EFFECTS OF BISPHENOL-A AND SELENIUM ON SOME OXIDATIVE STRESS MARKERS IN MALE WISTAR RATS

BY

NODUGBE CHEKWUBE PAUL
BMS2005104

DEPARTMENT OF PHYSIOLOGY
SCHOOL OF BASIC MEDICAL SCIENCES
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN

SUPERVISOR
PROF. F.O. AGOREYO

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PHYSIOLOGY SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE AWARD OF THE BACHELOR OF SCIENCE (B.Sc.) DEGREE IN PHYSIOLOGY

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CERTIFICATION

This is to certify that this project work on "**EFFECTS OF BISPHENOL-A AND SELENIUM ON SOME OXIDATIVE STRESS MARKERS IN MALE WISTAR RATS"** by **NODUGBE CHEKWUBE PAUL,** with matriculation number **BMS2005104** in partial fulfillment for the award of Bachelor of Science Degree (B.Sc.) in department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin city, Edo state, Nigeria.

_____ _____

NODUGBE CHEKWUBE PAUL Date
(Student)

_____ _____

PROF. F.O. AGOREYO Date
(Project Supervisor)

_____ _____

PROF. O.K. UCHE Date
(Head of Department)

_____ _____

PROF. S. A. ONASANWO Date
(External Examiner)

DEDICATION

This project work is dedicated to Almighty God, to my parents and siblings for support, wisdom and faithfulness.

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ABSTRACT

Bisphenol-A (BPA) is an industrial chemical primarily used in the production of polycarbonate plastics and epoxy resins. Selenium (Se) is an essential trace element, vital for the health of humans and other living organisms. The aim of this study was to investigate the effects of Bisphenol-A (BPA) on some oxidative stress markers in adult male rats and evaluate the potential effect of Selenium (Se) in BPA-induced oxidative damage. A total of twenty (20) male Wistar rats weighing between 180g and 200g were purchased and kept in standard cages for two weeks to enable them acclimatize to their new environment. After acclimatization period, the twenty-adult male Wistar rats were divided into four (4) different groups A, B, C, D: control, BPA-only, Se-only, and BPA+Se. Group A served as control, Groups B-D received 20mg/kg BPA, 2mg/kg Se, and both, respectively, for 54 days. Blood was collected and analyzed for oxidative stress parameters such as malondialdehyde (MDA), superoxide Distumates (SOD) and catalase (CAT). All statistical analyses were carried out using Graph Pad prism statistical software version 10.0. The data from all the groups were presented as Mean ± S.E.M (Standard Error of Mean), (n=5) in each group and analyze for statistical significance using one-way Analysis of Variance (ANOVA). Values were considered significant at $P<0.05$. The result shows that exposure to BPA resulted in significant ($p<0.05$) reduction in body weight. There was significant ($p<0.05$) increase in MDA levels in all groups compared with the control. On the other hand, SOD and CAT activities were significantly ($p<0.05$) reduced in all groups compared with the control, thereby indicating decreased antioxidant enzyme activities. In conclusion, these finding shows that Selenium (Se) supplementation did not mitigate the adverse effects of BPA and instead worsened oxidative stress, implying that Selenium (Se) may not provide protection against the harmful effect of Bisphenol-A (BPA) even at this dose but rather potentiated the effect of Bisphenol-A (BPA).

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Bisphenol-A (BPA), a chemical commonly used in making polycarbonate plastics and epoxy resins, is widespread in consumer products, leading to near-universal human exposure. Over time, increasing worries about its possible health risks have spurred considerable research. BPA is mainly recognized as an endocrine disruptor, a substance that interferes with the normal function of the hormone system. Because it resembles estrogen, it can cause hormone imbalances that disrupt various bodily functions (Diamanti-Kandarakis *et al*., 2009). These disturbances have been associated with several health problems, such as reproductive issues, metabolic imbalances, and neurological damage. Besides its endocrine-disrupting effects, BPA is also believed to trigger oxidative stress. Oxidative stress happens when the production of reactive oxygen species (ROS) of the body exceeds its capacity to neutralize them. New research indicates that exposure to BPA may increase ROS production and/or weaken antioxidant defenses, resulting in oxidative damage to cells and tissues (Rubio *et al*., 2012). Oxidative stress can play a role in the development of diseases like cancer, heart disease, and neurodegenerative conditions. While research increasingly suggests a connection between BPA and oxidative stress, more investigation is necessary to completely clarify the underlying processes and how significantly it affects human health.

Selenium is an essential trace element that is vital for maintaining human health (Rayman, 2012). It is categorized as a micronutrient, indicating that the body needs it in minimal quantities for optimal performance. One of its key functions is its participation in the antioxidant defense system. Selenium is integrated into a group of proteins called selenoproteins, which are crucial for numerous physiological functions (Labunskayte *et al*.,

2014). Among these, glutathione peroxidase (GPx) is perhaps the most well-known. GPx is an enzyme that neutralizes harmful reactive oxygen species (ROS), protecting cells from oxidative damage (Arthur and Beckett, 2003). Selenium, by being part of selenoproteins like GPx, is a crucial part of the antioxidant defense of the body, helping to keep a healthy balance and protect against diseases linked to oxidative stress.

Oxidative stress is an imbalance between the production of reactive oxygen species (ROS), which are free radicals, and antioxidant capacity of the body to neutralize them (Pizzino *et al.,* 2017). When this balance is disrupted, cells and tissues can be harmed, impacting vital components like proteins, lipids, and DNA. This damage plays a role in the development of long-term illnesses including cancer, diabetes, heart disease, and Alzheimer's (Janssen-Heininger *et al.,* 2008). Oxidative damage has been associated with the development of various chronic diseases, such as cardiovascular disease, neurodegenerative disorders, cancer, diabetes, and inflammatory conditions (Valko *et al*., 2007). In conditions like atherosclerosis, oxidative stress promotes the oxidation of LDL cholesterol, impairs the function of the endothelium (the lining of blood vessels), and contributes to the build-up of plaque (Libby, 2002). likewise, in neurodegenerative diseases such as Alzheimer’s and Parkinson’s, oxidative damage to neurons plays a critical role in disease progression (Sohrabji *et al*., 2000).

1.2. STATEMENT OF PROBLEM

Bisphenol-A (BPA), a widely used industrial chemical, poses significant health risks due to its role as an endocrine disruptor and its ability to induce oxidative stress. This oxidative stress can lead to cellular damage, apoptosis, and organ toxicity, particularly affecting reproductive tissues. Despite Selenium being known as having antioxidant property that can counteract such effects, the precise mechanisms by which it mitigates BPA-induced toxicity in adult male Wistar rats remain poorly understood.

1.3. JUSTIFICATION OF THE STUDY

This research is justified by the pressing need to understand the combined effects of Bisphenol-A (BPA) and selenium (Se) on oxidative stress, particularly in the context of male health, using an animal model.

