NEURO-PROTECTIVE EFFECT OF VITAMIN C IN BISPHENOL-A INDUCED TOXICITY IN DROSOPHILA MELANOGASTER

BY

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BMS1802609

DEPARTMENT OF PHYSIOLOGY

SCHOOL OF BASIC MEDICAL SCIENCES COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN.

OCTOBER, 2023

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PHYSIOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR OF SCIENCE (BSc) DEGREE IN PHYSIOLOGY

OCTOBER, 2023.

CERTIFICATION

This is to certify that this project work was carried out by **Laura Oghogho EHIGIATOR (Miss) i**n the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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MR. G. O. ORIBHABOR

DATE

(Project Supervisor)

DEDICATION

This project work is dedicated to God Almighty for his love, faithfulness and mercy and also to my parents for their financial supports and advice.

ACKNOWLEDGEMENT

I want to first appreciate God for His mercies and grace that has brought me so far, for helping me realize many of my dreams within this great citadel of learning. To him be all the glory and adoration. To my supervisor, DR. Churchill A. Inneh, for being very patient, for his guidance, advice and corrections I say thank you, may the Almighty continue to provide the best for you and your family.

My sincere appreciation and gratitude go to my father Mr. Godday Onojiolu Ikuenobe and mother Mrs. Rita Onyinye Ikuenobe for their care, moral and financial support. I also appreciate my project group members, lest I forget the family and friends I made here on my adventure, Iwinosa, Silas, Whitney, Angel, Ijeoma, Yolanda, Zino, Shalom, Destiny, Michael, Festus, Louisa, Kene, Anabel, Davido, as well as my study group. Finally, I would want to express my gratitude to my elder brother Mr. Great Enoch, my clan Doctors house and Parie Ravat for all of their support.

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ABSTRACT

A common industrial chemical, bisphenol-A (BPA) is connected to oxidative stress, memory loss, learning impairment, reduced cholinergic function, and neuronal degeneration. BPA is also utilized in the manufacturing of polycarbonate, epoxy resins, and plastics. Ascorbic acid, another name for vitamin C, is a necessary substance that is involved in several biological activities. It has been proposed as a potential therapeutic intervention for oxidative stress because of its strong antioxidant properties, which shield the body from oxidative damage brought on by free radicals. On the other hand, opinions about the protective role of vitamin C in bisphenol-A-induced toxicity are divided. This research looked at the neuroprotective effects of vitamin C in the context of toxicity caused by bisphenol-A in Drosophila melanogaster. Three to five days ago, flies were divided into groups. Group 2 received a diet containing 1 mM of bisphenol-A (BPA), whereas Group 1 acted as the control group. 200 mM of vitamin C was given to Group 3 by food, whereas Group 4 received 200 mM of vitamin C plus 1 mM of BPA through food. For six (6) days, the flies were kept on these treatments at room temperature. To evaluate locomotor performance, an open field research and negative geotaxis were conducted (climbing activity and exploratory movement). Additionally, a 15-day survival research was conducted to look at the effects of vitamin C and bisphenol A on fly survival rates. After the experiment was over, the flies were homogenized, and the supernatants were used to measure the activities of glutathione S-transferase (GST), acetylcholinesterase (AChE), superoxide dismutase (SOD), catalase (CAT), malonaldehyde (MDA), and catalase-catalase. The survival rate, motility, and climbing activity (negative geotaxis) of flies treated with BPA were all significantly reduced. Additionally, the activities of AChE, MDA, Catalase, SOD, and BPA-treated flies were reduced. Vitamin C was able to considerably raise the flies' survival rate, motility, and climbing activity throughout the co-treatment procedure. It also lessened the effects of the BPA increase on AChE activity and MDA levels in these flies. Furthermore, vitamin C inhibited and BPA-induced reduction in GST activity was observed.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Bisphenol-A (BPA), 2, 2-bis (4-hydroxyphenyl) propane) is a chemical that is widely used in the production of epoxy resins, polycarbonate, and polyvinyl chloride plastics according to (Mertz, 2016). It is also present in the epoxy layer of canned foods (Rudel *et al*., 2011). BPA can therefore leak into food and beverages coming from these containers. BPA comes into contact with food materials through a variety of processes, including heating, excessive use, interaction with acidic or alkaline liquids, and microwave exposure (Vandentorren *et al*., 2011). BPA can also be found in a variety of healthcare products, including composites and dental sealants (Drozdz *et al*., 2011). BPA is widely used in industries, which is concerning as it can be harmful to various biological systems (Halden, 2010).

BPA is structurally similar to phenols and this has been attributed to be responsible for its toxicity (Shirani *et al*., 2018). BPA has been documented to cause neurotoxicity, hepatotoxicity, and nephrotoxicity in a number of animal models (Zhou *et al*., 2017). Oxidative stress has been reported to be an integral pathological mechanism by which BPA induce- toxicity. Increase in oxidative stress has negative impact on antioxidant enzymes and mitochondrial activity. Additionally, (Yin *et al.*, 2017) have shown that BPA also modifies inflammatory cytokines, leading to inflammation and immunological dysfunction. Research has shown that BPA causes neuroinflammation and death in the cells of the central nervous system, which results in aberrant neurological development in zebrafish during their early life stages (Gu *et al*., 2022). Prenatal exposure to BPA impacts the mevalonate (MVA) pathway in developing rat brains, according to studies on animal models (Tonini *et al*., 2020). The MVA pathway is crucial for the growth and health of the brain (Tonini *et al.*, 2020). Additionally, research has shown that BPA exposure produces oxidative stress in the brains of *drosophila*, which results in the degeneration of different types of neurons (Adesanoye *et al*., 2020). Flies exposed to BPA had reduced longevity, behavioral deficits, developmental delays, and an imbalance between antioxidant

and oxidative stress (Sarkar *et al*., 2021). The Parkinsonian-like alterations in flies caused by BPA appeared to be directly related to the oxidative stress. BPA has been reported to cross the blood-brain barrier (BBB) in different concentrations due to its lipophilicity (Wang *et al.,* 2019). Emerging body of evidence is accumulating that BPA exerts effects on the central nervous system (CNS) (Masami and Yoshinori, 2014) leading to various behavioral changes associated with cognitive impairment along with increased aggression, hyper-reactivity learning deficits, and increased drug dependency (Inadera, 2015). A study had shown its interference with the growth of neuronal networks, which control brain functions (Negri-Cesi, 2015). Another study reported that BPA causes neurological injury by damaging protein and lipid structures through free radicals mediated mechanisms (Agarwal *et al.,* 2016). A public health study had also revealed that prenatal BPA intoxication can adversely affect the behaviors and cognitive abilities of babies resulting to such as autism neurobehavioral complications depression, anxiety, spectrum disorders (ASD), and communication deficits (Perera *et al.,* 2016). In recent decades, the bioactive compounds from natural sources have been attracting growing attention. Phytochemicals, such as polyphenols in fruit, vegetables and medicinal plants have a wide range of health-protective effects on heart, kidney, liver and brain (Nassiri-Asl and Hosseinzadeh, 2009). Vitamin C, also known as ascorbic acid, is an essential nutrient that plays a vital role in many biological processes. It is a water-soluble vitamin that is not synthesizedin the body, and therefore, must be obtained through the diet or supplements (Carr andMaggini, 2017). It acts as a potent antioxidant, protecting the body against oxidativedamage caused by free radicals. Vitamin C also plays a crucial role in the synthesis of collagen, a protein that is essential for the health of connective tissues such as skin,bones, and cartilage. It is used as a food additive and a dietary supplement for its antioxidant properties (Pandey and Nichols, 2011).

Drosophila melanogaster is a dipteran insect, belongs to the *drosophilidae* family (Reiter *et al.*, 2001). It is a significant model to understand not only how genes cause diseases but also the finding of the link between such genes and diseases (Poddighe *et al*., 2013). This is due to the presence of more than 65-70% of human illness genes in *drosophila melanogaster* (Fortini and Bonini, 2000). Due to its

small size and brief lifespan, *drosophila melanogaster* has a fast reproductive cycle and is easier to maintain in large numbers in the lab than other models it has become a valid

alternative model for toxicological research (Sudatti *et al*., 2013), neurodegenerative studies (Hirth, 2010)

1.2 JUSTIFICATION OF STUDY

Vitamin C, a water-soluble vitamin with strong antioxidant capabilities, is crucial for preventing oxidative cell damage. According to reports, one of the key mechanisms by which BPA causes damage in a variety of biological systems is oxidative stress. This study looked at the protective role of vitamin C against bisphenol-A (BPA) induced toxicity in drosophila melanogaster because it has been shown that oxidative stress plays a significant role in the development of neurological disorders such as Parkinson's disease and Alzheimer's disease.

1.3 RESEARCH QUESTIONS

1. Will poisoning from bisphenol-A impact flies' ability to move and cause a locomotor deficit in them?
2. Will flies' levels of antioxidant enzymes decline and oxidative stress be brought on by bisphenol-A toxicity?
3. Will acetylcholinesterase levels in flies be affected by bisphenol-A toxicity?
4. Will vitamin C help flies treated with bisphenol-A with their locomotor impairment and survival rate?
5. Will vitamin C help flies treated for bisphenol-A poisoning in terms of antioxidant status?

1.4 AIM OF STUDY

The aim of this study is to investigate the protective effect of vitamin c in bisphenol- A induced toxicity in *drosophila melanogaster.*

1.5 SPECIFIC OBJECTIVES

The objectives of this study are as follows:

to look at how vitamin C and bisphenol-A poisoning affect the survival rate of flies.

to ascertain how vitamin C and bisphenol-A poisoning affect flies' ability to move.

to ascertain the impact of vitamin C and bisphenol-A poisoning on flies' antioxidant enzymes and oxidative stress indicators.

