen (GCVG) (Keshvari *et al.,* 2020).
2. Basal Veins of Rosenthal: These veins drain the posterior hippocampus and join the Great Cerebral Vein of Galen (GCVG) (Liu *et al.,* 2015).
3. Transverse Sinus: The transverse sinus receives venous blood from the hippocampus via the Lateral Mesencephalic Vein (LMV) (Liu *et al.,* 2015).

Meanwhile the deep venous system, comprising the ICVs and GCVG, plays a crucial role in hippocampal venous drainage (Keshvari *et al.,* 2020). Occlusion of the ICVs can lead to hippocampal edema and cognitive impairment (Liu *et al.,* 2015). And the superficial venous system, including the basal veins of Rosenthal, drains the posterior hippocampus and is vulnerable to thrombosis (Anyachor *et al.,* 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 ANIMALS AND MANAGEMENT

Eighteen (18) adult wistar rats were used in this study. The rats were bred in the animal house of the department of Anatomy, University of Benin, Benin city, where they were kept in plastic cages under room temperature and were fed daily with Grower's mash (manufactured by Premier feed Mills Co LTD, a subsidiary of Flour Mills of Nigeria Plc) and water. They were weighed daily throughout the duration of the experiment using a digital weighing scale calibrated in gram and recorded to the nearest whole number. Protocols for this experiment were in accordance with the guide for care and use of laboratory animals.

3.2 EXPERIMENTAL DESIGN

In this study, eighteen (18) rats were assigned into three (3) groups A, B, C each containing six (6) rats, after acclimatization for 2 weeks with free access to feed and water. The administration of toxicant was then carried out for 28 days.

GROUPS	DOSAGE
GROUP A	Control group
GROUP B	5 mg/kg of Bisphenol A (low dose)
GROUP C	10 mg/kg of Bisphenol A (high dose)

All administration via oral route using an orogastric tube and lasted for 28 days

3.3 SACRIFICE OF ANIMALS AND SAMPLE COLLECTION

The rats were weighed at the beginning, during (on a daily basis) and at the end of the study using a weighing balance. They were sacrificed by cervical dislocation, the skulls were opened, brain organs harvested and blotted free of blood for morphometry which involved taking brain weight using digital weighing scale calibrated in grams. The harvested brain organs were fixed in formal saline and tissues processed for light microscopic examination.

3.4 OXIDATIVE STRESS

The harvested and weighed brains were homogenized with acid-washed sand and PBS in a porcelain mortar and pestle after being washed twice in cold phosphate-buffered saline (PBS). The homogenate was then centrifuged at 10,000 g for 15 minutes at 4 °C, and the supernatant was collected to estimate the results of several biochemical experiments.

3.4.1 Estimation of Catalase (CAT) activity

This was determined by the method of Cohen et al. (1970). Catalase is found in nearly all animal, plant, and bacterial cells, where it serves to prevent the accumulation of harmful hydrogen peroxide (H_2O_2), converting it into oxygen (O_2) and water (H_2O). For the preparation of reagents, a 0.01 M solution of potassium permanganate ($KMnO_4$) was made by dissolving 0.158 g of $KMnO_4$ in 100 mL of distilled water. A phosphate buffer at pH 7.4 was created by weighing and dissolving 0.426 g of sodium phosphate ($NaHPO_4$ and NaH_2PO_4) in 100 mL of distilled water. A 6 M sulfuric acid (H_2SO_4) solution was prepared by adding 32.3 mL of concentrated H_2SO_4 to 66.7 mL of distilled water. A 30 mM hydrogen peroxide (H_2O_2) solution was made by measuring 0.34 mL of 30% H_2O_2 and diluting it in 1001 mL of phosphate buffer. In the procedure, 5.0 mL of H_2O_2 was

added to a known volume of plasma (0.5 mL). This mixture was inverted to ensure thorough mixing and allowed to stand for 30 minutes. The reaction was then halted by adding 6 M H_2SO_4. Absorbance was measured at 480 nm within 30–60 seconds against distilled water.

Activity = [OD / × min × Vt] / [M × V × L ×Y]

OD= absorbance

L= light path =1cm

V_t = total volume of the reaction sample

M= molar extinction co-efficient of H_2O_2 (40/M/cm)

3.4.2 Estimation of Malondialdehyde (MDA) activity

Malondialdehyde (MDA) was determined using the thiobarbituric acid assay developed by Buege and Aust (1978). MDA, a product of lipid peroxidation, reacts with thiobarbituric acid to form a red chromophore. For the preparation of the reagent, a stock solution of trichloroacetic acid (TCA), thiobarbituric acid (TBA), and hydrochloric acid (HCl) was made by mixing 15 g of TCA, 0.375 g of TBA, and 0.25 N HCl. This solution was gently heated to facilitate the dissolution of the thiobarbituric acid. In the procedure, 1.0 mL of plasma was added to 2.0 mL of the TCA-TBA-HCl solution and mixed thoroughly. The mixture was then heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuging at 1000 g for 10 minutes. The absorbance was measured at 535 nm against a blank. The concentration of MDA was calculated using the appropriate formula:

MDA (unit/mg protein) = [A × Vt × 1000] / [M × V × l × Y]

A = absorbance of sample test at 535nm

Vt= total volume of the reaction = 3ml

M = molar extinction co-efficient of product= 1.56 × 105m-1cm-1 l = light path= 1cm

V= volume of tissue extract used = 1ml

Y = mg tissue in the volume of sample used

3.4.3 Estimation of Glutathione Peroxidase (GPx) activity

This was determined using the method established by Nyman (1959), which relies on the oxidation of pyrogallol to purpurogallin through peroxidase activity, resulting in a deep brown color that was measured at 430 nm. For reagent preparation, a 20 mM solution of pyrogallol was made by dissolving 0.2552 g of pyrogallol in 100 mL of distilled water. In the procedure, an aliquot of plasma (0.2 mL) was combined with 2.5 mL of phosphate buffer, 2.5 mL of hydrogen peroxide (H_2O_2), 1.5 mL of distilled water, and 2.5 mL of pyrogallol. The reaction mixture was allowed to stand for 30 minutes at room temperature, resulting in the formation of a deep brown color, which was then read at 420 nm.

$$\text{Activity} = [OD/Min \times VtDf] / [E \times Vs \times Y]$$

OD = Absorbance of test

Vt = Total volume of reaction of reaction mixture

Df = Dilution factor = 1

E = Molar extinction coefficient (12/M/cm)

Vs= volume of sample

Y = mg of protein used

3.4.4 Estimation of Superoxide Dismutase (SOD)

This was determined following the method of Misra and Fridovich (1972). Adrenaline rapidly undergoes autoxidation to form adrenochrome, which can be measured at 420 nm using a spectrophotometer. The autoxidation process depends on the presence of superoxide anions. Superoxide dismutase (SOD) inhibits this reaction by catalyzing the breakdown of superoxide anions. The degree of inhibition indicates the activity of SOD, which is measured at 420 nm.