1.4. AIM

The aim of this study was to investigate the effects of Bisphenol-A (BPA) and Selenium (Se) on some oxidative stress markers in adult male Wistar rats.

1.5. RESEARCH QUESTIONS

1. Does exposure to Bisphenol-A (BPA) affect oxidative stress markers in male Wistar rats?
2. Does selenium supplementation mitigate BPA-induced oxidative stress in male Wistar rats by enhancing antioxidant defense?

1.6. SPECIFIC OBJECTIVES

1. To determine the effect of Bisphenol-A and selenium on oxidative stress markers in adult male Wistar rats.
2. To evaluate the potential effect of selenium against BPA-induced oxidative stress.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bisphenol-A

Bisphenol-A (BPA) is a chemical primarily used in the production of polycarbonate plastics and epoxy resins in an industry (Rahman *et al*., 2017). it was discovered by a Russian chemist Aleksandr Dianin in 1891, the widespread use of BPA began in the 1950s when it was discovered to be an important monomer in the production of resilient, clear, and durable plastics (Kang *et al.*, 2006).

Structurally, its chemical Formula is $C_{15}H_{16}O_2$. it is made up of two phenol functional groups connected by a propane bridge (Skinner, 2007). It is synthesized by condensing acetone with two equivalents of phenol in an acidic environment (Skinner, 2007). After purification, the resulting product serves as a foundational component for manufacturing different kinds of plastics and resins (Tyl, 2009).

BPA is utilized in the manufacturing of a diverse range of products such as water bottles, sports equipment, CDs, DVDs, and eyewear lenses (Vitku *et al*., 2015). Appreciated for its expectional strength, optical clarity, and heat resistance, this material is frequently utilized in coatings for food and beverage cans to offer corrosion protection. Additionally, it serves as a protective barrier in dental sealants and composites. (Wang *et al*., 2019). BPA is not only present in thermal paper used for receipts and tickets. But also plays a role in the manufacture of medical devices and electronics (Williams *et al*., 2014).

2.1.1 Mechanism of action of BISPHENOL-A

Endocrine Disruption

BPA is recognized for its capacity to mimic the effects of estrogen, a naturally occurring hormone in the body (Xi *et al*., 2011). It can bind to estrogen receptors, specifically (ERα and ERβ), though its affinity is significantly lower than that of the natural estrogens of the body (Xi *et al*., 2011). This disruption of normal hormonal function can result in a range of negative health consequences (Ylenia *et al.*, 2023).

Genomic and Non-Genomic Pathways

In genomic pathways, BPA binds to estrogen receptors, which then interact with DNA to modulate gene expression (Claudia *et al*., 2020). BPA can modify the transcription of genes related to growth, development, and metabolism. In non-genomic pathways, BPA may also activate membrane-bound estrogen receptors, triggering rapid cellular responses that do not require changes in gene expression (Chiara *et al*., 2023).

Interaction with Other Receptors

Beyond estrogen receptors, BPA can also interact with other hormone receptors, such as androgen receptors, thyroid hormone receptors, and peroxisome proliferator-activated receptors (PPARs) (Giovanni *et al*., 2020). These interactions can interfere with several endocrine pathways, leading to disruptions in hormone regulation (Giovanni *et al*., 2020).

Epigenetic Modifications

BPA exposure is associated with epigenetic alterations, including DNA methylation and histone modifications, these changes can influence gene expression without altering the underlying DNA sequence (Kevin *et al*., 2021). These changes can lead to long-term impacts on development and increase susceptibility to diseases (Nathalie *et al*., 2023).

Oxidative Stress and Inflammation

BPA can trigger oxidative stress by producing reactive oxygen species (ROS), which results in cellular damage (Rachel and Michelle, 2023). This oxidative stress can trigger inflammatory responses, which contribute to the development of various diseases, including cancer, cardiovascular diseases, and metabolic disorders (David and Paul, 2023).

Disruption of Cell Signaling Pathways

BPA can disrupt multiple cell signaling pathways, including those that regulate cell proliferation, apoptosis, and differentiation (Laura and Mark, 2023). This disruption can impact the development and function of tissues (Laura and Mark, 2023).

Neurotoxicity

BPA is capable of crossing the blood-brain barrier and affect the central nervous system (Emily and John, 2023). It has been demonstrated to disrupt neurotransmitter systems, alter brain development, and impair cognitive functions (Sarah and Michael, 2023).

Metabolic Disruption

BPA can impact metabolic processes, contributing to the development of insulin resistance, obesity, and type 2 diabetes (Karen and David, 2023). It can interfere with adipogenesis, lipid metabolism, and glucose homeostasis (Karen and David, 2023).

2.1.2 Health Effects of BISPHENOL-A

There has been growing concern about the health effects of BPA exposure due to its widespread use and its potential to leach into food and beverages (Covaci *et al*., 2023). Some of it's effects on the body are:

Endocrine Disruption: BPA is known to mimic estrogen, a hormone that plays an important role in the endocrine system (Xi *et al*., 2011). As an endocrine disruptor, BPA can interfere

with the hormonal balance of the body, which could cause a range of health problems (Liao and Kannan, 2023).

Reproductive Health:

Female Reproductive Health: Exposure to BPA has been associated with negative impacts on female fertility, such as irregular periods, impaired ovarian function, and decreased ability to conceive (Diana and Carlos, 2023).

Male Reproductive Health: Exposure BPA can affect male reproductive health by reducing sperm quality and concentration, which may lead to infertility (Gore *et al*., 2023).

Developmental Effects: Prenatal and early life exposure to BPA has been associated with developmental issues in children (Konieczna *et al.*, 2023). These can include behavioral problems, altered brain development, and increased risk of certain disorders such as attention deficit hyperactivity disorder (ADHD) (Konieczna *et al.*, 2023).

Metabolic Effects: Exposure to BPA has been connected to metabolic disorders, such as obesity, insulin resistance, and type 2 diabetes (Rochester, 2023). This is due to its ability to disrupt normal metabolic processes and glucose regulation (Vandenberg *et al*., 2020).

Cardiovascular Effects: Research has shown that exposure to BPA may increase the risk of cardiovascular diseases, including hypertension, coronary artery disease, and heart attacks (Rubin and Soto, 2018).

Cancer: Some studies suggest that exposure to BPA may increase the risk of certain cancers, particularly breast and prostate cancer, due to its estrogen-like activity (Maffini *et al.*, 2013).

Immune System Effects: BPA may also affect the immune system, potentially leading to immune dysregulation and increased susceptibility to autoimmune diseases and allergies (Vom Saal *et al*., 2023).

2.1.3 Regulatory and Safety Measures:

Due to these potential health risks, many regulatory agencies around the world have taken steps to limit BPA exposure (Bae *et al*., 2019). For example, the European Union and Canada have banned the use of BPA in baby bottles, and the U.S. Food and Drug Administration (FDA) has restricted its use in certain products (FDA, 2012). Additionally, there has been a push towards BPA-free products, with manufacturers using alternative materials such as Bisphenol-S (BPS) and Bisphenol-F (BPF), though these alternatives also have raised health concerns (Kang *et al*., 2021).