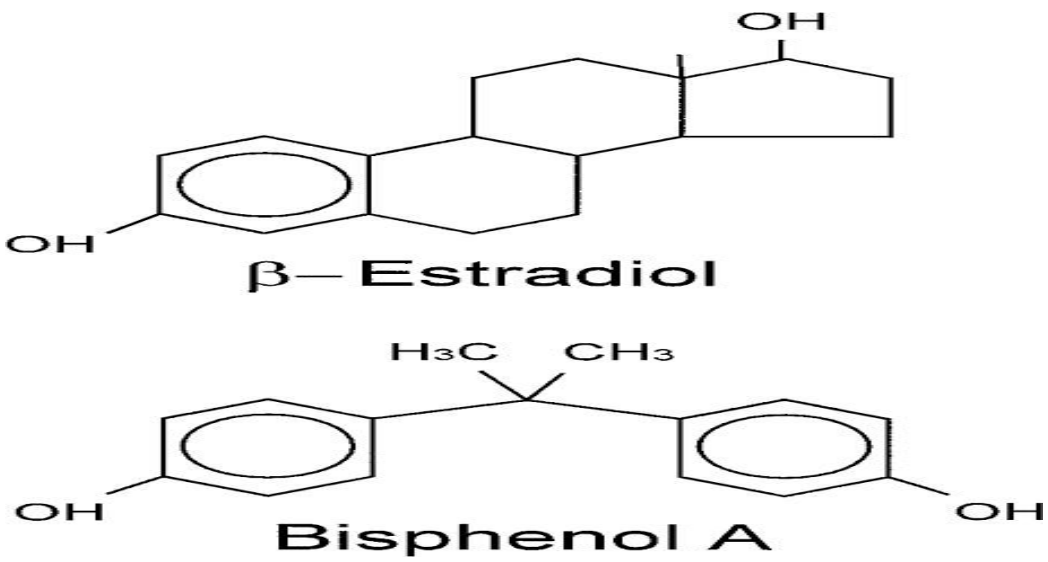
To look at how vitamin C and bisphenol-A poisoning affect the amount of acetylcholinesterase

CHAPTER TWO

LITERATURE REVIEW

2.1 BISPHENOL-A

Bisphenol-A (BPA) is a white, crystalline solid with the molecular formula $C_{15}H_{16}O_2$ that belongs to the organic chemical family. The most well-known use of BPA is in the manufacturing of polycarbonate plastics and epoxy resins, which are primarily utilized in the creation of water bottles, baby bottles, and other food and beverage containers. Manufacturers create around 8 billion pounds of BPA annually, making it one of the most commonly utilized industrial chemicals produced worldwide (Rubin 2011).



Chemical structure of bisphenol A and estradiol

2.1.1 BIOCHEMICAL PROPERTIES OF BISPHENOL-A

In 1891, Russian scientist A.P. Dianin made the first batch of BPA. Acetone and two equivalents of phenol condense to form the chemical, which is why the name ends with an A. It is made up of two linked functional groups of phenol. BPA is added to polycarbonate plastic to improve the end product's strength, clarity, and durability. BPA is included in epoxy resins, which extend the shelf life of canned goods, and dental composite resins, which are used to fill most cavities (Kingman et al., 2012).

2.1.2 PREVALENCE OF THE USE OF BPA

In the 1930s, it was discovered that the monomer bisphenol A (BPA) could stimulate the female reproductive system in rats much like estrone. BPA was initially created as a synthetic estrogen in the 1890s (Dodds and Lawson, 1936). According to Konieczna et al. (2015), 2.8 million metric tons of BPA were manufactured in total in 2002, while an estimated 5.5 million metric tons were produced in 2011. A data assessment conducted in 2009 estimated that at least 2.2 million tons of BPA were produced annually worldwide in 2009, with the United States contributing a fifth of the total. Since its discovery in 1891, BPA has been produced in large quantities, at least since the interwar period (Dodds and Lawson, 1936). As a result, BPA has ingrained itself into our ecology, exposing people to it almost everywhere. 93% of Americans over the age of six have measurable amounts of BPA in their urine, according to research by Calafat et al. (2008). According to Arnold et al. (2013), the highest measured BPA levels in drinking water in Europe was discovered to be 0.014 μg/1. They also noticed that the exposure levels were much lower than the claimed BPA harmful thresholds. This is in line with the UK's Food and Safety Agency's (www.gov.uk) current stance.

2.1.3 ENDOCRINE DISRUPTING CHEMICALS

As a well-known endocrine disruptor, BPA has been shown in lab experiments to bind to estrogen receptors and have estrogenic effects. BPA is as potent as 17-beta estradiol (E2) in terms of its estrogenic effects on responses mediated by non-nuclear estrogen receptors, although having a lower affinity for nuclear estrogen receptors (Arnold et al., 2013). Furthermore, by competing with endogenous E2, BPA may function as an antiestrogen and prevent the estrogenic response. BPA has the ability to bind directly to androgen receptors and may have antiandrogenic properties by inhibiting the activity of natural androgens (Vogel, 2005). It has been shown that BPA binds to thyroid receptors and affects thyroid function in both agonistic and antagonistic ways (Vogel, 2005; Arnold et al., 2013). The growing central nervous system, the endocrine pancreas, and the immunological system are among the various organs and physiological systems that BPA interacts with (Rubin, 2011). A significant amount of study is aimed at identifying which of the several molecular processes mediates the effects of BPA on various aspects of human health (Rochester, 2013).

2.1.4 EFFECTS OF BPA ON NEUROLOGICAL SYSTEMS

The biological effects of BPA have been the subject of several published research, and a thorough summary of the risks to human health has been provided (Vandenberg et al, 2009). BPA was first investigated for its effects on sexual dysfunction, malformations, and malignancies of the reproductive system due to its influence on the endocrine system.

BPA has been associated with metabolic diseases including obesity and diabetes as well as cardiovascular disease, according to recent population-based epidemiological research. Prior research has connected exposure to BPA to reproductive system anomalies and an increased risk of cardiovascular disease (Melzer et al., 2012). Over the last several decades, there has been a rise in the occurrence of neurodevelopmental diseases along with the surge in the manufacturing of harmful substances. There is growing evidence that environmental chemicals, such as BPA, may lead to neurodevelopmental abnormalities. After all, the National Toxicology Program (NTP) in the United

States came to the conclusion that "there is some concern for effects on the brain, behavior, and prostate gland in fetuses, infants, and children at current human exposures to BPA". Therefore, it is thought that brain systems are significant BPA targets.

Additionally, there is growing evidence that BPA may disrupt the development of the neurological system, as seen by the recent rise in the incidence of neurobehavioral problems (Visser et al., 2014). For instance, increased BPA levels in the urine during pregnancy have been linked to worse behavioral outcomes in kids (Harley et al., 2013). Nonetheless, there are still a lot of unknowns, and contentious debates are going on (Braun et al., 2009).

According to Saal et al. (2007), there is a connection between blood BPA levels in adult humans and a number of metabolic diseases, such as type 2 diabetes, abnormal liver function, breast and prostate cancers, endometrial hyperplasia, polycystic ovarian syndrome, and neurobehavioral issues like attention deficit and hyperactivity disorder.

According to research on animals, exposure to BPA has been shown to cause decreased sperm motility and count as well as increased prolactin hormone secretion (Steinmetz et al., 1997). Furthermore, a few studies have shown how BPA affects experimental animals' memory. Furthermore, it was shown that the hippocampal cholinergic system in mice raised in utero and neonatal chronic exposure to BPA decreased memory (Xu et al., 2010). According to recent proposals, exposure to specific BPA may be linked to the development of neurodegenerative illnesses and disorders (Solleiro et al., 2020). N-methyl-D-aspartate receptors (NMDARs) are a particular kind of ionotropic glutamate receptor located in the hippocampus area of the midbrain. They are essential in regulating synaptic plasticity and cognition, and this is one of the fundamental mechanisms in the central nervous system (CNS) (Gilmour et al., 2012). The primary location for memory and learning is the hippocampus. It has been shown that BPA reduces the expression of the estrogen receptor and NMDAR subunits in the hippocampal regions (Xu et al., 2011). While many research on the effects of BPA in vivo and in vitro have been published about its impacts on biological systems, there is

disagreement over whether exposure to BPA at normal levels might be harmful to people. On the other hand, growing data suggests that BPA negatively impacts brain development, which is concerning given the rising prevalence of developmental problems (Inadera, 2015).

2.1.5 EFFECT OF BPA EXPOSURES IN DROSOPHILA MELANOGASTER (FRUIT FLY)

disrupting chemical (EDC) (Wetherill et al., 2007; Vandenberg et al., 2009). BPA's endocrine-disturbing effects in vertebrates are caused by its structural resemblance to estradiol, which facilitates its binding to different ER subtypes (Wetherill et al., 2007). Nevertheless, drosophila lacks genetic encoding for ERs. They do contain an estrogen-related receptor (dERR), however, which does not bind estrogen despite belonging to the same nuclear receptor superfamily as ERs (Tennessen et al., 2011; Giguere, 2002). However, ERRs have the ability to bind several ER ligands, suggesting that their binding specificity may overlap (Giguere, 2002). According to Tennessen et al. (2011) and Misra et al. (2017), dERR activity is required for metabolism, mitochondrial function, testicular development, and testicular function in drosophila. Although research using the harlequin fly Chironomus riparius showed that BPA may boost the expression of genes important to ERR activity, no binding experiment has directly examined BPA's capacity to bind ERRs in any creature (Park and Kwak, 2010).

BPA has the ability to affect how an embryo or fetus develops neurologically because of its lipophilic makeup, which allows it to easily permeate cell membranes, including the placenta and the blood-brain barrier (Nishikawa et al., 2010). Throughout pregnancy and the first few years of life, BPA exposure has shown connected in research to children's behavioral problems. For example, positive correlations between BPA and diagnoses of autism spectrum disorder (ASD) were found by detecting BPA in household items and in the urine of pregnant women exposed to such products, then tracking the developmental outcomes of their offspring (Braun et al., 2011). Studies have shown that BPA

exposure during development influences the behavioral and neurological characteristics of progeny in both fruit flies and rats (Kinch et al., 2015).