Carbonate buffer(0.05M, Ph 10.2) prepared by dissolving 0.2014 g of Na_2CO_3, 0.2604 g of $NaHCO_3$, and 0.0372 g of EDTA in 100 mL of distilled water.

Hydrochloric acid (0.005)prepared by adding 0.044 mL of concentrated HCl to 99.96 mL of distilled water.

Adrenalin solution (0.3Mm)prepared by dissolving 0.01098 g of adrenaline in 100 mL of 0.005 M HCl. A plasma volume of 0.2 mL was mixed with 2.5 mL of carbonate buffer and 0.3 mL of adrenaline solution. For the reference sample, 0.2 mL of distilled water was mixed with 2.5 mL of carbonate buffer and 0.3 mL of adrenaline. The mixtures were thoroughly combined, and the absorbance was read at 420 nm.

% inhibition = [(O.D test – O.D ref) x 100] / O.D test

Enzyme activity was calculated thus:

SOD activity (Unit/ mg protein) = [% inhibition] / 50 x Y

Where Y = mg of protein in the volume of sample used.

3.5 HISTOLOGICAL PROCEDURE

3.5.1 PARAFFIN TISSUE PROCESSING OF DRURY AND WALLINGTON (1980).

Following fixation, the tissues were processed as follows:

Dehydrate in ascending grades of ethanol: 70 % ethanol, 90 % ethanol and 100 % (absolute) ethanol for one hour each.

Dehydrated tissues were cleared in three changes of xylene for 60 minutes each.

Infiltration of tissues was done in three changes of paraffin wax at 60ºC for 60 minutes each.

Paraffin wax was used in embedding the tissue. The paraffin blocked tissues were trimmed and mounted on wooden block for a rotary microtome.

3.5.2 HAEMATOXYLIN AND EOSIN STAINING METHOD OF DRURY AND WALLINGTON (1980).

Sections were dewaxed in Xylene

Sections were rehydrated in decreasing grades of alcohol (100 %, 90 %, and 70 %) and then transferred to water.

Staining of sections was carried out in Iron Haematoxylin for 10-15 minutes.

Excess stain was removed by washing under tap water.

Differentiated in 1% acid alcohol for 10 seconds.

Blued in running tap water for 5minutes.

Sections were counter-stained in 1% Eosin for 5-10minutes.

Sections were rinsed in water.

Dehydrated in ascending grades of alcohol (50% to absolute alcohol).

Cleared in Xylene and mounted in DPX (Distrene Plasticizer and Xylene).

Result: Nuclei stained blue while Cytoplasm stained pink or red.

3.6 PHOTOMICROGRAPHY

A Leica DM750 research microscope with attached digital camera was used to examine the sections. Photomicrograph of the tissue sections were taken at different magnifications.

3.7 STATISTICAL ANALYSIS

In this study, all values were presented as mean ± standard error of the mean for all groups. The significance of differences in the means of all parameters was determined using one way analysis of variance (ANOVA). All statistical analysis were carried out using statistical package for social sciences (SPSS) product of the International Business Machine Corporation (IBM) in Armonk, New York.

CHAPTER FOUR

RESULTS

4.1 WEIGHT RESULTS

Figure 4.1- 4.5 Shows the initial body weight, Final body weight, Weight change, Brain weight and Relative brain weight across the experimental groups respectively. There was no significant difference ($p>0.05$) in initial body weight, final body weight, weight change, brain weight and relative brain weight across the experimental groups.

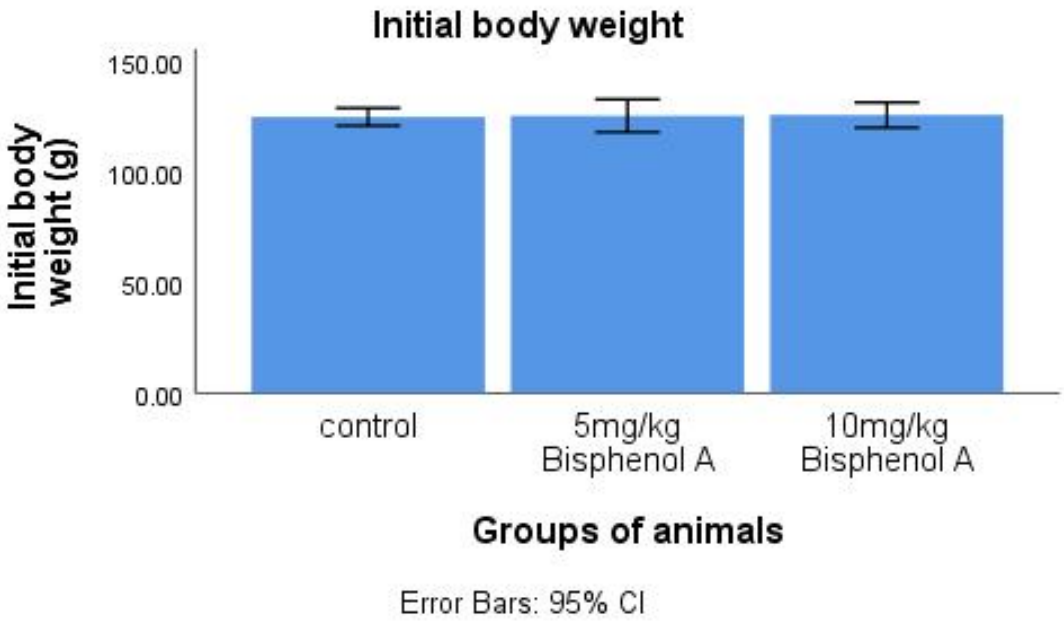

Figure 4.1: Initial body weight across the experimental groups.