2.1.4 Recommendations

Reduce Use of Plastics: Opt for glass, stainless steel, or BPA-free plastic containers for food and beverages (Rochester and Hardell, 2022).

Avoid Heating Plastics: Do not microwave food in plastic containers, as heat can increase the leaching of BPA (Khan *et al*., 2021).

Check Product Labels: Look for products labeled as BPA-free (Boulanger *et al*., 2023).

Limit Consumption of Canned Foods: Choose fresh or frozen foods over canned ones, as the linings of cans often contain BPA (Martins *et al*., 2022).

2.2 Selenium

Selenium (Se) is a trace mineral essential for various biological functions in the human body (Rayman, 2010). It is primarily known for its role in antioxidant defense, thyroid function, and immune system support (Haug *et al*., 2007).

Chemical Nature

Selenium (Se) is a non-metal and exists in several forms, including selenocysteine and selenomethionine, which are incorporated into proteins as selenoproteins (Shreenath *et al*., 2013).

Biological Functions

Selenium is a crucial component of the enzyme glutathione peroxidase, which protects cells from oxidative damage by reducing peroxides (Steinbrenner and Sies, 2013). It is vital for the synthesis and metabolism of thyroid hormones (Roman *et al*., 2014). Selenium-dependent enzymes, such as iodothyronine deiodinases, convert thyroxine (T4) to the active form, triiodothyronine (T3) (Kryukov and Gladyshev, 2012).

Selenium supports the immune system by enhancing the proliferation of activated T cells and the production of antibodies (Rayman, 2012).

Dietary Sources

Selenium is found in foods like Brazil nuts, seafood, meat, eggs, and cereals (Ruz and Codoceo, 2013). The selenium content in food depends on the selenium concentration in the soil where plants are grown or animals are raised (Ruz and Codoceo, 2013).

Recommended Intake

The recommended daily allowance (RDA) for selenium varies by age, sex, and life stage (Schomburg, 2016). For adults, it is generally around 55 micrograms per day. Higher intakes are recommended during pregnancy and lactation (Schomburg, 2016).

Deficiency

Selenium deficiency is relatively rare but can lead to health problems, such as Keshan disease (a type of cardiomyopathy) and Kashin-Beck disease (a type of osteoarthropathy) (Steinbrenner and Sies, 2013). Deficiency can also impair immune function and increase susceptibility to infections (Steinbrenner and Sies, 2013).

Toxicity

While selenium is essential, excessive intake can lead to toxicity, known as selenosis (Cardoso *et al*., 2016). Symptoms include gastrointestinal distress, hair loss, white blotchy nails, and in severe cases, neurological damage (Cardoso *et al*., 2016).

Health Benefits and Research

Some studies suggest that selenium may have a protective effect against certain types of cancer due to its role in DNA repair and apoptosis (Fairweather-Tait *et al*., 2011). Selenium's antioxidant properties may help reduce the risk of cardiovascular disease, though evidence is mixed (Hatfield *et al.*, 2014).

Adequate selenium levels are crucial for preventing thyroid disorders, such as Hashimoto's thyroiditis (Zhao *et al*., 2018).

Supplementation

Selenium supplements are available in various forms, including selenomethionine and sodium selenite (Chen *et al.*, 2021). However, supplementation should be approached cautiously, as both deficiency and excess can have significant health impacts (Kumar and Sharma, 2022).

Global Selenium Status

Selenium levels in the population vary widely depending on geographical factors, particularly soil selenium content (Lemire *et al.*, 2010). In some regions, both selenium deficiency and excess are public health concerns (Qazi *et al*., 2018).

2.2.1 Absorption of selenium

Selenium is primarily obtained through dietary intake in the forms of organic selenium (selenomethionine, selenocysteine) and inorganic selenium (selenite, selenate) (Meltzer *et al.*, 2021). It is absorbed primarily in the small intestine, particularly in the duodenum. Absorption rates depend on the chemical form. Organic selenium (e.g., selenomethionine) is absorbed more efficiently (up to 90%) compared to inorganic forms (Meltzer *et al.*, 2021). Organic selenium is absorbed via active amino acid transport mechanisms, while inorganic selenium relies on passive diffusion (Meltzer *et al.*, 2021).

2.2.2 Metabolism

Incorporation into Proteins

Selenomethionine is non-specifically incorporated into proteins in place of methionine, while selenocysteine is specifically incorporated into selenoproteins during protein synthesis (Zhang *et al*., 2020).

2.2.3 Reduction of Inorganic Selenium

Selenate (SeO_4^{2-}) is reduced to selenite (SeO_3^{2-}), then to elemental selenium (Se^0) and selenide (H_2Se), which serves as a precursor for selenocysteine synthesis (Meltzer *et al.*, 2021).

2.2.4 Selenoprotein Synthesis

Selenocysteine is synthesized on its specific transfer RNA (tRNA^[Sec]) and incorporated into selenoproteins, such as glutathione peroxidase and thioredoxin reductase (Kieliszek and Błażejak, 2013).

2.2.5 Transport

Selenium circulates in the blood bound to selenoprotein P, which delivers selenium to tissues (Gladyshev and Hatfield, 2022).

2.2.6 Excretion

Excess selenium is excreted via urine (as methylselenol and other metabolites) and feces, with minor amounts lost through sweat and breath (as dimethylselenide, causing a garlic-like odor in cases of high intake) (Rayman *et al*., 2021).

2.3 Oxidative Stress

2.3.1 Definition of Oxidative Stress

Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the ability of the body to detoxify these reactive intermediates or repair the resulting damage (Sies, 2015). This imbalance leads to oxidative damage to biomolecules such as DNA, lipids, and proteins, disrupting normal cellular functions (Liguori *et al.*, 2018).

2.3.2 Mechanism of Oxidative Stress

ROS and RNS attack biomolecules, initiating chain reactions like lipid peroxidation and oxidative modification of proteins and DNA (Pizzino *et al*., 2017). When ROS/RNS exceed the capacity of antioxidant defenses, oxidative stress occurs (Schieber and Chandel, 2014).

Oxidation alters protein structure, function, and enzymatic activity (Sies and Jones, 2020). Oxidation also lead to the induction of strand breaks, base modifications (e.g., 8-oxo-2'-deoxyguanosine) (Sies and Jones, 2020).

2.3.3 Cellular Consequences

Other consequences of Oxidative stress are the activation of stress-sensitive signaling pathways (e.g., NF-κB, MAPK), impairment of mitochondrial function and bioenergetics and the induction of apoptosis or necrosis in severe cases (Sies, 2017).

2.3.4 Diseases Associated with Oxidative Stress

They range from cardiovascular diseases (e.g., atherosclerosis), to neurodegenerative disorders (e.g., Alzheimer's, Parkinson's), diabetes and metabolic syndromes, cancer and aging-related disorders (Forman and Zhang, 2021).