2.1.6 BPA TOXICITY MARKED BY OXIDATIVE STRESS

Oxidative stress has been associated with aging, cardiometabolic diseases, immunological disorders, neurodegenerative illnesses, neuronal degeneration, and the beginning and progression of cancer, via a variety of poorly understood pathways (Lenaz, 2012). Redox equilibrium has to be controlled in order to maintain cellular homeostasis, development, growth, and survival. Actually, created by a healthy cellular metabolism, reactive oxygen species (ROS) include superoxide anions, peroxides, and hydroxyl radicals. These radicals are essential to the well-coordinated process of redox equilibrium. Reactive nitrogen species (ROS) include molecules containing nitrogen in addition to radical and non-radical oxygen derivatives (e.g., peroxynitrite and nitric oxide). Many parts of the cell cooperate to keep the redox balance in check so that excessive ROS and its detrimental consequences (mutations, uncontrolled cell division, and heightened sensitivity to signals of cell death) are avoided. BPA may cause direct or indirect oxidative balance disruption by upregulating oxidative mediators, downregulating antioxidant enzymes, and identifying mitochondrial apoptotic activation, alterations to cell signaling pathways, and dysfunction (Tavakkoli et al., 2020). BPA induces oxidative stress in rat liver and epididymal sperm by increasing lipid peroxidation and hydrogen peroxide while decreasing the antioxidant enzymes catalase, glutathione reductase (GR), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px).

Because postmenopausal women have such low hormone levels, xenoestrogen BPA may bind to the ER more widely, which can produce undesirable cellular responses including oxidative damage and inflammation. Chronic inflammation is aided in its growth by immune cells that release nitrogen species and reactive oxygen species, such as neutrophils and macrophages. Numerous studies have indicated an increase in these compounds in organisms exposed to BPA (Rochester, 2013).

Malondialdehyde (MDA), a marker of lipid peroxidation, and 8-hydroxy-deoxyguanosine (8-OHdG), a marker of DNA oxidation, have both been widely considered as potential biomarkers for oxidative stress Kasai, (1997); Tsikas, (2017).

According to Wang et al. (2019), exposure to BPA also raises the production of pro-inflammatory cytokines in a variety of tissues and organs, including serum, the colon, the liver, and interleukin (IL)-1, IL-6, IL-8, and TNF. BPA produces hydrogen peroxide species and ROS, which trigger NF-B. (Cho and others, 2018). (Moon et al., 2012) discovered that BPA exposure at low levels results in hepatic damage and liver mitochondrial malfunction. Furthermore, this pollutant causes cytochrome c release, ATP depletion, loss of mitochondrial mass, and membrane potential as well as changes in the expression of genes connected to metabolism and mitochondrial function (Lin et al., 2013).

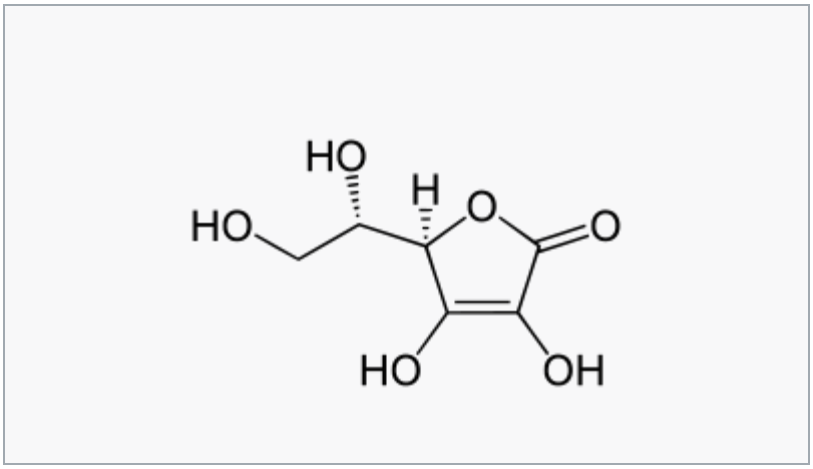
2.2 VITAMIN C

Vitamin C, also known as ascorbic acid, an organic compound with formula C6H8O6, originally called hexuronic acid. It is a white solid, but impure samples can appear yellowish. It dissolves well in water to give mildly acidic solutions. It is a mild reducing agent.

Ascorbic acid exists as two enantiomers (mirror-image isomers), commonly denoted "L" (for "levo") and "D" (for "dextro"). The L isomer is the one most often encountered: it occurs naturally in many foods, and is one form ("vitamer") of vitamin C, an essential nutrient for humans and many animals. Deficiency of vitamin C causes scurvy, formerly a major disease of sailors in long sea voyages. It is widely recognized for its antioxidant properties and its involvement in collagen synthesis, immune function, and iron absorption (The American Society of Health-System Pharmacists, 2016).It is a micronutrient required for multiple biological functions, it is necessary for normal growth and development and is an essential enzyme cofactor for several enzymes in the post-translational hydroxylation of collagen, biosynthesis of carnitine, conversion of the neurotransmitter dopamine to norepinephrine, peptide amination, and in tyrosine metabolism. It is also an antioxidant that helps

protection against infection and iron absorption. Some animal species have lost the capacity for l-ascorbate synthesis, for that reason, they are dependent upon diet to ensure adequate levels of vitamin C for metabolism and oxidative protection. The high l-ascorbate contents found in plants make them the primary source of vitamin C intake for humans (Hacışevki, 2009). Vitamin C is one of the potent reducing agents and scavenger of free radicals in biological systems, working as a scavenger of oxidizing free radicals and harmful oxygen-derived species, such as hydroxyl radical, hydrogen peroxide

(H_2O_2), and singlet oxygen (Arrigoni et al., 2002).



Structure of Vitamin C

2.2.1 HISTORY OF VITAMIN C

Vitamin C was discovered in 1912, isolated in 1928 and synthesized in 1933, making it the first vitamin to be synthesized (Squires, 2011), Shortly thereafter Tadeus Reichstein succeeded in synthesizing the vitamin in bulk by what is now called the Reichstein process (Stacey*et al*., 1978). This made possible the inexpensive mass- production of vitamin C. In 1934 Hoffmann–La Roche trademarked synthetic vitamin C under the brand name Redoxonand began to market it as a dietary supplement (Wang and Xu H,2016). In 1907 a laboratory animal model which would

help to identify the antiscorbutic factor was discovered by the Norwegian physicians Axel Holst and Theodor Frølich, who when studying shipboard beriberi, fed guinea pigs their test diet of grains and flour and were surprised when scurvy resulted instead of beriberi. By luck, this species did not make its own vitamin C, whereas mice and rats do (Norum and Gray, 2002). In 1912, the Polish biochemist Casimir Funk developed the concept of vitamins. One of these was thought to be the anti- scorbutic factor. In 1928, this was referred to as "water-soluble C", although its chemical structure had not been determined (Rosenfeld, 1997).From 1928 to 1932, Albert Szent-Györgyi and Joseph L. Svirbely's Hungarian team, and Charles Glen King's American team, identified the anti-scorbutic factor. Szent-Györgyi isolated hexuronic acid from animal adrenal glands, and suspected it to be the antiscorbutic factor (Svirbely and Szent-Györgyi, 1932). In 1957, J. J. Burns showed that some mammals are susceptible to scurvy as their liver does not produce the enzyme L-gulonolactone oxidase, the last of the chain of four enzymes that synthesize vitamin C (Burns and Evans, 1956). American biochemist Irwin Stone was the first to exploit vitamin C for its food preservative properties. He later developed the idea that humans possess a mutated form of the L-gulonolactone oxidase coding gene (Henson et *al.*, 1991). In 2008, researchers at the University of Montpellier discovered that in humans and other primates the red blood cells have evolved a mechanism to more efficiently utilize the vitamin C present in the body by recycling oxidized L-dehydroascorbic acid (DHA) back into ascorbic acid for reuse by the body. The mechanism was not found to be present in mammals that synthesize their own vitamin C (Montel *et al*., 2008).

2.2.2 Sources of Vitamin C

For vitamin C, fruits and vegetables are the finest sources. The main sources of vitamin C include potatoes, tomatoes, and tomato juice, as well as citrus fruits. Cantaloupe, broccoli, strawberries, Brussels sprouts, kiwifruit, red and green peppers, and broccoli are some more nutritious dietary options. Granted, grains don't natively contain vitamin C, but certain fortified morning cereals do. Because ascorbic acid is water soluble and degraded by heat, cooking and extended storage may lower

the vitamin C concentration of food. Microwaving or steaming might reduce cooking losses. Luckily, a lot of the greatest vitamin C-containing foods, such fruits and vegetables, are best eaten uncooked. Over 200 mg of vitamin C may be obtained by consuming five different portions of fruits and vegetables each day (U.S. Department of Agriculture, 2019)

Table 2: Vitamin C Content of Selected Foods (U.S. Department of Agriculture, 2019)

Food	**Milligrams (mg) per serving**	**Percent (%) DV***
Red pepper, sweet, raw, ½ cup	95	106

Table 2: Vitamin C Content of Selected Foods (U.S. Department of Agriculture, 2019)

Food	Milligrams (mg) per serving	Percent (%) DV*
Orange juice, ¾ cup	93	103
Orange, 1 medium	70	78
Grapefruit juice, ¾ cup	70	78
Kiwifruit, 1 medium	64	71
Green pepper, sweet, raw, ½ cup	60	67
Broccoli, cooked, ½ cup	51	57
Strawberries, fresh, sliced, ½ cup	49	54
Brussels sprouts, cooked, ½ cup	48	53
Grapefruit, ½ medium	39	43

Table 2: Vitamin C Content of Selected Foods (U.S. Department of Agriculture, 2019)

Food	Milligrams (mg) per serving	Percent (%) DV*
Broccoli, raw, ½ cup	39	43
Tomato juice, ¾ cup	33	37
Cantaloupe, ½ cup	29	32
Cabbage, cooked, ½ cup	28	31
Cauliflower, raw, ½ cup	26	29
Potato, baked, 1 medium	17	19
Tomato, raw, 1 medium	17	19
Spinach, cooked, ½ cup	9	10
Green peas, frozen, cooked, ½ cup	8	9

2.2.3 Antioxidant Activity of Vitamin C

The antioxidant properties of vitamin C are among its most well-known uses. Reactive oxygen species (ROS) and free radicals may be scavenged by vitamin C, shielding cells from oxidative damage. Supplementing with vitamin C has been shown in many studies to improve antioxidant defense systems and lower oxidative stress (Carr and Maggini, 2017). According to research by Carr et al., vitamin C supplements improved overall antioxidant capacity and decreased oxidative stress indicators in healthy people (Carr et al., 2009). To enhance its total antioxidant activity, vitamin C has also been shown to regenerate other antioxidants, including vitamin E (Packer et al., 2001).