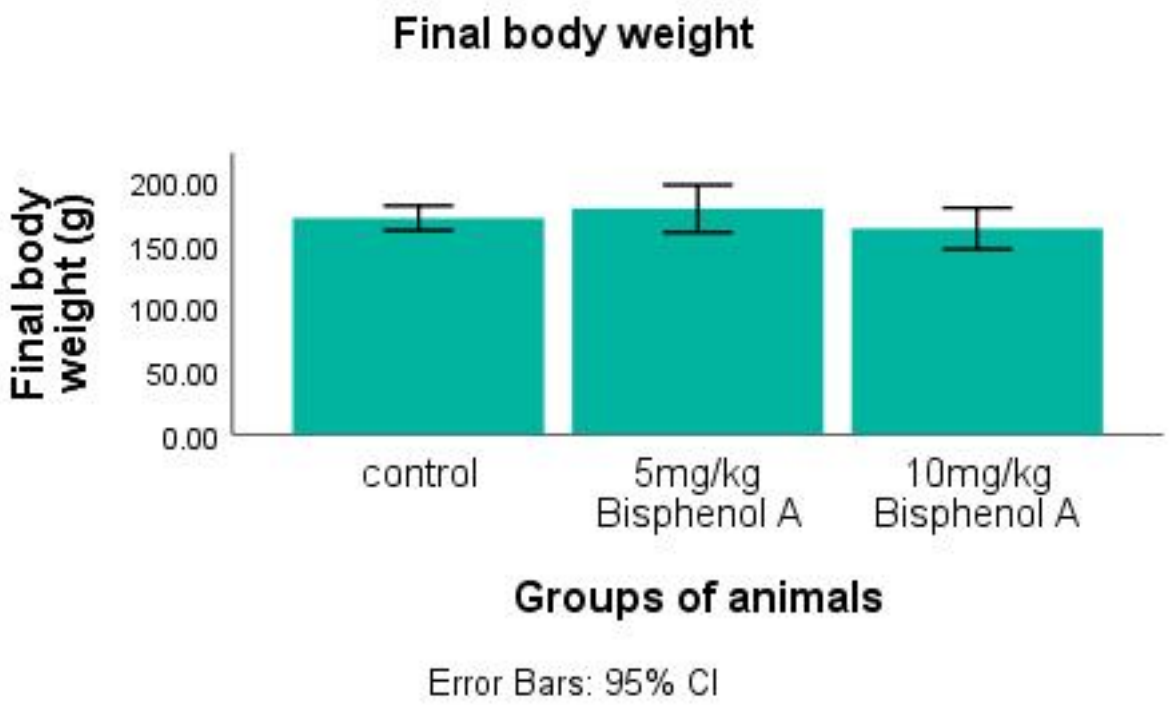

Figure 4.2: Final body weight across the experimental groups.

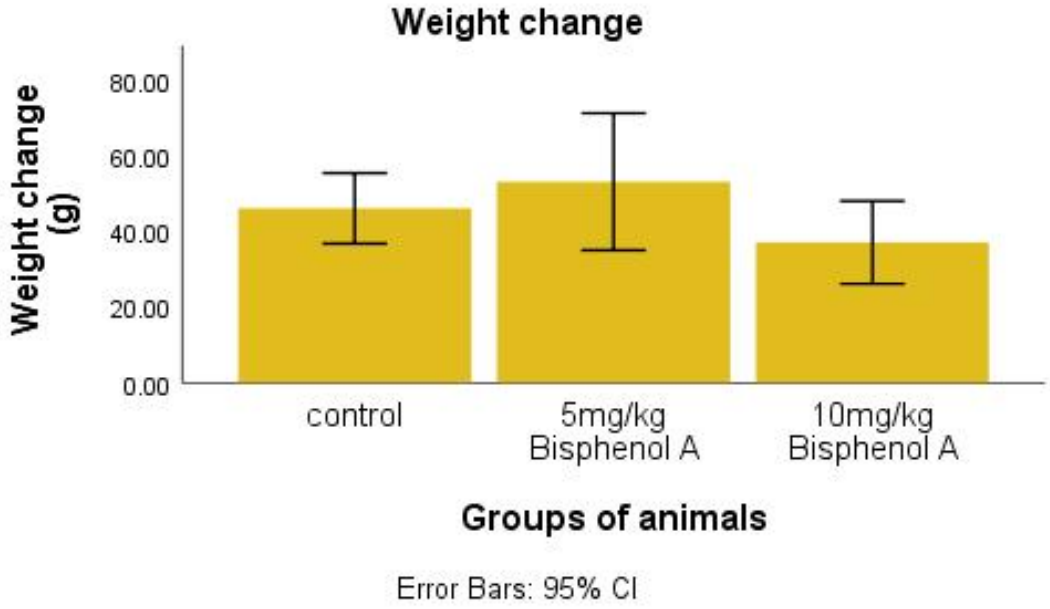

Figure 4.3: Weight change across the experimental groups.

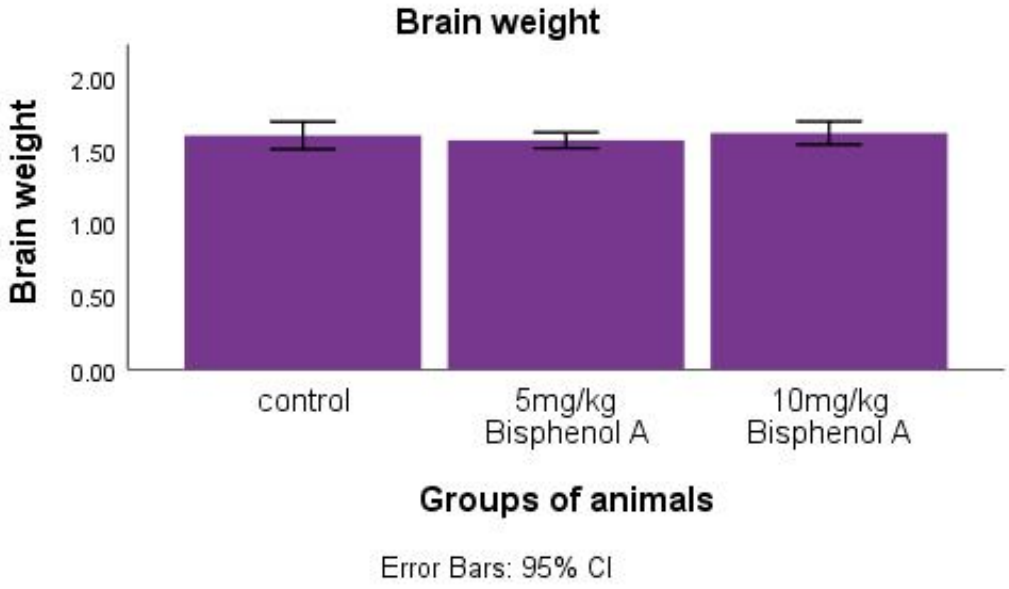

Figure 4.4: Brain weight across the experimental groups.