2.3.5 Sources of oxidative stress

Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the ability of the body to detoxify these reactive intermediates or repair the resulting damage (Valko *et al*., 2016). This imbalance can lead to cellular and tissue damage, impacting both human and animal health (Liguori *et al.*, 2018).

Exposure to pollutants, toxins, ultraviolet (UV) radiation, and radiation can increase ROS production (Birben *et al*., 2015). Lifestyle choices such as smoking, excessive alcohol consumption, and diets high in fats or carbohydrates can also contribute to oxidative stress (Pizzino *et al.*, 2017).

Normal cellular metabolism, particularly within mitochondria, naturally produces ROS as byproducts (Sies *et al*., 2017). However, excessive ROS generation can overwhelm antioxidant defenses (Sies *et al*., 2017).

Also, the immune response of the body to infections involves the production of ROS to combat pathogens, which can inadvertently cause oxidative stress (Reuter *et al.*, 2015).

2.3.6 Consequences on Health

ROS can damage DNA, proteins, and lipids, leading to cellular dysfunction and death (Liguori *et al.*, 2018). Oxidative stress is associated with the aging process, potentially accelerating cellular senescence and age-related decline in brain function (Forman and Zhang, 2021). It also play a role in the development of various diseases, including cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders (Forman and Zhang, 2021). Oxidative stress can lead to sperm DNA fragmentation, contributing to male infertility (Pham-Huy *et al.*, 2015).

In animals, oxidative stress can impair growth, reproduction, and immune function, affecting overall health and productivity (Liguori *et al.*, 2018).

2.3.7 Mitigation Strategies

Consuming foods high in antioxidants, such as fruits, vegetables, and nuts, can help neutralize ROS (Kruk and Aboul-Enein, 2017). Regular exercise, adequate sleep, and reducing exposure to environmental pollutants can also lower oxidative stress levels (Nimse and Pal, 2015).

In certain cases, antioxidant supplements or therapies may be recommended, though it's essential to consult with a healthcare provider before starting any supplementation (Liguori *et al.*, 2018).

2.3.8 Bisphenol-A (BPA): Role in Oxidative Stress

Exposure to BPA has been shown to increase the production of ROS, which are chemically reactive molecules containing oxygen (Rochester and Bolden, 2015). Elevated ROS levels can damage cellular components, including lipids, proteins, and DNA (Soleimani *et al*., 2018).

Studies also suggest that BPA may deplete antioxidant reserves in the body, weakening the natural defense mechanisms against oxidative damage (Tiwari *et al.*, 2016).

2.3.9 Health Implications

Animal studies have demonstrated that BPA-induced oxidative stress can lead to degenerative changes in the liver and kidneys, impairing their function (Krivoshiev *et al*., 2020).

Research has also indicated that exposure to BPA may result in oxidative stress in the brain, potentially leading to neurobehavioral disorders (Nesan *et al*., 2016). Furthermore, BPA-induced oxidative stress has been linked to adverse effects on cardiac tissues, potentially contributing to cardiovascular diseases (Siracusa *et al*., 2018).

2.3.10 Mitigation Strategies

Some studies suggest that antioxidants, such as silybin, may mitigate BPA-induced oxidative damage by enhancing the antioxidant defenses of the body (Zhou *et al.,* 2020). Limiting the use of BPA-containing products, especially in food and beverage storage, can reduce the risk of oxidative stress-related health issues (Zhou *et al.,* 2020).

2.3.11 Studies on BPA's effects on oxidative stress markers

Bisphenol-A (BPA) exposure has been extensively studied for its impact on oxidative stress markers, revealing significant alterations in various physiological systems (Vázquez-Gómez *et al*. 2021). Studies have observed that exposure to BPA leads to elevated MDA levels, a marker of lipid peroxidation, indicating increased oxidative stress (Liu *et al.*, 2020).

Another research indicates that exposure to BPA can disrupt the balance of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, leading to oxidative stress (Goh *et al*., 2022).

Exposure to BPA has been linked to increased oxidative DNA damage, as evidenced by elevated levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage (Naseer *et al*., 2020).

BPA exposure has also been associated with increased oxidative stress in the brain, potentially leading to neurodegenerative changes (Nesan *et al*., 2016). Studies have demonstrated that BPA induces liver damage through oxidative stress pathways, affecting the oxidant/antioxidant balance (El-Sheikh *et al*., 2017).

2.3.12 Selenium: Role in Oxidative Stress

Selenium is an essential trace element that plays a pivotal role in protecting the body against oxidative stress (Rayman *et al.*, 2018). It is a key component of selenoproteins, which are integral to the antioxidant defense mechanisms of the body (Steinbrenner *et al*., 2015).

Selenium is incorporated into selenoproteins, such as glutathione peroxidases and thioredoxin reductases, which are crucial for neutralizing reactive oxygen species (ROS) and maintaining redox balance within cells (Steinbrenner *et al*., 2015).

2.3.13 Impact on Health

By mitigating oxidative stress, selenium helps protect cells from damage that can lead to chronic diseases such as cancer and cardiovascular disorders (Forman and Zhang, 2021). Selenium is also essential for the proper functioning of the thyroid gland, aiding in the production of thyroid hormones and protecting the gland from oxidative damage (Forman and Zhang, 2021).

Adequate selenium levels support the immune system by enhancing the defense against infections and modulating inflammation of the body (Steinbrenner *et al*., 2015).

2.3.14 Dietary Sources

Selenium must be obtained through diet, as the body cannot synthesize it (Hatfield *et al*., 2016). Rich dietary sources include Brazil nuts, seafood, meats, and grains (Hatfield *et al*., 2016). The recommended dietary allowance varies by age, gender, and physiological status, with adults typically requiring about 55 micrograms per day (Hatfield *et al*., 2016).

2.3.15 Considerations

While selenium is vital for health, both deficiency and excess can have adverse effects (Cardoso *et al.*, 2017). Deficiency may lead to weakened antioxidant defenses and increased susceptibility to oxidative stress, while excessive intake can result in toxicity (Steinbrenner *et al*., 2015). Therefore, maintaining selenium levels within the recommended range is crucial for optimal health (Cardoso *et al.*, 2017).

2.3.16 Interactive Effects of BPA and Selenium on Oxidative Stress Markers

Bisphenol-A (BPA) exposure has been linked to increased oxidative stress, leading to cellular damage and various health issues (Liguori *et al.*, 2018). Studies have explored the potential of selenium (Se) to mitigate these effects (Liguori *et al.*, 2018).

Research indicates that selenium nanoparticles can attenuate BPA-induced testicular toxicity by inhibiting oxidative stress in male rats (Gonçalves *et al.*, 2021). Another study on mice demonstrated that selenium supplementation reduced mitochondrial oxidative stress and improved sperm motility affected by BPA exposure (Zhou *et al*., 2020). Selenium has been shown to counteract apoptosis in rat testes induced by BPA, suggesting its role in protecting against oxidative damage (Zhou *et al*., 2020).