2.2.4 Biosynthesis and molecular structure of vitamin C

In animals, biosynthesis of vitamin C is included in the glucuronic acid metabolic pathway, which is involved in the metabolism of sugars under normal and disease conditions, and in regulation of physiological functions (Figure 1a). Glucuronic acid metabolic pathway is also an important pathway for detoxification processes (Hacışevki, 2009). While most animals can convert d-glucose into l-ascorbic acid, humans and other primates, guinea pigs, some fish and birds, and insects are unable to produce ascorbic acid endogenously. The major plant pathway is different from the animal l-ascorbate synthesis pathway that involves 10 enzymatic steps from d-glucose to l-ascorbate via the intermediate formation of GDP-d-mannose and l-galactose (Hacışevki, 2009) (Figure 1a).

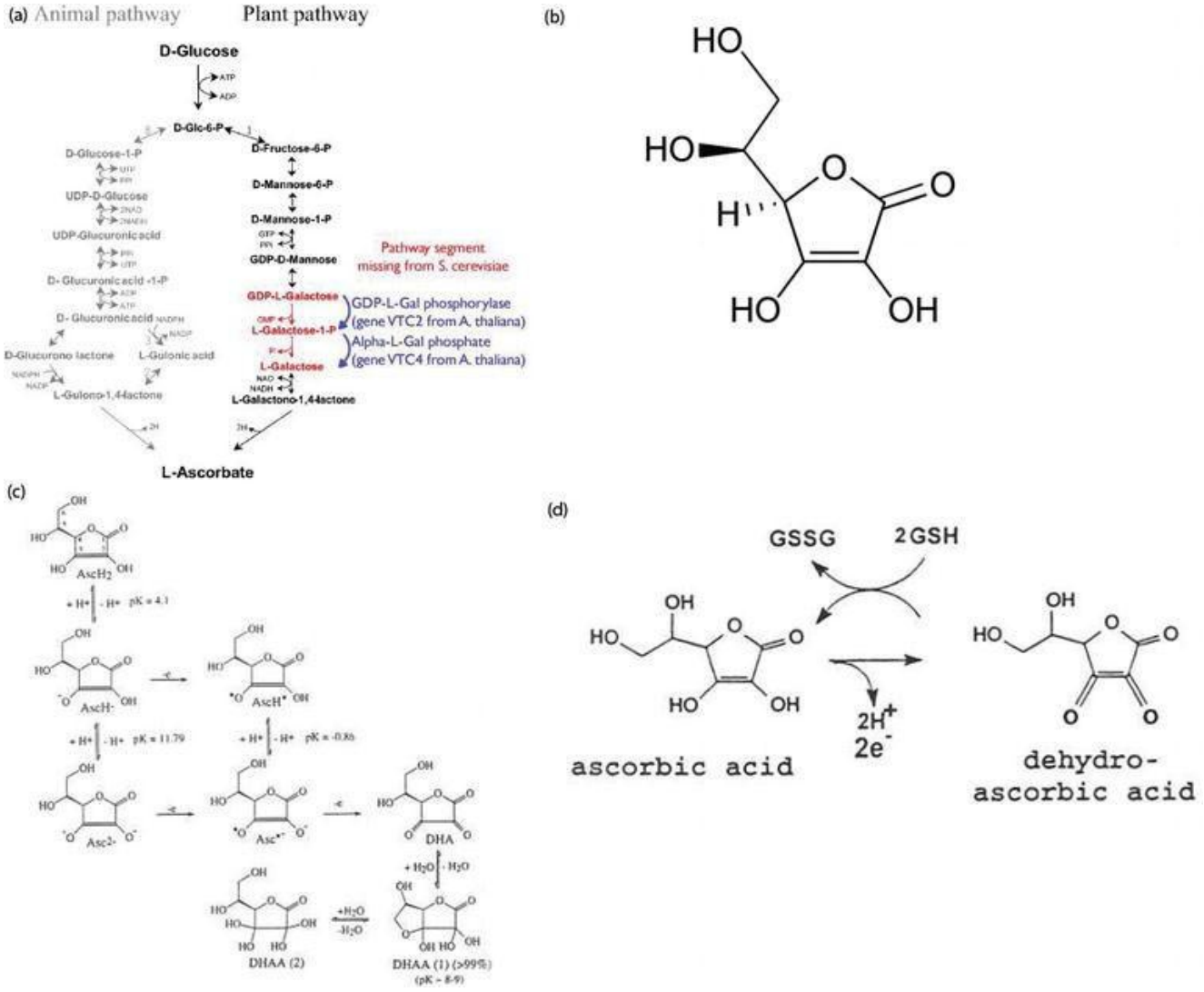


Figure 1.

(a) Diagram for the vitamin C pathway (Sauer *et al*., 2004); (b) molecular structure of l-ascorbic acid (vitamin C); (c) the equilibrium and redox species in the ascorbic acid-dehydroascorbic acid system; (d) ascorbic acid and dehydroascorbic acid. The oxidized form, dehydroascorbic acid, can be reduced back to ascorbic acid by glutathione (GSH).

Vitamin C (l-ascorbic acid) is a dibasic acid with an enediol group built into a five- membered heterocyclic lactone ring (Figure 1b). The chemical and physical properties of ascorbic acid are related to its structure (Aguirre and May, 2008). The structure of dehydroascorbic acid, the first oxidation product of ascorbic acid, has been analyzed by X-ray crystallography to be a dimer (Figure

1c). Electrochemical studies have indicated that ascorbic acid and dehydroascorbic acid form a reversible redox couple (Figure 1d).

2.2.5 Immune Function of Vitamin C.

Numerous studies have been conducted on the possible effects of vitamin C on the immune system. It is essential for maintaining both the innate and adaptive immune systems. Numerous immune cells, such as neutrophils, lymphocytes, and phagocytes, have been shown to be stimulated in number and activity by vitamin C (Hume and Weyers, 1973). Furthermore, it increases the synthesis of chemokines and cytokines, which control the activity of immune cells (Hemilä et al., 2004). Regular vitamin C intake was shown to minimize the length and intensity of common cold symptoms, according to a systematic study by Hemilä et al. (Fowler, 2017). Additionally, it has been proposed that vitamin C may help with wound healing and sepsis, among other illnesses (Wilson, 2017).

2.2.6 Collagen Synthesis and Tissue Repair

Collagen production, which is necessary for tissue repair, wound healing, and connective tissue maintenance, depends on ascorbic acid. According to Carr and Frei (1999), vitamin C functions as a cofactor in the enzymatic events that result in the production of collagen. Numerous

Research has highlighted the role that vitamin C plays in the healing of wounds. For instance, a randomized controlled experiment conducted by Marik et al. showed that surgical patients' recovery rates were enhanced by high-dose vitamin C supplementation (Marik et al., 2020). Furthermore, low vitamin C levels may cause problems with collagen formation and lead to diseases like scurvy.

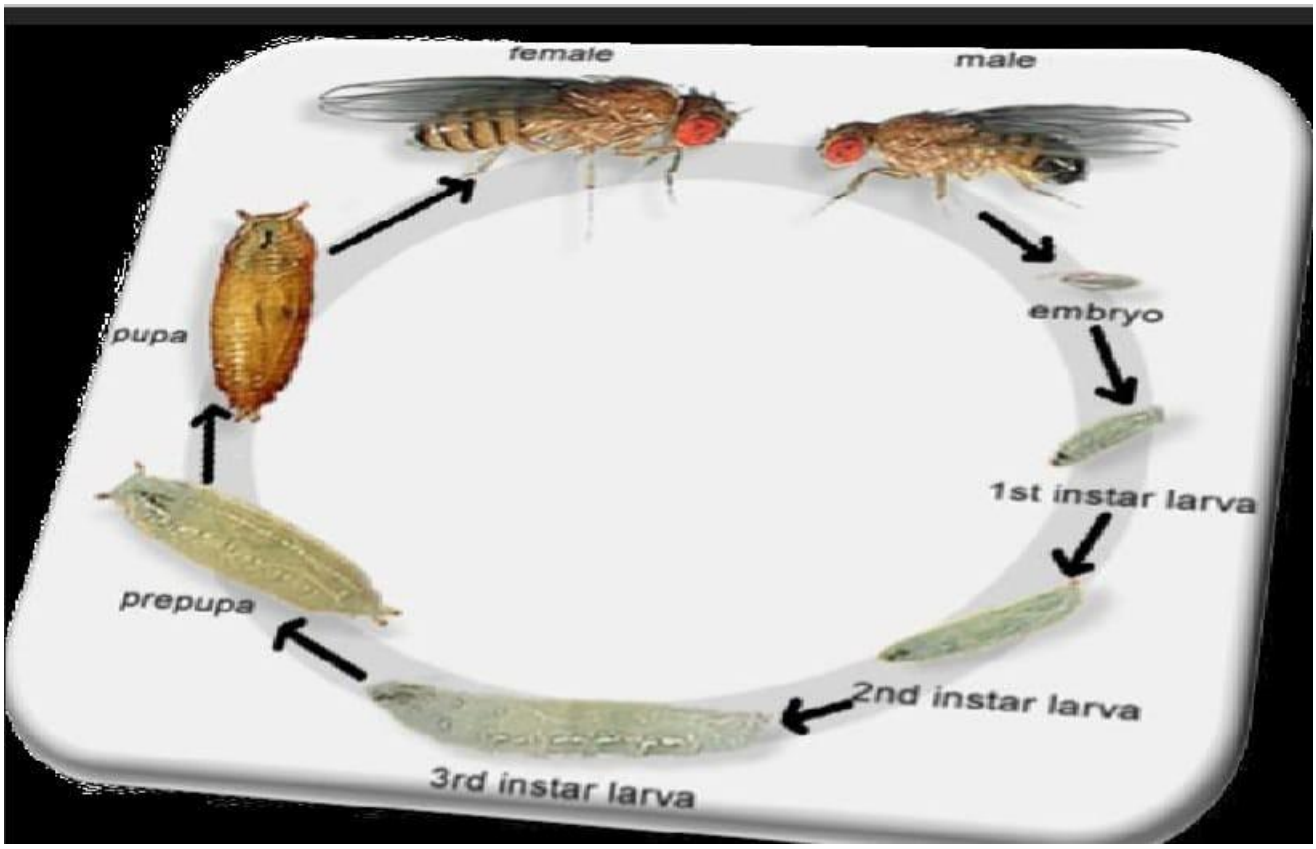
2.2.7 Effect of Vitamin C on Cardiovascular Health

Vitamin C's possible impact on cardiovascular health has drawn attention. According to epidemiological research, the risk of cardiovascular illnesses and vitamin C consumption are inversely correlated (Ye et al., 2013). According to Lykkesfeldt and Tveden-Nyborg (2019), vitamin C may have cardioprotective benefits via a variety of mechanisms, such as its antioxidant activity, enhancement of endothelial function, and decrease in inflammation linked to oxidative stress. Clinical investigations on the impact of vitamin C supplementation on cardiovascular outcomes,

however, have produced conflicting findings; some have shown no appreciable advantages (Levine et al., 1996)).