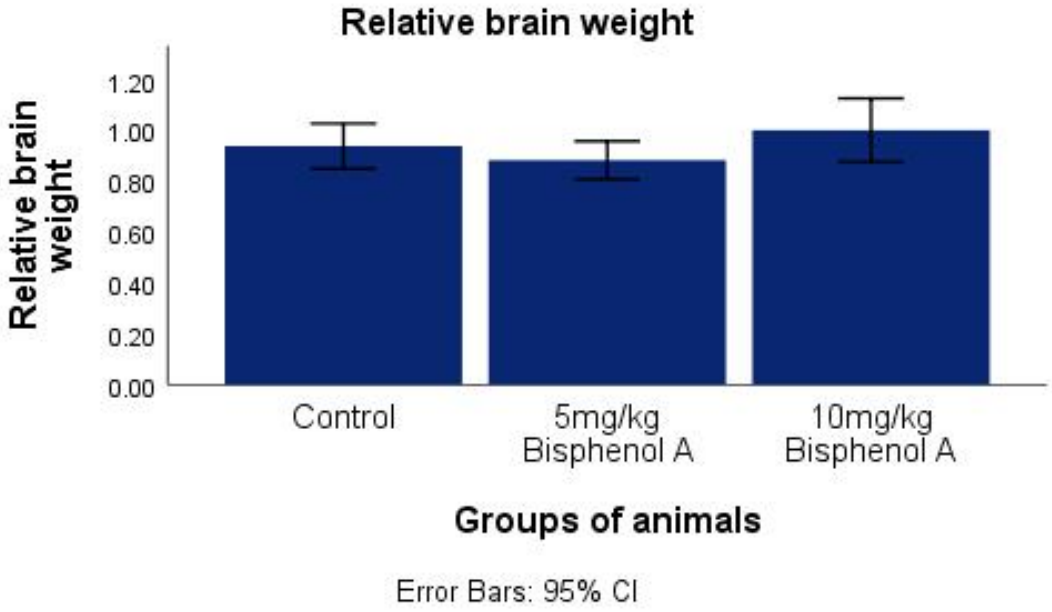

Figure 4.5: Relative brain weight across the experimental group.

4.2 OXIDATIVE STRESS RESULTS

Figure 4.6-4.10 showed the activity of SOD, CAT, GPx. GSH and MDA concentration respectively, across the experimental groups. There was a significant decrease ($p<0.05$) in SOD, CAT, GPx and GSH activity in the 10mg/kg Bisphenol A group when compared to control. There was a significant increase ($p<0.05$) in MDA concentration in the 5mg/kg and 10mg/kg Bisphenol A groups when compare to control.

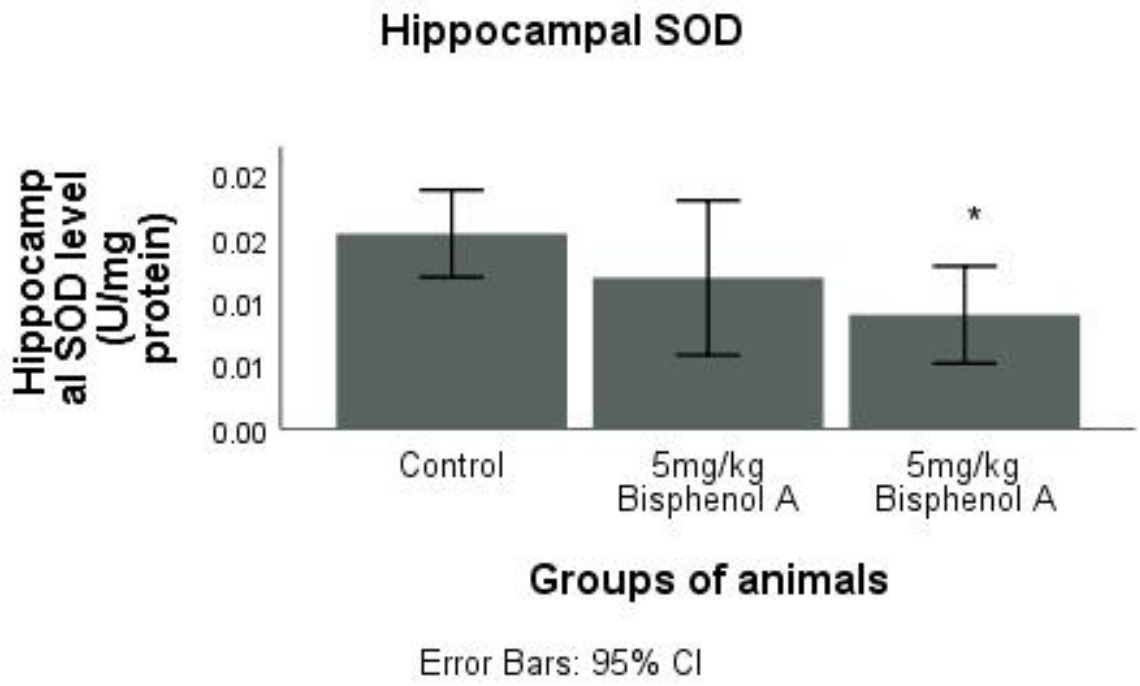

Figure 4.6. SOD activity across the experiment al groups.
Values are given as Mean ±SEM, *: control compared to bisphenol A

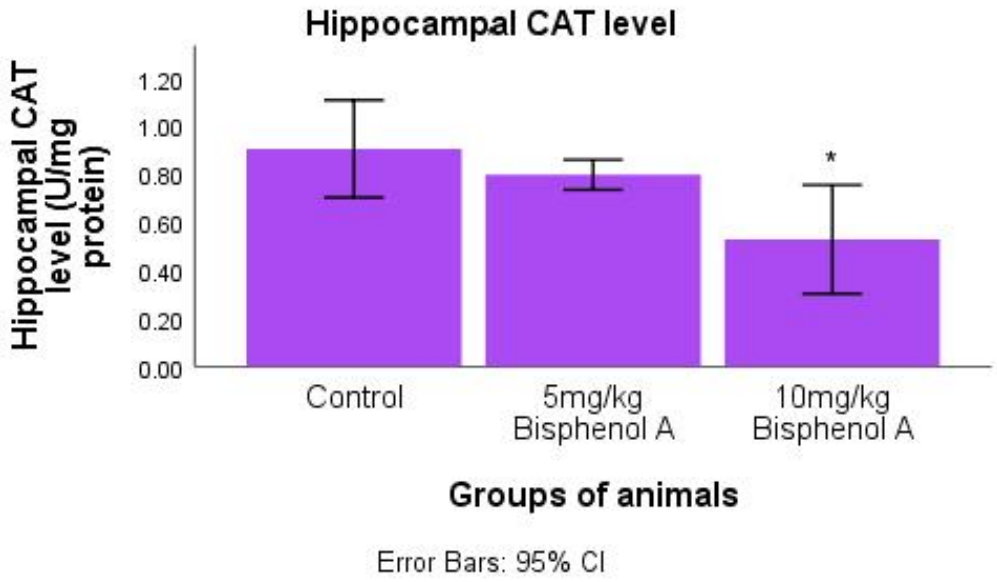

Figure 4.7. CAT activity across the experiment al groups.
Values are given as Mean ±SEM, *: control compared to bisphenol A