Supplementation with selenium and vitamin E has been found to restore antioxidant enzyme activities and reduce stress-activated kinases in rats exposed to BPA (Al-Bogami *et al*., 2020). Selenium and vitamin E have also demonstrated protective effects against BPA-induced liver damage in male rats, highlighting their role in mitigating oxidative stress (Al-Bogami *et al*., 2020).

2.4 Antioxidants

Antioxidants are compounds that inhibit oxidation, a chemical reaction that produces free radicals, potentially damaging cells and contributing to diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders (Gupta *et al*., 2014).

2.4.1 Definition and Role

Antioxidants neutralize free radicals and reactive oxygen species (ROS), preventing oxidative stress and protecting biomolecules such as DNA, lipids, and proteins (Gupta *et al*., 2014).

2.4.2 Types of Antioxidants

Antioxidants are divided into endogenous Antioxidants (Produced by the body) and exogenous antioxidants (obtained from diet (Shafe *et al.*, 2024). Endogenous antioxidants are divided into enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic antioxidants (glutathione, uric acid and coenzymes), while Exogenous antioxidants are obtained from vitamin C (ascorbic acid), Vitamin E (tocopherols). Minerals (selenium, zinc, manganese) and Phytochemicals Flavonoids, carotenoids, polyphenols (Shafe *et al.*, 2024).

2.4.3 Mechanism of ROS generation

Antioxidants donate electrons to stabilize free radicals, halting chain reactions that cause cellular damage (Tumilaar, 2024). For example, Vitamin E neutralizes lipid peroxyl radicals in cell membranes. Vitamin C regenerates Vitamin E to its active form and scavenges ROS in the aqueous phase (Nwozo *et al*., 2023).

2.4.4 Health Benefits

Antioxidants reduces risk of cancer, cardiovascular diseases, and diabetes. It also protects against UV-induced damage and aging (Lobo *et al*., 2014). Antioxidants mitigates neurodegenerative diseases such as Alzheimer's and Parkinson's and enhances immune defense mechanisms (Pisoschi and Pop, 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Male wistar rats, weighing scale, Plastic cages, feeding and drinking bowls, clean water, feed, Syringe, Surgical gloves, Oral gastric tube, Chloroform, Beaker, Cotton wool, Plain blood sample bottles

3.2 Experimental Animals and Research Design

A total of twenty (20) male wistar rats weighing between 180g and 200g were purchased and kept in a standard cage for two weeks to enable them acclimatize to their new environment. Pelletized commercial rat feed and water was given to the rats *ad libitum* within this period. All conditions of animal use were adhered to as obtained from National Institute of Health (NIH) Guide for Care and Use of Laboratory Animals. After acclimatization period, the rats were allocated into 4 groups of five rats each designated as groups A, B, C, D. Group A serves as the control and group B-D serves as the experimental test groups. Group B received 20mg/kg body weight of Bisphenol-A, Group C received 2mg/kg body weight of Selenium and Group D receive 20mg/kg body weight of Bisphenol-A + 2mg/kg body weight of Selenium for fifty-four days.

3.3 Collection of Samples

At the end of the administration period of fifty-four days (54 days), rats from various groups were re-weighed, anesthetized with chloroform vapor, and dissected. Blood was collected by cardiac puncture into clean plain blood sample bottles for oxidative stress markers analysis.

3.4 Laboratory Analysis

Reagents

The chemicals and reagents used in this study were of analytical grade. The 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH) standard, thiobarbituric acid (TBA), trichloroacetic acid (TCA), were products of Merck (Germany). Ethylene diamine tetra acetic acid (EDTA), epinephrine (adrenaline), hydrogen peroxide (H_2O_2), potassium hydroxide (KOH), sodium carbonate (Na_2CO_3), sodium citrate, sodium chloride (NaCl), sodium dihydrogen phosphate (NaH_2PO_4), sodium hydrogen carbonate ($NaHCO_3$), sodium hydrogen phosphate ($NaHPO_4$), sodium hydroxide (NaOH), and sulphuric acid (H_2SO_4) were purchased from British Drug House (BDH) (England). Hydrochloric acid (HCl), potassium permanganate ($KMnO_4$), and pyrogallol were obtained from May and Bayer (England).

Determination of MDA Concentration

The concentration of MDA was determined according to the method of Buege and Aust (1978). The principle that underlies this assay is that MDA – a product of lipid peroxidation when heated with thiobarbituric acid (TBA), in the presence of an acid, forms a pink or reddish complex that is measured spectrophotometrically at 532 nm. The table below clearly illustrates the procedure adopted in the determination of the level of malondialdehyde.

Assay Procedure

An aliquot of the liver homogenate was added to 3.0 mL of TCA – TBA – HCl reagent and mixed thoroughly by swirling. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed via centrifugation at 1000 g for 10 min. The absorbance of the clear supernatant was measured against a reference blank at 535 nm.

Calculation

The MDA concentration of each sample was calculated as follows:

$$\frac{O.D \times V_t \times 1000}{a \times V \times L \times Y}$$

where,

O.D = Absorbance of sample test at 535 nm

V_t = Total volume of the reaction mixture = 3.6 mL

a = Molar extinction coefficient of product = $1.56 \times 10^5\ M^{-1}cm^{-1}$

L = Light path = 1.0 cm

V = Volume of sample homogenate used = 0.6 mL

Y = mg of tissue in the sample used

The unit of MDA is moles/mg wet tissue

Determination of Superoxide Dismutase (SOD) Activity

Principle

The activity of SOD was assessed based on the method of Misra and Fridovich (1972). Adrenaline auto-oxidizes rapidly in aqueous solution to adrenochrome whose concentration can be determined spectrophotometrically at 420 nm. The auto-oxidation depends on the presence of superoxide anions (O^{2-}). Superoxide dismutase (SOD) inhibits this auto-oxidation by catalyzing the breakdown of superoxide anions. The degree of inhibition is thus a measure of SOD activity. The amount of enzyme producing 50 % inhibition is defined as one unit of the enzyme activity.

Assay Procedure

Sample homogenate (0.2 mL) was added to 2.5 mL of 0.05 M carbonate buffer (p^H 10.2) and allowed to equilibrate. The reaction was initiated by the addition of 0.3 mL of freshly prepared 0.03 mM adrenaline as substrate. The solution was mixed by inversion. The reference tube contained 2.7 mL of carbonate buffer and 0.3 mL of adrenaline, while the blank contained 2.5 mL of carbonate buffer, 0.2 mL of distilled water and 0.3 mL of 0.03 mM adrenaline. The increase in absorbance at 420 nm due to the formation of adrenochrome was monitored every 30 sec for 120 sec. One unit of SOD activity was taken as the amount of SOD necessary to cause 50 % inhibition of the oxidation of adrenaline to adrenochrome within 120 sec.