2.3 DROSOPHILA MELANOGASTER

An anthropod belonging to the Drosophilidae family is the fruit fly, or Drosophila melanogaster. It is a member of the same insect order as the dipteran, or two-winged. This insect was first used as a model in biology around a century ago, and it has subsequently inspired many developments in genetics and related fields (Sepel and Loreto, 2010). The anatomy of the fruit fly makes it unique, with its wings and complex eyes among its features. Depending on its diet, it may live for 40 to 120 days and the degree of stress posed by its environment, including temperature and human density. Fruit flies provide a fascinating model because of the significant genetic similarities between the two species and the comparable molecular pathways that impact longevity and aging in humans and fruit flies. Since around 65-70% of human disease genes are found in fruit flies, fruit flies are a useful model to research not only how genes create illnesses but also the relationship between such genes and diseases (Pandey and Nichols, 2011; Poddighe et al., 2013). The fruit fly is widely used as a model organism in the domains of genetics, biochemistry, cell biology, and developmental biology, according to Fortini and Bonini (2000). In recent decades, it has been used as a model to explain human disorders and as a starting point for toxicological research (Paula et al., 2013; Sudatti et al. 2013).



Life cycle of the fruit fly

2.3.1 HISTORY OF FRUIT FLY

Fruit flies have a brief life cycle; at 25°C, ten to twelve days may pass between a viable mating pair and hundreds of genetically identical progenies. Another interesting feature of the bug is its ability to take on several forms. Thus, its embryo, larva, pupa, and adult may all be used as examples in various situations. For instance, although the embryo, pupa, and adult may all be utilized as models in developmental research, the larva can be used to study physiological and behavioral processes. It is noteworthy that the adult fly has complex and sophisticated systems. Its anatomical features are capable of carrying out functions similar to those of the lung, kidney, stomach, and heart in mammals, which resulted in discoveries ranging from the definition of gene structure to the identification of crucial genes involved in embryogenesis. These genes have since been linked to typical mammalian development. The fruit fly is the first significant complex organism whose entire genome has been sequenced (Adams *et al*., 2000). When the human genome was sequenced a few years later,

homologies between the fly genome and the human genome were found as a direct result of this incident. The use of the fly as a model to comprehend human biology and disease processes was later supported by this. Because molecular investigations are frequently first clarified in straightforward creature models, like the fly, and then translated to mammalian systems, the fly has continued to draw interest.

2.3.2 FRUIT FLY CULTURE AND MEDIUM

Fruit flies are short-lived, thus when employing them for study, it's important to have fly cultures on hand in case of emergency. It is best to use consistent bottles and vials for fly cultivation and transfer. The vials are carefully cleaned and sterilized to prevent the transmission of illness. There are several typical media compositions for fruit flies. In our lab, for example, we maintain and develop flies on a cornmeal medium (1% w/v brewer's yeast, 2% w/v sucrose, 1% w/v powdered milk, 1% w/v agar, 0.08% v/w nipagin) with a 12-hour light/dark cycle at a constant temperature and humidity (23+1oC and 60% relative humidity, respectively). The diets used by Li et al. (2007) and Peng et al. (2009) were based on 105g of cornmeal, 21g of yeast, 105g of glucose, and 13g of agar; 0.4% of ethyl 4-hydroxybenzoate was then added to the diet to stop the formation of mold (Hugo and Peter, 2008.

2.3.3 IT'S USE IN ASSESSING OXIDATIVE STRESS AND ANTI-OXIDANT BIOMARKERS

In the field of biomedical research, we are living in a time of free radical and oxidative stress. Actually, oxidative stress has been connected by experimental evidence to the pathophysiology of many health issues (Hwa, 2013); nevertheless, the precise mechanisms by which oxidative stress and free radicals contribute to disease pathology remain unknown. For this reason, studying oxidative stress and free radicals in animal models is crucial right now. Numerous studies that have been reported in the literature have evaluated antioxidants and oxidative stress indicators using fruit flies.

Each test may call for a different procedure based on the quantity of the indicators the toxicologist is interested in. To identify biochemical and molecular markers of interest to the toxicologist, for example, the procedure of homogenizing the treated and control flies in appropriate buffers, centrifuging at an appropriate speed and temperature, and using the separated supernatants can be employed (Sudayi et al., 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 CHEMICALS AND REAGENT

Analytical grade materials and reagents were used in this experiment. In Benin-City, Edo state, Nigeria, Pyrex Chemicals was the supplier of vitamin C and bisphenol-A. Pyrex Chemicals in Benin-City, Edo state, Nigeria, is the source of other chemicals used, including phosphate buffer, acetylthiocholine iodide, 5,5'-dithiobis-2-nitro-benzoicacid (DTNB), and adrenaline.

3.2 THE FRUIT FLY STOCK CULTURE

A wild-type fly (Harwich) stock culture was acquired from the Central Research Laboratory, University of Benin, Benin City. The stock culture was originated from the National Species Stock Culture, Bowling Green, Ohio, USA. The flies were raised in the Drosophila laboratory of the Central Research Laboratory, University of Benin, Benin City, at room temperature (24 °C), with a 12-hour light/dark cycle, on a cornmeal medium containing 1% w/v agar, 1% w/v brewer's yeast, 1%, 2% w/v sucrose, and 0.08% w/v nipagin. Over the duration of the experiment, the same strain of Drosophila melanogaster was useda.

3.3 SURVIVAL STUDY

In order to determine appropriate dose of vitamin C to utilize for this study and the effect of BPA toxicity on survival of flies, a 15 days survival study was carried out Flies were divided into 6 groups with 30 flies per group. Group 1 served as our control and receive their normal corn meal diet; Group 2 were treated with 1 mM BPA while Group 3 and 4 were treated with 100mM and 200mM of vitamin C respectively (via their diet) and Group 5 and 6 were treated with 100mM of vitamin C + 1BPA and 200mM of vitamin C + 1BPA respectively for 15days. The flies were observed daily for mortality, and the survival rate was determined by counting the number of dead flies during the 15

days period (Abolaji *et al*., 2014). The survival rates were analyzed, 200mM concentration of Vitamin C produced the least mortality in flies than 100mM which informed the choice of 200mM concentration of vitamin C used for the ameliorative part of this study.

3.4 EXPERIMENTAL DESIGN

The meal preparation is as follows: For a standard meal, 850 ml of water was measured, and 150 ml of this was measured out to mix with the 52g measured corn meal. The remaining 700ml of water was poured into a clean pot. 7.9g of agar was measured and poured slowly into the pot and stirred to ensure a smooth meal. This is followed by the addition of the corn meal with continuous stirring, 3.5g of glucose was added and stirred. Then add the measured 5g of dissolved yeast and stir. Then dissolve 1g of the measured nipagin in 2ml of ethanol. After a few minutes, the corn meal was poured into the desired containers. The flies were fed with the standard formulated diet of corn meal medium.

The flies were allowed to mate in vials monitored under a regulated temperature until the eggs metamorphosed into young adult fruit flies under a natural photoperiod of about 12 hours light and dark daily for a period of administration of BPA and vitamin c.

The flies were divided into six groups with each treatment tube containing 30 flies.

Group 1: Control flies fed on 9.8g of basal diet.

Group 2: Flies fed on basal diet + 1mM of BPA

Group 3: Basal diet + 100mM of Vitamin C

Group 4: Basal diet + 200mM of Vitamin C

Group 5: Basal diet + 100mM of Vitamin C + 1mM of BPA

Group 6: Basal diet + 200mM of Vitamin C+ 1mM of BPA

The experiment lasted about 22 days and the flies were monitored daily and the mortality rate were recorded for plotting survival curve graph. Negative geotaxis was observed to check locomotor deficit. This process involves immobilization using mild ice anesthesia and a 6cm marked treatment

tube. The number of flies capable of crossing above the 7cm mark are recorded and repeated three times to take the average and also field study was carried out to also check for locomotor deficit. This process also involves immobilization using mild ice anesthesia and paper which contains boxes which measures 1cm by 1cm. The number of flies capable of crossing the number of squares per seconds. Eventually, the flies were homogenized and centrifuged and the supernatant were collected for biochemical analysis.

3.5 NEGATIVE GEOTAXIS ASSAY

This test is used to measure the flies' climbing activity or locomotor performance. As previously mentioned by Abolaji et al., 2018, this test was conducted. Each group's ten (10) flies were rendered immobile using a little ice anesthetic. After that, they were arranged individually in vertical glass columns with labels (11.5 cm in length and 2.9 cm in diameter). The flies were permitted to ascend after they had recovered from their exposure to the ice by lightly tapping the bottom of the column. The number of flies that stayed below the 9 cm mark of the column after this time was recorded, as well as the number of flies that climbed up to and over this mark in 5 seconds. To score climbing activity, one had to indicate the percentage of flies that climbed over the 8.5cm barrier. This process was repeated one minute later. Three repetitions in all were carried out.