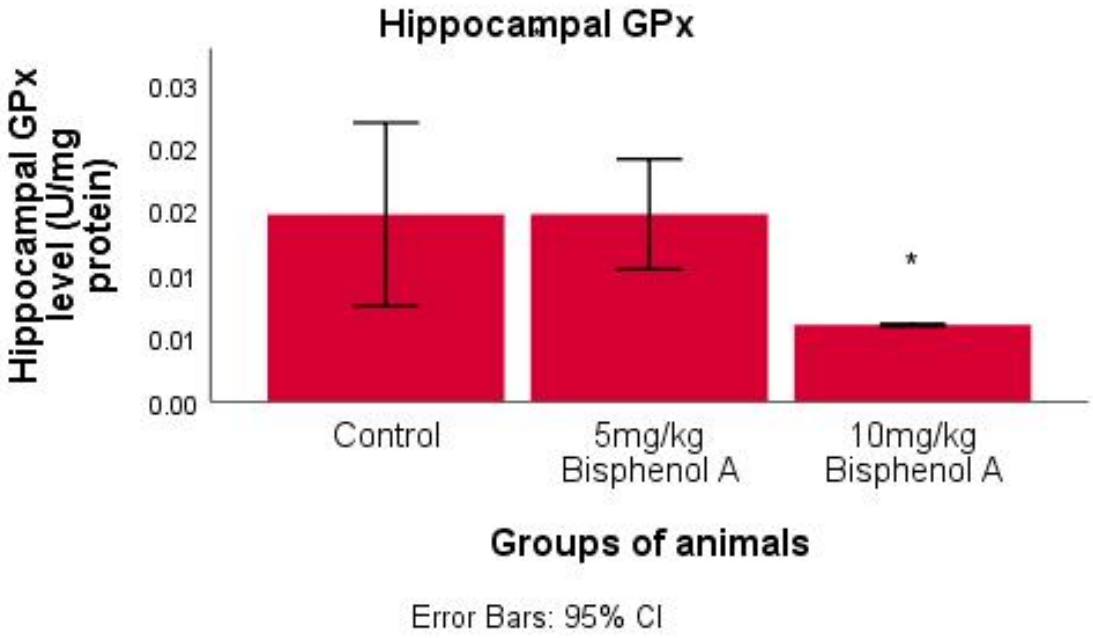

Figure 4.8. GPx activity across the experiment al groups.
Values are given as Mean ±SEM, *: control compared to bisphenol A

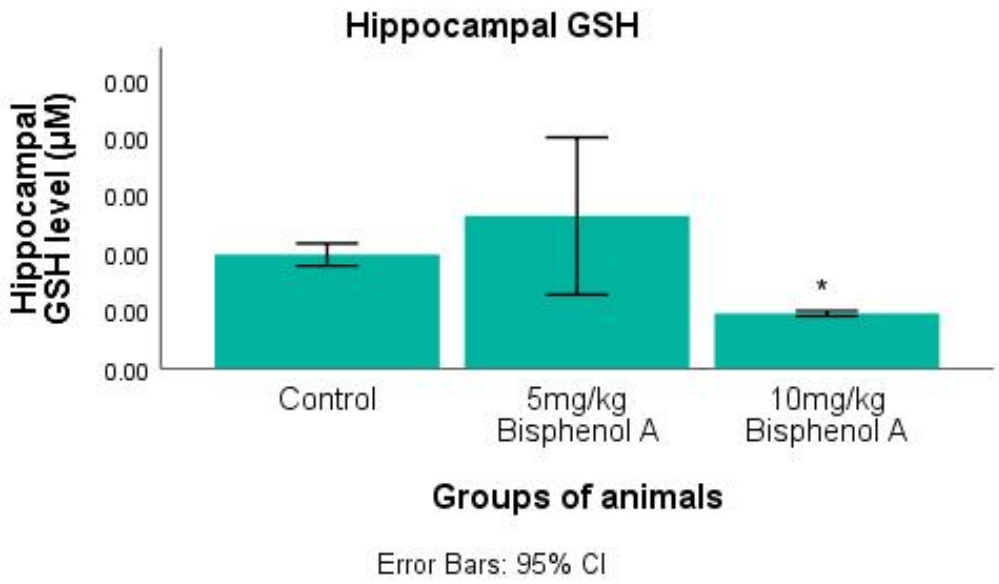

Figure 4.9. SOD activity across the experiment al groups.
Values are given as Mean ±SEM, *: control compared to bisphenol A