Calculation

$$\% \text{ Inhibition} = \frac{O.D_{test} - O.D_{reference}}{O.D_{test}} \times \frac{100}{1}$$

$$\text{Enzyme Activity (units/mg protein)} = \frac{\% \text{ inhibition}}{50 \times Y}$$

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

A unit of SOD activity was taken as the amount of SOD required to cause 50 % inhibition of the auto-oxidation of adrenaline to adrenochrome per minute.

Determination of Catalase Activity

Principle

This is based on the method of Cohen, *et al.,* (1970). This estimation is based on the measurement of the rate of decomposition of hydrogen peroxide (H_2O_2), after the addition of the material containing the enzyme.

Catalase catalyses the reaction: $2H_2O_2 \xrightarrow{} 2H_2O + O_2$

The quantity of hydrogen peroxide decomposed is directly proportional to the concentration of the enzyme in the sample. The hydrogen peroxide produced in tissues is measured by reacting it with excess potassium permanganate ($KMNO_4$) and then measuring the residual $KMNO_4$ spectrophotometrically at 480 nm.

Assay Procedure

Sample homogenate (0.5 mL) was placed in ice – cold test tubes, the blank contained 0.5 mL distilled water. Cold phosphate-buffered H_2O_2 (30 mM, 5 mL) was added to both blank and sample tubes at fixed intervals, and were mixed by inversion. After 3 min, the reaction was stopped by rapid addition of 1 mL of 6 M H_2SO_4. The tubes were mixed thoroughly by inversion after which 7 mL of 0.01 M $KMNO_4$ was added. Absorbance was read at 480 nm within 3 min.

Calculation

The activity of catalase in each sample is calculated thus:

$$\frac{O.D/min \times Vt \times 1000}{M \times V \times L \times Y}$$

where,

O.D = Absorbance of sample test at 480 nm

Vt = Total volume of the reaction mixture = 13.5 mL

M = Molar extinction coefficient of $H_2O_2 = 43.6M^{-1}\ cm^{-1}$

L = Light path = 1.0 cm

V = Volume of sample homogenate used = 0.5 mL

Y = mg of protein in tissue used

3.4 Statistical Analysis

All statistical analyses were carried out using Graph Pad prism statistical software version 10.0. The data from all the groups were presented as Mean ± S.E.M (Standard Error of Mean), (n=5) in each group and analyze for statistical significance using one-way Analysis of Variance (ANOVA). Values were considered significant at $P<0.05$.

CHAPTER FOUR

4.0 RESULTS

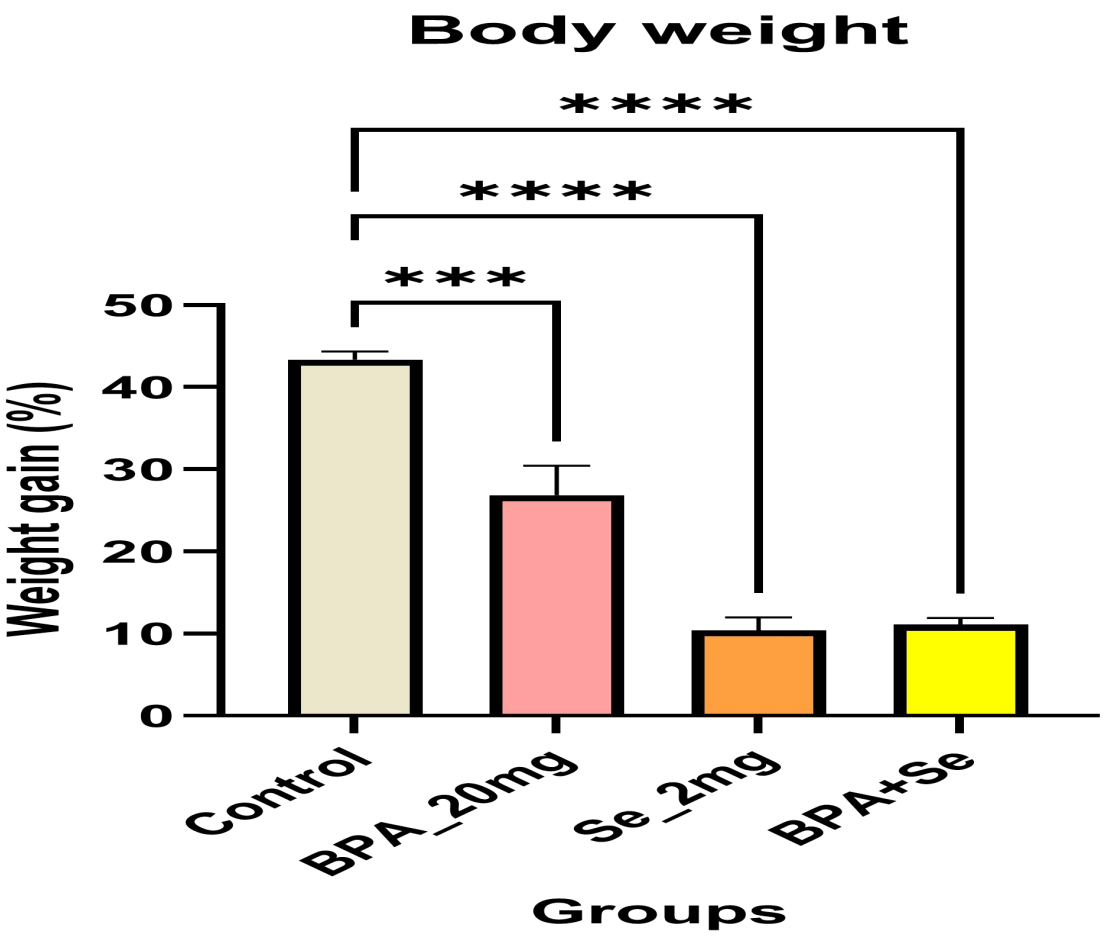

Figure 1: Effect of BPA and Se in singlet and combined forms on the body weight of rats.

Bars represents the body weight of rats exposed to BPA and Se in singlet and combined form.

(*) shows point of significant difference relative to the control ($p< 0.05$).