3.6 OPEN FIELD STUDY ASSAY

Through the open field test, it is possible to evaluate the spontaneous locomotor capacity of the flies. The test was performed according to the methodology established by Connolly (1966), with some adaptations. one fly from each group were used, totalling 12 flies used for the test application. The flies were immobilized under mild ice anesthesia and separately added to a transparent polycarbonate petri dish (9 mm in diameter) with the aid of a soft bristle brush. The plate cover was graduated with squares measuring 1 cm each. Thus, in order to evaluate the spontaneous locomotor activity of the flies, the number of quadrants walked by the flies was observed and visually counted with the help of a manual counter, triggered at each crossed square by the fly, during 60 s timed with the aid of a stop watch. The test was performed in duplicate and the mean values were calculated.

3.7 DETERMINATION OF BIOCHEMICAL INDICES.

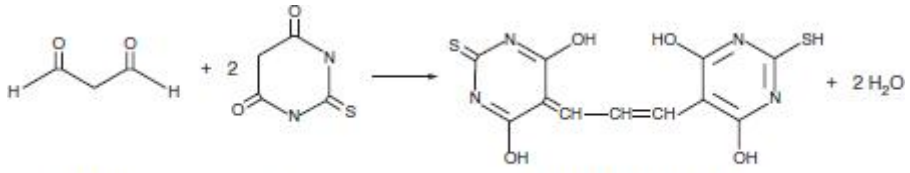
3.7.1 Assay of Lipid Peroxidation Biomarker: Malondialdehyde (MDA)

Concentrations of MDA in the samples are estimated by the thiobarbituric acid reactive substance (TBARS) assay. Assay of TBARS measures malondialdehyde (MDA) present in the sample, as well as malondialdehyde generated from lipid hydroperoxides by the hydrolytic conditions of the reaction. Assay of TBARS was done according to the method of Varshney and Kale (1990).

3.7.1.1 Relevance and Principle of TBARS assay;

MDA which is formed from the breakdown of polyunsaturated fatty acids serves as a convenient marker for the determination of the extent of lipid peroxidation. Assay is based on the reaction of MDA with thiobarbituric acid (TBA), forming a MDA-2TBA adduct (Pink-red coloured complex) that absorbs light at 535nm.

Reaction equation;



Reagents: Trichloroacetic acid/Thiobarbituric acid (TCA/TBA) Solution, distilled water.

3.7.1.2 Preparation of TCA/TBA solution

I. 63ml of concentrated HCL was added to 37.5g TCA and 0.94g TBA in a clean beaker

II. The volume was made up to 250ml with distilled water

III. The solution was placed in water bath at 100ºC and stirred evenly to dissolve

the particles

IV. The reagent was allowed to cool and stored in brown bottle.

3.7.1.3 Procedure for TBARS Assay;

- 25µl of serum sample was dispensed into a clean test tube
- 50µl of TCA/TBA working solution was added
- 0.925ml of distilled water was added
- The mixture was placed in a boiling water bath for 15 minutes
- This was centrifuged after cooling
- The absorbance of the supernatant fluid was read at 535nm using reagent blank.

Protocol Table

	Sample	Blank
Serum	25µl	
TBA/TCA Solution	50µl	100µl
Distilled water	925µl	950 µl

Calculation:

$$\text{TBARS activity (mmol/ml)} = \frac{\text{Absorbance of test x V x 1000}}{\text{A x v x L x Y}}$$

Where;

V = Total volume to which test was diluted

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

v = Volume of serum sample used

Y = Total protein estimated for a particular sample

L =Length path = 1cm.

3.7.2 Assay of Superoxide Dismutase (SOD)

Activity of serum Superoxide dismutase enzyme was assayed according to the kinetic method of Misra and Fridovich, 1972.

Relevance of assay:

Superoxide dismutase is class of enzymes that catalyse the dismutation of superoxide into oxygen and hydrogen peroxide. As such they are an important antioxidant defence in nearly all cells exposed to oxygen.

$$2O_2^- + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

3.7.2.1 Assay Principle:

Adrenaline auto-oxidises rapidly in aqueous solution to adrenochrome, whose concentration can be determined at 420nm. The auto-oxidation of adrenaline depends on the presence of superoxide anions. The enzyme, SOD inhibits the auto-oxidation of adrenaline by catalysing the breakdown of superoxide anions. The degree of inhibition is thus a reflection of the acyivity of SOD, and is determined at one unit of the enzyme activity.

3.7.2.2 Reagents and preparation:

Adrenaline; 0.1g of adrenaline salt was dissolved in solution of 25ml concentrated HCl and volume was made up to 100ml with distilled water.

Phosphate buffer (Ph = 8.0)

3.7.2.3 Assay Procedure Protocol:

	Blank	Reference	Test
Distilled water	1500 µl	100µl	
Sample			25µl
Phosphate buffer		1200µl	1250µl
Adrenaline		150µl	150µl

Solutions were mixed properly and the absorbance were read at 420nm.

Calculation;

$$\% \text{ Inhibition} = \frac{ABS_{ref} - ABStest \times 100}{ABS_{ref}}$$

$$\text{Thus, SOD (Unit/ml)} = \frac{\% \text{ Inhibition}}{50 \times S}$$

Where: ABS_{ref} = Absorbance of reference

ABS_{test} = Absorbance of Test

S = Total protein (g/dl) for each sample

Conversion: Units/mL (nmol/min/mL) x 1000 = 1 µmol/min/mL

3.7.3 Assay of Glutathione Peroxidase (GPx)

GPx assay was done according to the kinetic method described by Flohe and Gunzler, 1984.

3.7.3.1 Relevance of assay:

The biochemical role of Glutathione Peroxidase is to reduce lipid hydroxyl-peroxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. Glutathione donates the hydrogen atoms used in the reduction reaction, while it will be oxidized.

$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2H_2O$

$2GSH + ROOH \xrightarrow{GPx} GSSG + ROH\ H_2O$

3.7.3.2 Assay principle:

GPx catalyses the reaction of pyrogallol with hydrogen peroxide to form purpurogallin (purple to black coloured) whose absorbance is read at 420nm.

$2pyrogallol + 3H_2O_2 + purpurogallen + 5H_2O + CO_2$

3.7.3.3 Reagents and Preparation;

Phosphate buffer (Ph = 8.0): 0.13g Na_2HPO_4, 0.019g NaH_2PO_4 and 0.8g NaCl were dissolved in 100ml distilled water.

1% H_2O_2: 1ml concentrated H_2O_2 was diluted to 100ml with cold distilled water.

Pyrogallol solution: 5g of dehydrated pyrogallol was dissolved in 100ml of cold distilled water. This was stored in a brown bottle to prevent photo-reactivity of pyrogallol.

3.7.3.4 Assay Procedure and Protocol:

	Blank	Sample
Sample		20µl
Phosphate buffer	250µl	250µl
H_2O_2	250µl	250µl
Distilled water	170µl	150µl
Pyrogallol solution	250µl	250µl

Absorbance of each sample was read at 420nm and at 0sec, 20sec, 40sec, 60sec, 80sec and 100sec.

Calculation:

Concentration of GPx was calculated using the formula below

$$GPx\ (Unit/ml) = \frac{\Delta ABS \times V \times Df}{A \times v \times L \times Y}$$

Where; ΔABS = mean of the differences in the absorbance

V = Total volume of the reaction mixture

Df = Dilution factor

A = Molar extinction coefficient of purpurogallin = 12.0 $M^{-1}cm^{-1}$

v = Volume of serum sample used

L = Light path = 1cm

Y = Total protein (g/dl) for each sample.

Expression of result: The result was expressed in Units/ml of serum, where 1 unit = mole of pyrogallol oxidized per minute.

Conversion: Units/mL (nmol/min/mL) x 1000 = 1 μmol/min/mL

3.7.4 DETERMINATION OF CATALASE ACTIVITY

Catalase activity was determined according to the method of Claiborne (1985). Principle The method is based on the loss of absorbance observed at 340 nm as catalase splits hydrogen peroxide. Despite the fact that hydrogen peroxide has no absorbance maximum at this wavelength, its absorbance correlates well enough with concentration to allow its use for a quantitative assay. An extinction coefficient of 0.0436 mM-1 cm-1 (Noble and Gibson, 1970) was used.

Reagents

1. **Phosphate buffer (0.05 M, pH 7.4)**

Dipotassium hydrogen phosphate trihydrate (0.696 g) and potassium dihydrogen phosphate (0.265 g) were dissolved in 90 ml of distilled water, the pH adjusted to 7.4 and the volume made up to 100 ml with distilled water.

2. **Hydrogen peroxide (19 mM)**

194 µl of 30% H2O2 was added to 50 ml of 0.05 M phosphate buffer, pH 7.4 and the volume made up to 100 ml with the same.

Procedure:

Hydrogen peroxide (590 µl of 19 mM solution) was pipetted into a 1 cm quartz cuvette and 10 µl of sample added. The mixture was rapidly inverted to mix and placed in a spectrophotometer. Change in absorbance was read at 340 nm 10 seconds for 2 min.

Protocol for determination of catalase activity

Table 2. 2: Protocol for determination of catalase activity

	H2O2 (µL)	Sample (µL)
Blank	600	_
Sample	590	10

Read for 2 minutes (10 sec interval) at 340nm Catalase activity was expressed as

µmol/min/mg protein using the extinction coefficient of hydrogen peroxide at 240nm. Calculation

$$\text{Catalase activity} = \frac{\triangle A340/\text{Min} \times \text{reaction volume} \times \text{dilution factor}}{0.0436 \times \text{sample volume} \times \text{mg protein/ml}}$$

3.7.5 DETERMINATION OF ACETYLCHOLINESTERASE ACTIVITY

Acetylcholinesterase activity was assayed using the method of Ellman (1961).