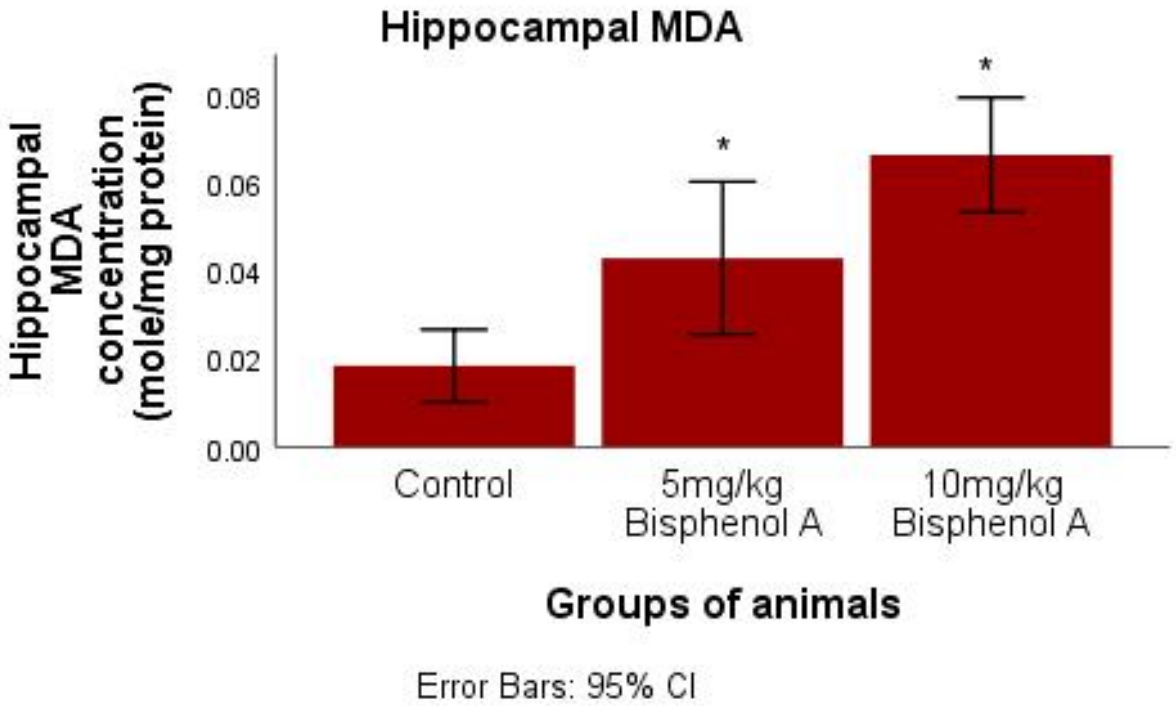

Figure 4.10. MDA concentration across the experiment al groups.
Values are given as Mean ±SEM, *: control compared to bisphenol A

4.3HISTOLOGY RESULTS

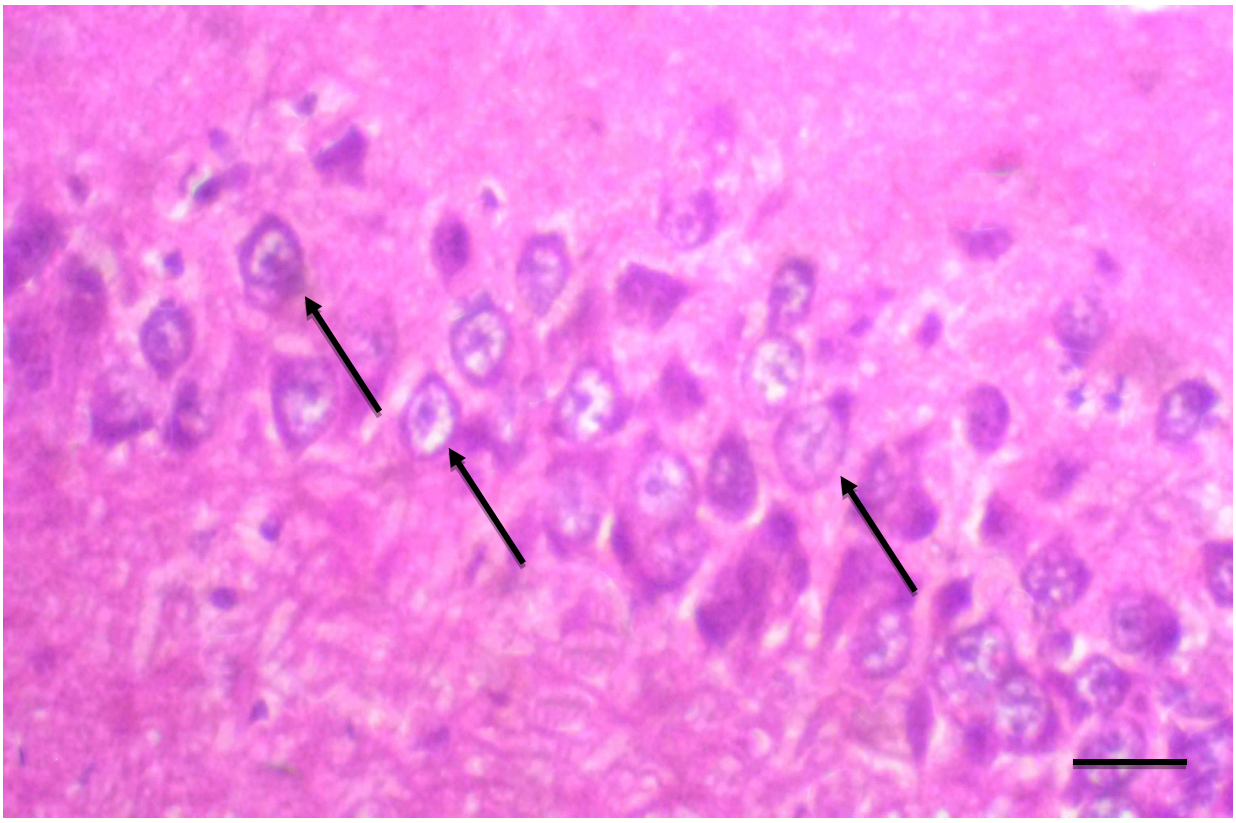

Plate 1: Representative histology of the hippocampus CA1 in control and treatment rats.

(A) Control group revealing normal structure of pyramidal cells (arrows) (H&E; Scale bar: 25µm)