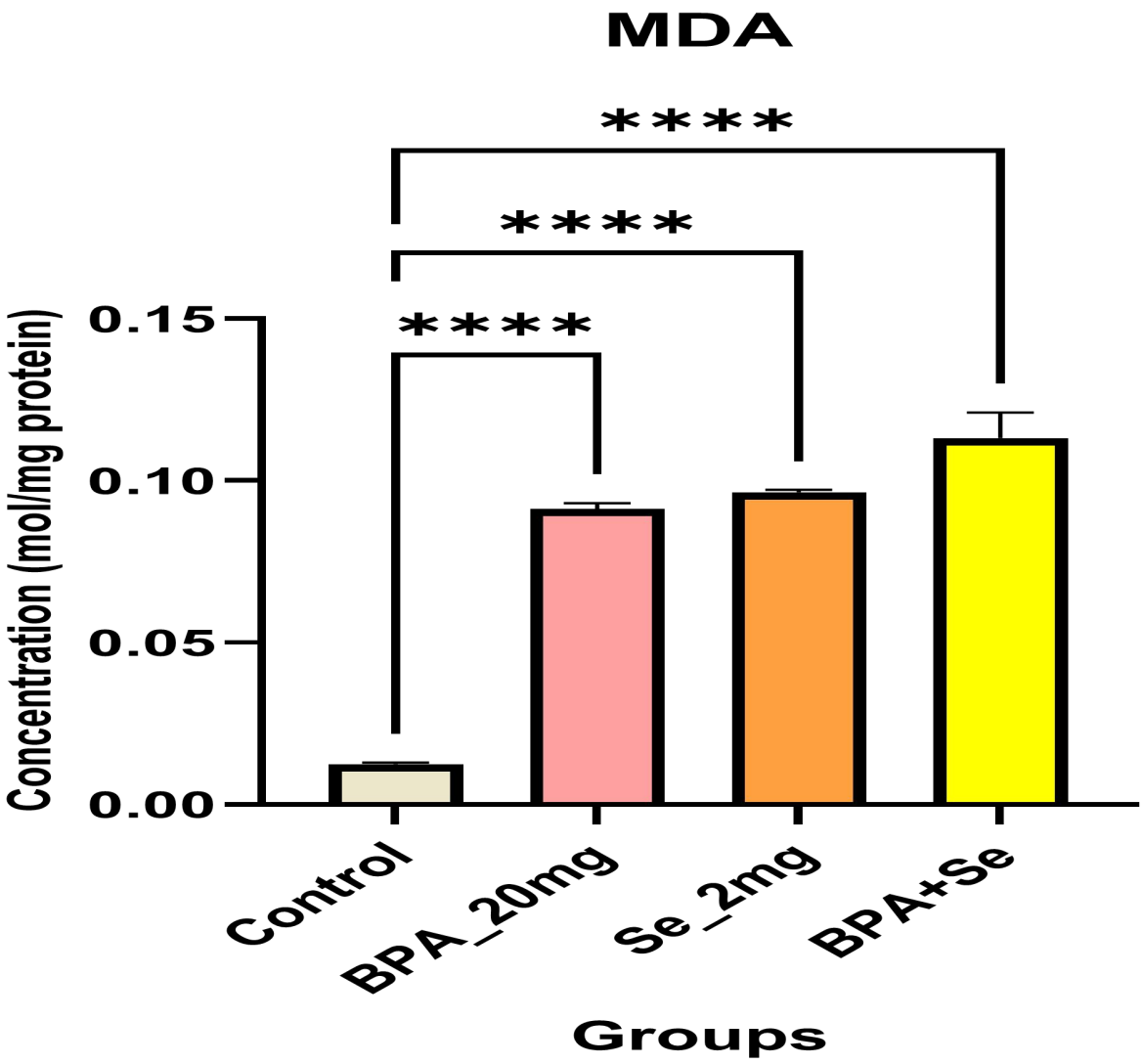

Figure 2: Effect of BPA and Se in singlet and combined forms on the concentration of MDA in male rats.

Bars represent the concentration of MDA in rats exposed to BPA and Se in singlet and combined form. (*) shows point of significant difference relative to the control ($p< 0.05$).

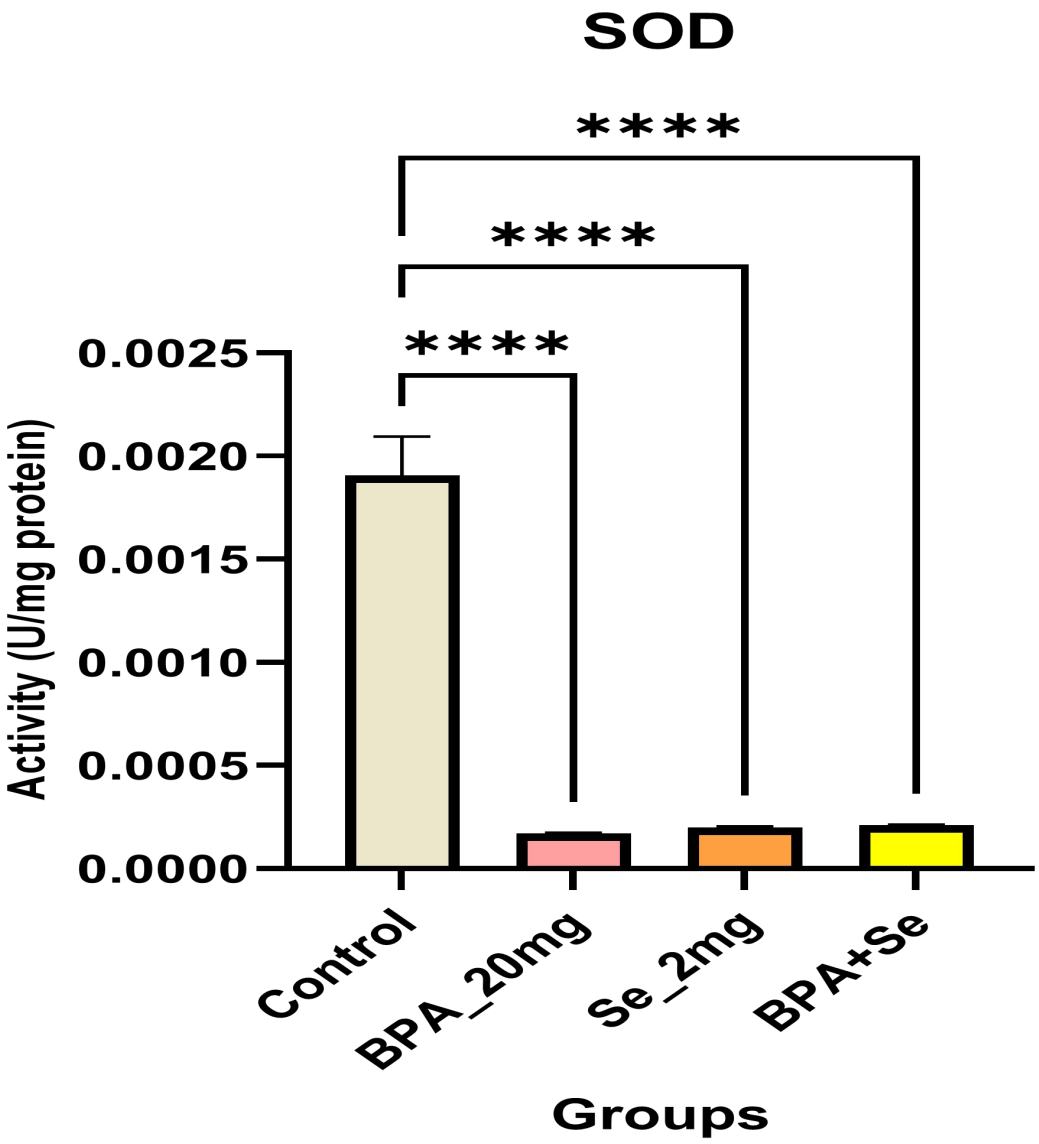

Figure 3: Effect of BPA and Se in singlet and combined forms on the activity of SOD in male rats.