Principle: Acetylcholinesterase (EC 3.1.1.7, AChE), also known as RBC cholinesterase, is found primarily in the blood and neural synapses. Low serum cholinesterase activity may relate to exposure to insecticides or to one of a number of variant genotypes. AChE catalyzes hydrolyses of acetylcholine to acetate and choline necessary to allow a cholinergic neuron to return to its resting state after activation. Cholinesterase levels of cells and plasma are used as a guide in establishing safety precautions relative to exposure and contact with the organic phosphate insecticides. Simple,

direct and ellman method, in which thiocholine produced by the action of acetylcholinesterase forms a yellow colour with 5', 5'- dithiobis (2-nitro-bezoic acid). The intensity of the product colour, measured at 412 nm, is proportionate to the enzyme activity in the sample.

Reagent (the working reagent should be prepared freshly and used within 30 minutes).

1. Phosphate buffer (0.1M, pH 7.4) 10.677 g of K_2HPO_4 and 5.267 g of KH_2PO_4 were dissolved in 800 ml of distilled water and made up to 1 litre and then stored at 40 C.
2. DTNB (5, 5'- dithiobis (2-nitro-benzoic acid) 0.03964 g of DTNB was dissolved in 10 ml of phosphate buffer.
3. ACETYLTHIOCHOLINE (8 mM, 10 ml) 0.0231g of acetylcholine iodide was weighed and dissolved in 10 ml of distilled water.

3.7.5.1 PROTOCOL FOR DETERMINATION OF ACETYLCHOLINESTERASE ACTIVITY.

ACETYLCHOLINESTERASE ACTIVITY.

	H_2O	**Phosphate buffer (0.1 M, pH 7.4)**	**DTNB**	**Sample**	**Acetylthiocholine**
Blank	200	120	40	----	40
Sample Blank	230	120	40	10	---
Sample (undiluted)	190	120	20	10	40

Read for 2 minutes (15 seconds interval) at 412nm

3.8 STATICAL ANALYSIS`

Statical analysis was carried out using the graph pad prism software. All data were represented as mean ± standard error of mean (SEM). Values obtained were examined using analyses of variance (ANOVA), and the least significance difference (LSD) analysis, to evaluate the differences among groups and for multiple comparisons among different groups. $p < 0.05$ was the level of significance used. SPSS/PC software was used in all calculations.

CHAPTER FOUR

RESULT OF STATISTICAL ANALYSIS

Effect of Vitamin C on Survival rate in BPA Induced toxicity in *drosophila melanogaster*.

The effect of Vitamin C on survival rate in BPA induced toxicity in *drosophila melanogaster* is shown in figure

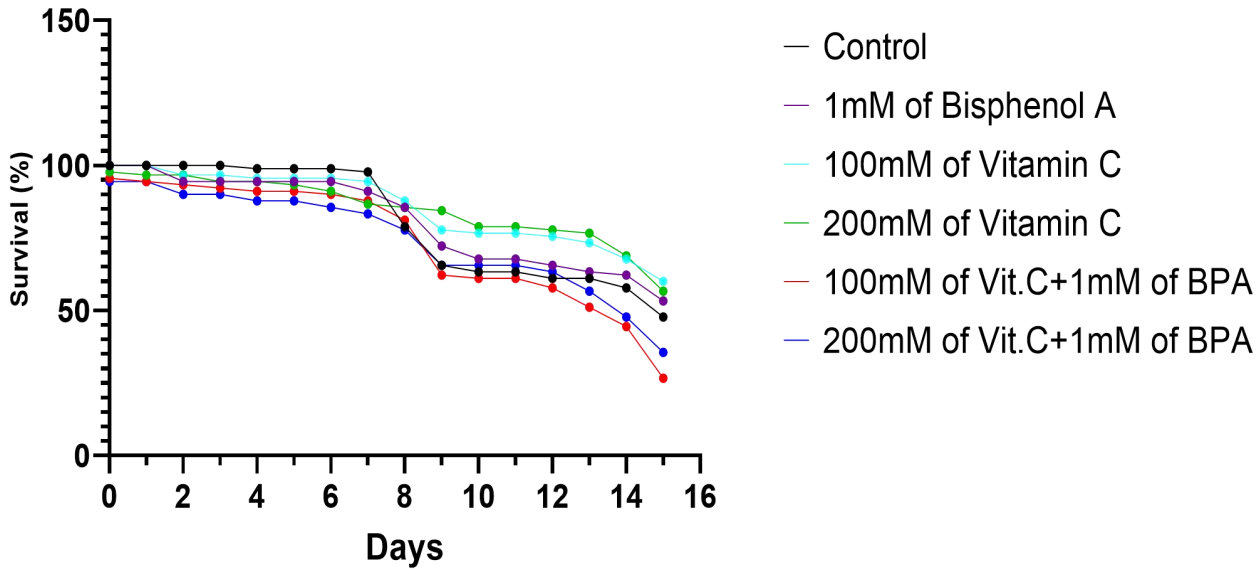
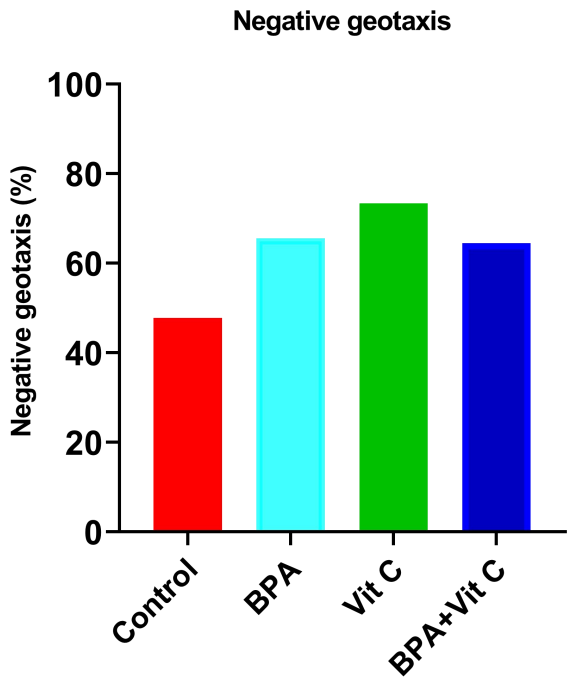


Figure 1: Graph representation for survival studies on Drosophila Melanogaster for the period of 15 days.

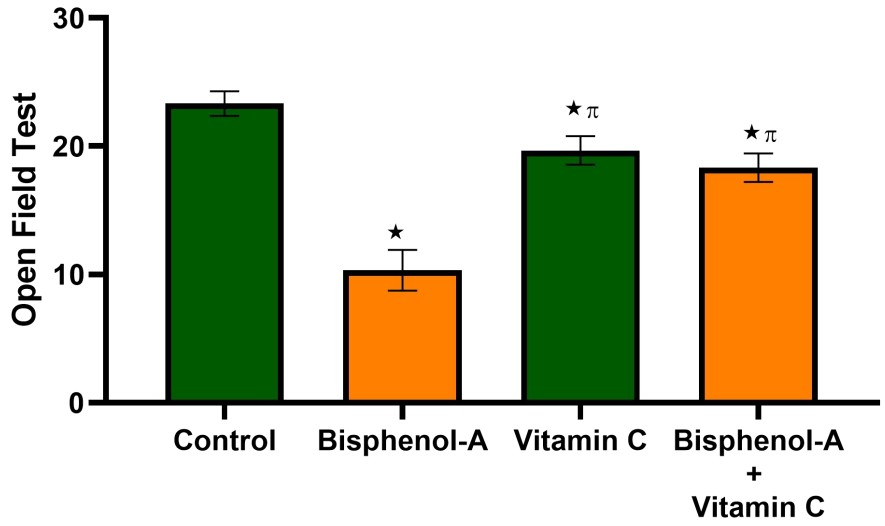
The effect of Vitamin C on Negative geotaxis in BPA induced toxicity in *drosophila melanogaster*



Graphical Representation of Negative Geotaxis of Drosophila Melanogaster expressed in percentage.

Open field test of drosophila melanogaster with Bisphenol-A induced toxicity in treated with Vitamin C.

There were significant decreases in BPA, Vitamin C and BPA+Vitamin C groups compared with control. There was also a significant increase in BPA+Vitamin C compared with BPA only.

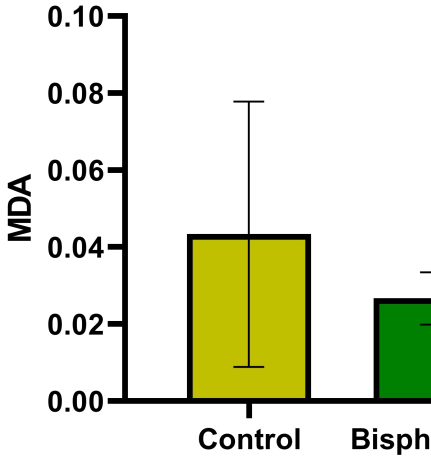


MDA levels in BPA induced toxicity in *drosophila melanogaster* treated with Vitamin C.

There was a significant increase in bisphenol-A toxicity induced treated with vitamin C (bisphenol-A + vitamin C) group compared with bisphenol. However, there were no significant differences in the different groups compared with control.