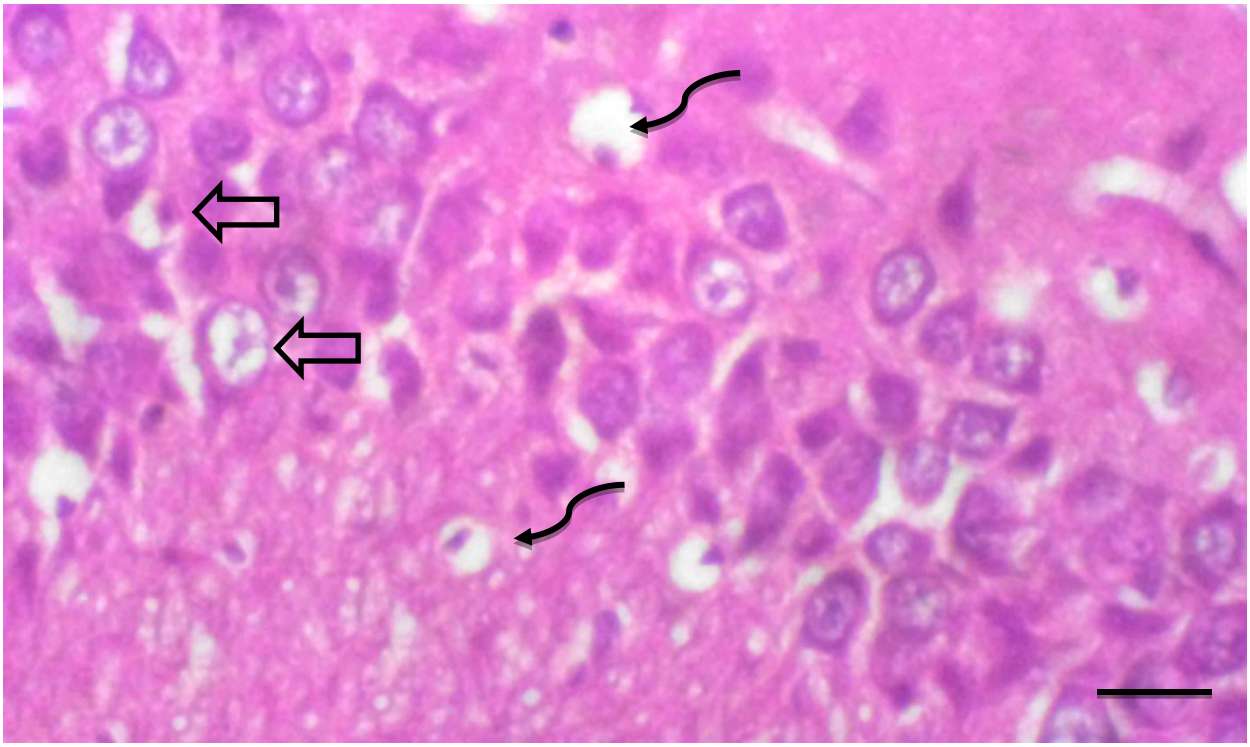

Plate 2: Representative histology of the hippocampus CA1 in (B) – 5mg/kg of Bisphenol A-treated rats showing atrophy and vacuolated pyramidal cells (double arrows) and astrocytes [curved arrows] (H&E; Scale bar: 25µm)

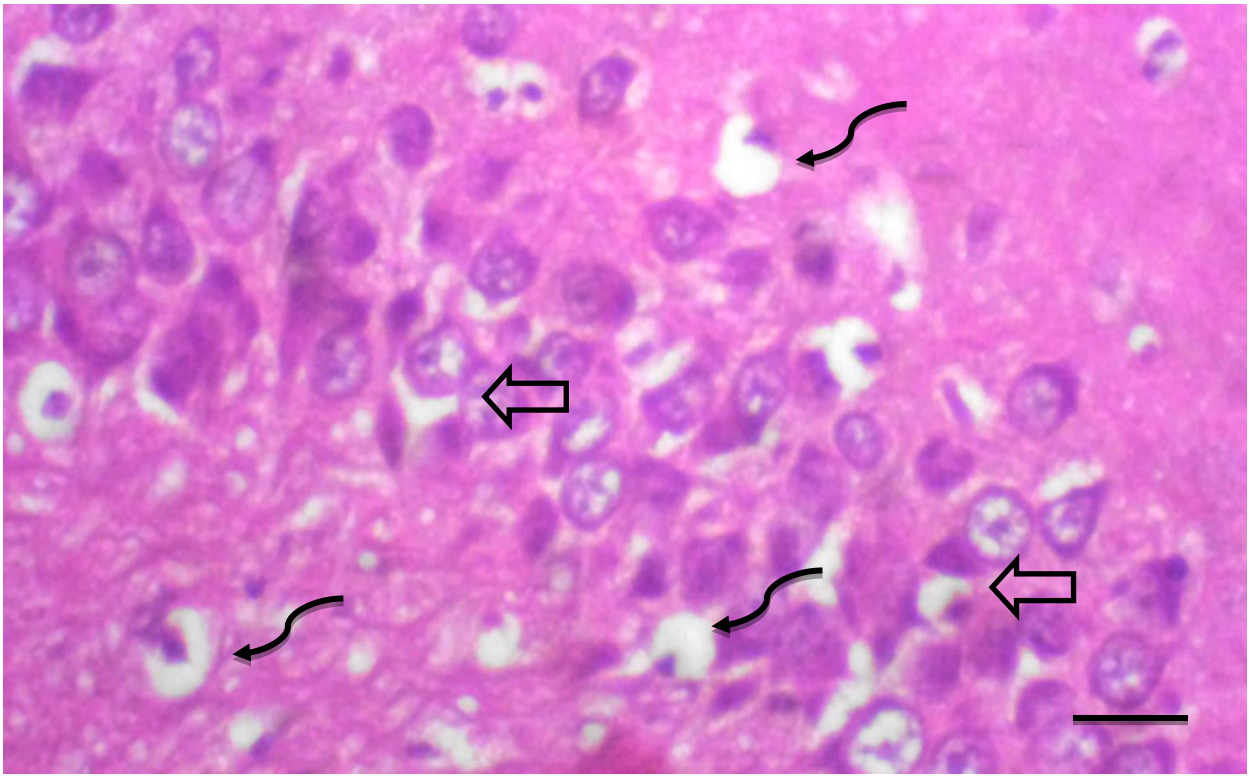

Plate 3: Representative histology of the hippocampus CA1 in control and treatment rats (C) -10mg/kg of Bisphenol A-treated rats showing atrophy and vacuolated pyramidal cells (double arrows) and astrocytes [curved arrows] (H&E; Scale bar: 25µm)

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Humans are frequently exposed to various endocrine-disrupting chemicals, among which Bisphenol A (BPA) is particularly prevalent and likely accumulates over the course of a lifetime. BPA has the ability to cross the blood-brain barrier, as demonstrated in studies (Denuzière and Ghersi-Egea *et al*., 2022; Hyun and Ka, 2024), and once it enters the body, it can easily accumulate within the brain (Hyun and Ka, 2024). This accumulation may pose significant long-term health risks due to its potential effects on brain function and overall endocrine system regulation. Hence the aim of this study was to assess the effect of Bisphenol A on the hippocampus of Wistar rats.

Findings from this study showed that initial Body Weight, final Body Weight, weight Change, brain Weight, and relative Brain Weight, indicate no significant differences ($p > 0.05$) across the experimental groups. This suggests that the treatments did not significantly impact the overall body mass or brain weight during the study period. The lack of differences in initial and final Body Weight and weight Change aligns with studies where uniformity in baseline measures is crucial to eliminate confounding factors (Almutairi *et al*., 2024).

Figures 4.6 to 4.10 illustrate the activities of SOD, CAT, GPx, GSH, and MDA concentration across the experimental groups. The results show a significant decrease ($p < 0.05$) in antioxidant enzymes (SOD, CAT, GPx) and GSH levels in the 10 mg/kg Bisphenol A (BPA) group compared to the control. Additionally, MDA concentration significantly increased ($p < 0.05$) in both the 5 mg/kg and 10 mg/kg BPA groups. The

decline in antioxidant enzyme activity and GSH levels suggests oxidative stress, as these enzymes play a crucial role in neutralizing reactive oxygen species (ROS)(Singh,2022). Similar findings have been reported in studies showing that BPA exposure impairs antioxidant defense mechanisms, leading to cellular damage (Mączka *et al*., 2022; Sadek *et al*., 2024). The increase in MDA, a marker of lipid peroxidation, further confirms oxidative stress, consistent with studies linking BPA to increased free radical production and membrane damage (Sadek *et al*., 2024). These results indicate that BPA exposure, particularly at higher doses, disrupts oxidative balance, increasing vulnerability to oxidative damage. This has significant implications, as oxidative stress is linked to neurodegenerative diseases and metabolic disorders(Sharma,2022).