Bars represent the activity of SOD in rats exposed to BPA and Se in singlet and combined form. (*) shows point of significant difference relative to the control ($p< 0.05$).

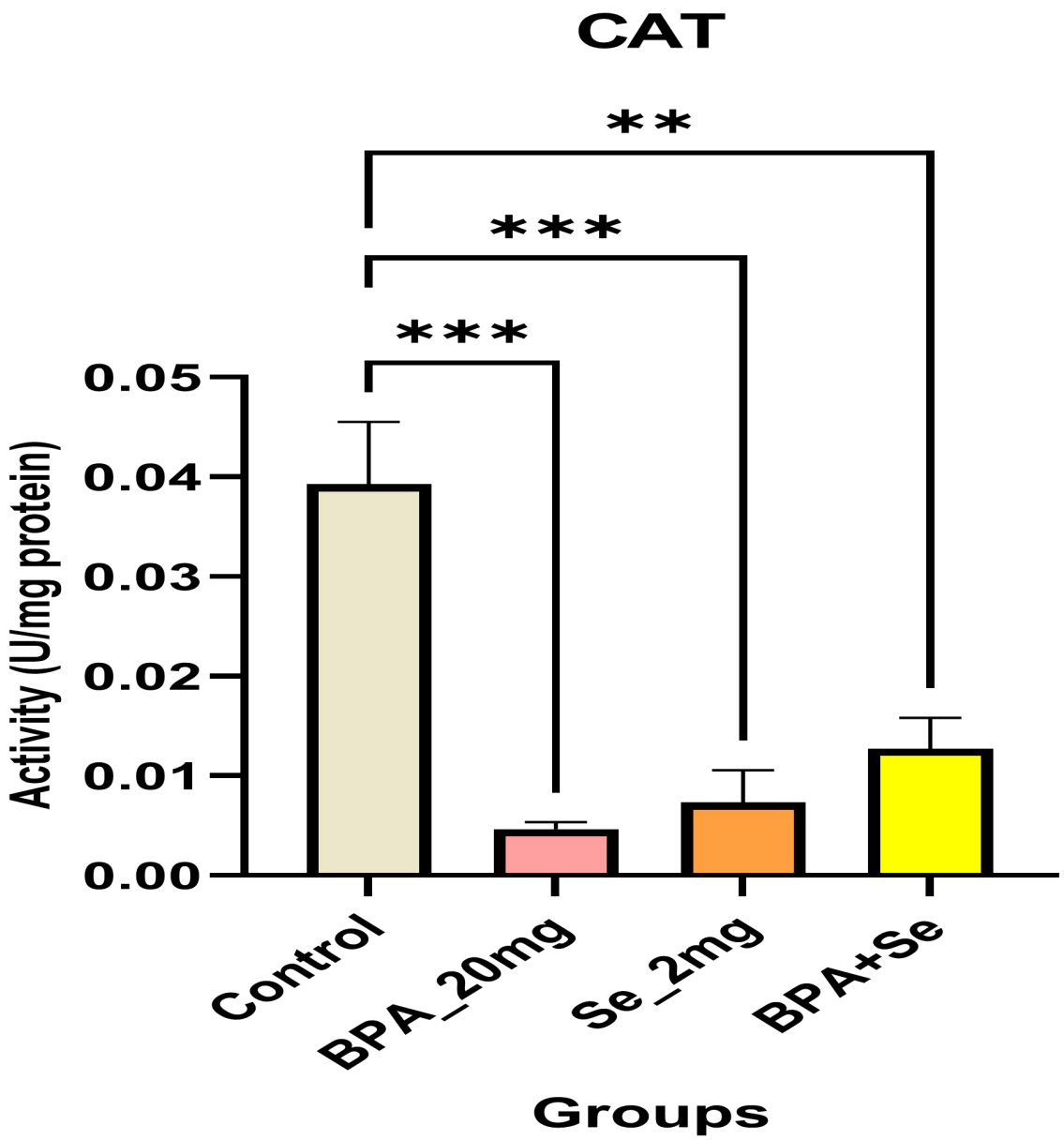

Figure 4: Effect of BPA and Se in singlet and combined forms on the activity of catalase in male rats.

Bars represent the activity of catalase in rats exposed to BPA and Se in singlet and combined form. (*) shows point of significant difference relative to the control ($p< 0.05$).

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The findings of this study indicate that exposure to Bisphenol-A (BPA) has detrimental effects on the body weight and oxidative stress status of adult male wistar rats. This is consistent with previous studies that have shown BPA to cause growth impairment and oxidative stress in rodents (Cabaton *et al*., 2011; Chitra *et al*., 2012). The significant decline in percentage weight change observed in rats administered with BPA, suggests that BPA may interfere with normal growth and development processes. This is supported by a study that found BPA to disrupt the hypothalamic-pituitary-adrenal axis, leading to growth impairment in rats (National Toxicology Program, 2008).

Furthermore, the inability of selenium (Se) to mitigate the harmful effects of BPA on body weight is noteworthy. This suggests that selenium (Se) may not be effective in counteracting the adverse effects of BPA on growth and development. In constrast, a study found that selenium supplementation reduced BPA-induced oxidative stress in mice (Kumar *et al*., 2014).

Also, the increased concentration of malondialdehyde (MDA) in rats exposed to BPA indicates enhanced lipid peroxidation and oxidative stress. This is consistent with previous studies that have shown that BPA to induce oxidative stress in rodents (Li *et al*., 2011; Chitra *et al*., 2012). The further increase in MDA levels observed in rats co-administered with BPA and Se suggests that Se may exacerbate the oxidative stress induced by BPA. This is in contrast to a study that found Se to reduce oxidative stress in rats exposed to cadmium (Khan *et al*., 2017).

Lastly, the significant reduction in reduced activities of oxidative stress enzymes (SOD, and catalase) in rats exposed to BPA and Se indicates impaired antioxidant defenses. This suggests that BPA and Se may disrupt the normal antioxidant mechanisms, leading to increased oxidative stress and damage. This is consistent with previous studies that have shown BPA to impair antioxidant defenses in rodents (Li *et al*., 2011; Chitra *et al*., 2012).

5.2 CONCLUSION

In conclusion, these findings show that Selenium (Se) supplementation did not mitigate the adverse effects of Bisphenol-A and instead worsened oxidative stress, implying that selenium may not provide protection against Bisphenol-A harmful effect even at this dose but rather potentiate the effect of Bisphenol-A.

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