The histological analysis of the hippocampus CA1 region revealed dose-dependent neurotoxic effects of Bisphenol A (BPA) exposure. In the 5 mg/kg BPA-treated group, notable histopathological alterations were observed, including neuronal atrophy, vacuolated pyramidal cells, and an increased presence of astrocytes. These changes indicate neuronal degeneration and glial activation, key markers of neuroinflammation and oxidative stress((Zhang,2022). Studies suggest that BPA exposure induces neurotoxicity by increasing reactive oxygen species (ROS) production, leading to neuronal damage and astrocyte proliferation as a protective response (Costa and Cairrao, 2024; Sadek *et al*., 2024). The presence of vacuolated neurons further suggests cytoplasmic degeneration, which may impair hippocampal function, potentially affecting memory and learning(Xu,2020).At a higher dose (10 mg/kg BPA), these alterations were more pronounced, with increased neuronal atrophy, more extensive vacuolization, and a

greater presence of astrocytes. The severity of these changes supports a dose-dependent neurotoxic effect of BPA, with higher exposure levels leading to more extensive hippocampal damage(Perera,2020). The increased astrocytosis suggests an ongoing neuroinflammatory response, possibly as an attempt to counteract neuronal injury(Kim,2022). These findings are consistent with previous studies indicating that higher BPA doses exacerbate hippocampal degeneration, contributing to cognitive impairments and increased neuronal cell death (Suresh *et al*., 2022, Meng *et al*., 2024; Al-Shami *et al*., 2024). Overall, these results highlight the potential neurotoxic impact of BPA on hippocampal structure, emphasizing the need for further studies on its long-term effects and possible protective interventions.

5.2 CONCLUSION AND RECOMMENDATION

Findings from this study demonstrates that Bisphenol A (BPA) exposure induces oxidative stress and neurotoxic effects on the hippocampus of Wistar rat in a dose-dependent manner. Recommendations include that further studies to explore the potential protective effects of antioxidants and neuroprotective agents against BPA-induced oxidative stress and neurodegeneration.

REFERENCES

Almutairi, A. H., Almutairi, N. S., Mousa, N., Elsayed, A., El-Sehrawy, A., & Elmetwalli, A. (2024). Aerobic exercise as a non-pharmacological intervention for improving metabolic and hemodynamic profiles in type 2 diabetes. *Irish Journal of Medical Science (1971-)*, 1-10.

Al-Shami, A. S., Abd Elkader, H. T. A. E., Moussa, N., Essawy, A. E., & Haroun, M. (2024). Early-life bisphenol A exposure causes neuronal pyroptosis in juvenile and adult male rats through the NF-κB/IL-1β/NLRP3/caspase-1 signaling pathway: exploration of age and dose as effective covariates using an in vivo and in silico modeling approach. *Molecular and Cellular Biochemistry*, 1-30.

Costa, H. E., & Cairrao, E. (2024). Effect of bisphenol A on the neurological system: A review update. *Archives of Toxicology*, *98*(1), 1-73.

Denuzière, A., & Ghersi-Egea, J. F. (2022). Cerebral concentration and toxicity of endocrine disrupting chemicals: The implication of blood-brain interfaces. *NeuroToxicology*, *91*, 100-118.

Hyun, S. A., & Ka, M. (2024). Bisphenol A (BPA) and neurological disorders: an overview. *The International Journal of Biochemistry & Cell Biology*, 106614.

Mączka, W., Grabarczyk, M., & Wińska, K. (2022). Can antioxidants reduce the toxicity of bisphenol?. *Antioxidants*, *11*(2), 413.

Meng, L., Ouyang, Z., Chen, Y., Huang, C., Yu, Y., & Fan, R. (2024). Low-dose BPA-induced neuronal energy metabolism dysfunction and apoptosis mediated by PINK1/parkin mitophagy pathway in juvenile rats. *Science of The Total Environment*, *929*, 172655.

Sadek, K. M., Khalifa, N. E., Alshial, E. E., Abdelnour, S. A., Mohamed, A. A. R., & Noreldin, A. E. (2024). Potential hazards of bisphenol A on the male reproductive system: Induction of programmed cell death in testicular cells. *Journal of Biochemical and Molecular Toxicology*, *38*(9), e23844.

Suresh, S., Singh, A., & Vellapandian, C. (2022). Bisphenol A exposure links to exacerbation of memory and cognitive impairment: A systematic review of the literature. *Neuroscience & biobehavioral reviews*, *143*, 104939.

Biedermann, S., Tschudin, P., & Grob, K. (2010). Transfer of bisphenol A from thermal printer paper to the skin. *Analytical and Bioanalytical Chemistry, 398*(1), 571-576.

Gould, J. C. (2006). Bisphenol A interacts with the estrogen receptor to affect endocrine function. *Molecular and Cellular Endocrinology, 253*(1-2), 79-86.

Richter, C. A., Birnbaum, L. S., Farabollini, F., Newbold, R. R., Rubin, B. S., Talsness, C. E., ... & Vom Saal, F. S. (2007). In vivo effects of bisphenol A in laboratory rodent studies. *Reproductive Toxicology, 24*(2), 199-224.

Rochester, J. R. (2013). Bisphenol A and human health: A review of the literature. *Reproductive Toxicology, 42*, 132-155.

Rochester, J. R., & Bolden, A. L. (2015). Bisphenol S and F: A systematic review and comparison to bisphenol A. *Environmental Health Perspectives, 123*(7), 643-650.

Rubin, B. S. (2011). Bisphenol A: An endocrine disruptor with widespread exposure and multiple effects. *The Journal of Steroid Biochemistry and Molecular Biology, 127*(1-2), 27-34.

Staples, C. A., Dorn, P. B., Klecka, G. M., O'Block, S. T., & Harris, L. R. (1998). A review of the environmental fate, effects, and exposures of bisphenol A. *Chemosphere, 36*(10), 2149-2173.

Vandenberg, L. N., Hauser, R., Marcus, M., Olea, N., & Welshons, W. V. (2007). Human exposure to bisphenol A (BPA). *Reproductive Toxicology, 24*(2), 139-177.

Vandenberg, L. N., Maffini, M. V., Sonnenschein, C., Rubin, B. S., & Soto, A. M. (2009). Bisphenol-A and the great divide: A review of controversies in the field of endocrine disruption. *Endocrine Reviews, 30*(1), 75-95.