Below are MDA levels in BPA induced toxicity in *drosophila melanogaster* treated with Vitamin C

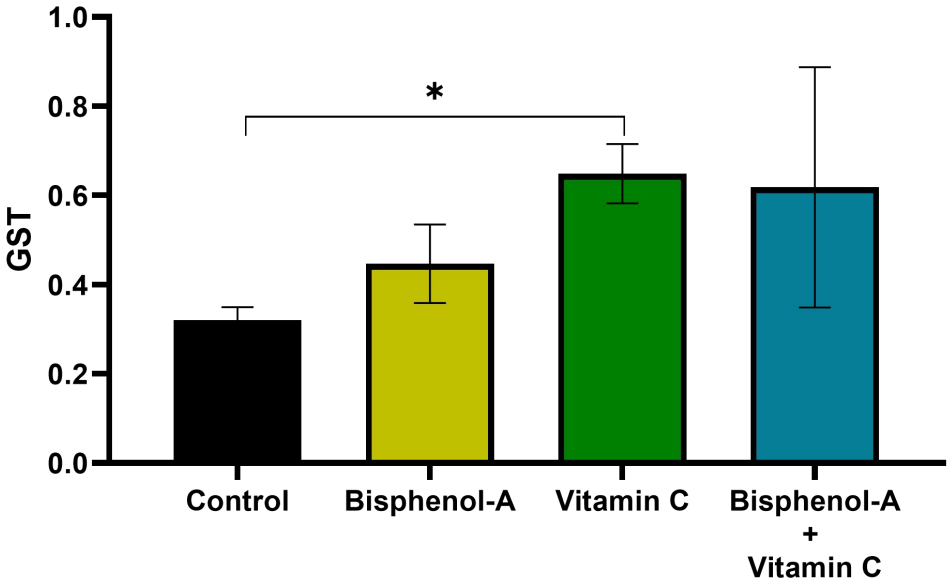
Groups	MDA mMol/ml
Control	0.0670 ± 0.0110
1mM BPA	0.02667 ± 0.007
200mM of Vitamin c	0.0460 ± 0.0006
1mM BPA +200mM of Vitamin c	0.0670 ± 0.0110
P	0.0141



GST levels in BPA induced toxicity in *drosophila melanogaster* treated with Vitamin C.

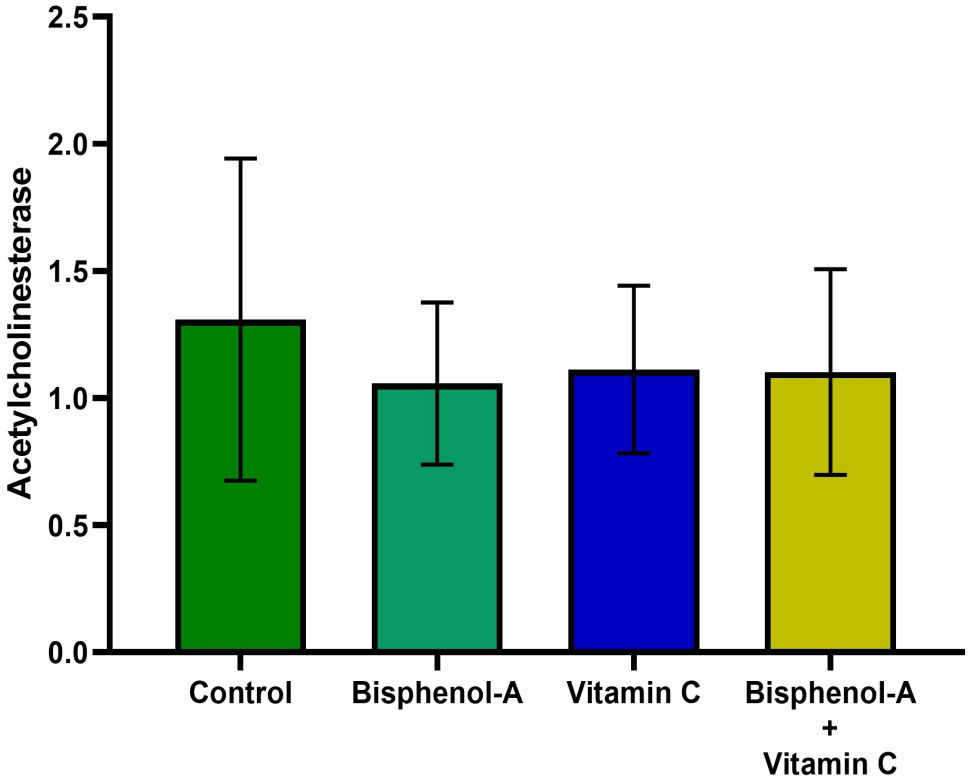
Groups	GST mMol/ml
Control	0.3207 ± 0.017
1mM BPA	0.4467 ± 0.051
200mM of Vitamin c	0.6487 ± 0.039
1mM BPA +200mM of Vitamin c	3 ± 0.156
P	0.0014

There was a significant increase in vitamin C compared with control, but there were no significant changes in bisphenol groups and bisphenol induced treated with vitamin C compared with control.



AChE levels of Bisphenol-A induced toxicity in drosophila melanogaster treated with Vitamin C.

Groups	AChe mMol/ml
Control	1.308 ± 0.634
1mM BPA	1.058 ± 0.319
200mM of Vitamin c	1.112 ± 0.33
1mM BPA +200mM of Vitamin c	1.102 ± 0.41
P	0.9775

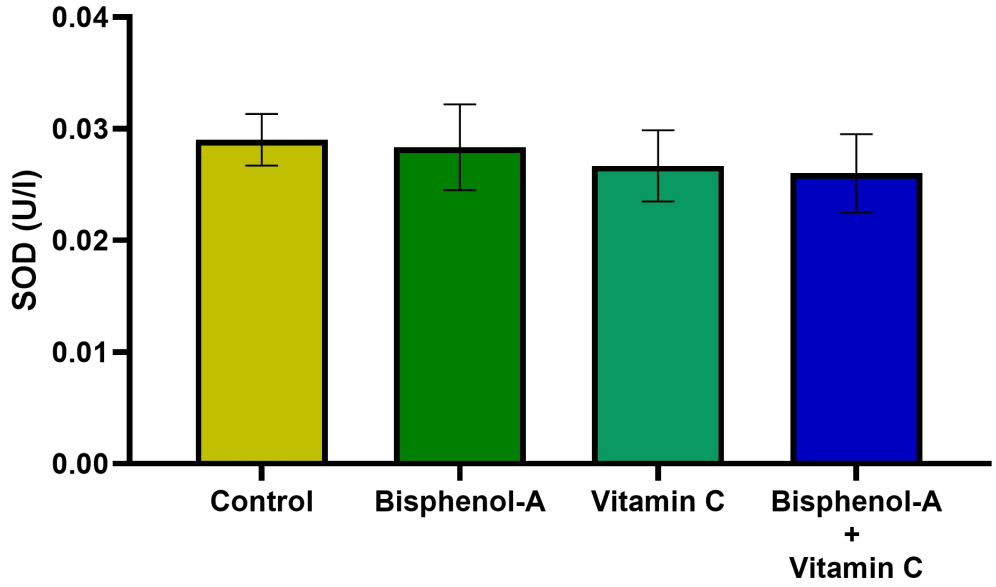


There were no significant changes in the bisphenol-A toxicity induced, vitamin C compared with control.

Superoxide dismutase levels of bisphenol-A induced toxicity in drosophila melanogaster treated with vitamin C.

Groups	SOD mMol/ml
Control	0.0290 ± 0.002
1mM BPA	0.02833 ± 0.004
200mM of Vitamin C	0.02667 ± 0.003
1mM BPA +200mM of Vitamin c	0.0260 ± 0.004

P	0.9040

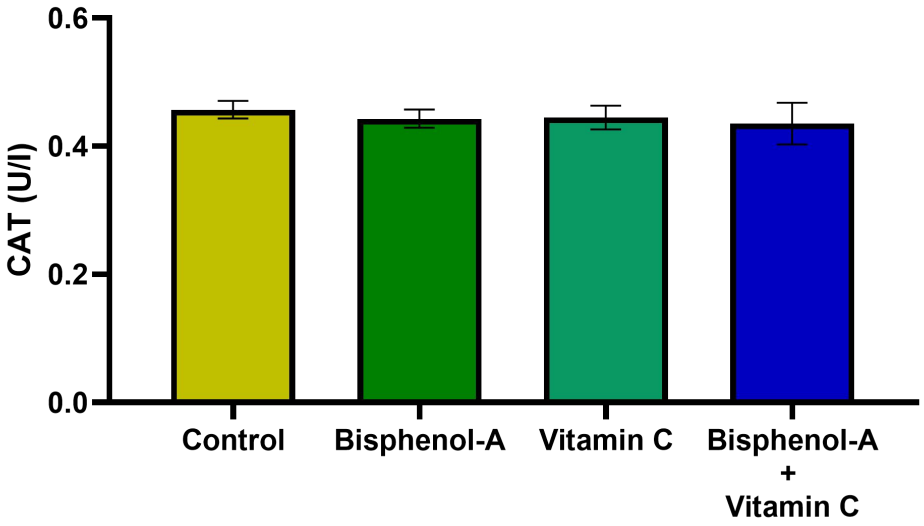


There were no significant changes in the bisphenol-A toxicity induced, vitamin C compared to control

The catalase of bisphenol-A induced toxicity in drosophila melanogaster treated with vitamin C.

Groups	CAT mMol/ml

Control	0.4570 ± 0.014
1mM BPA	0.4430 ± 0.014
200mM of Vitamin C	0.4447 ± 0.0185
1mM BPA +200mM of Vitamin c	0.4353 ± 0.033
P	0.9081



There were no significant changes in the bisphenol-A toxicity induced, vitamin C compared with control.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

DISCUSSION

Ishido and Masuo (2014) claim that a variety of neurological conditions have been linked to bisphenol-A exposure. There is strong evidence connecting oxidative stress and cholinergic dysfunction to the development of AD. Because cholinergic impairment causes acetylcholine levels in brain synapses to decline, current therapeutic approaches have concentrated on raising acetylcholine levels in the brain either by blocking its hydrolysis with ace inhibitors or by administering cholinomimetic substances (Prena, 2010). The undesirable side effects of ace inhibitors have led researchers to hunt for alternatives that include anticholinesterase and antioxidant properties. Since oxidative stress also plays a significant role in the etiology of Alzheimer's disease, this study was carried out to determine Vitamin C's potential to inhibit cholinesterase activity and its antioxidant effects in a Drosophila melanogaster model of toxicity caused by Bisphenol A. Oxidative stress indicators and endogenous antioxidant enzymes (SOD, CAT, and GST) were examined in flies.

Free radicals trigger a process called lipid peroxidation, which oxidatively degrades the polyunsaturated fatty acids contained in cellular membranes. Normal conditions result in a decreased degree of lipid peroxidation, which is seen in tissue and organs at low quantities (Mahalingam and Krishan (2010)). MDA is an oxidative stress biomarker and a sign of lipid peroxidation (Ayalaas well as 2014).

Malondialdehyde (MDA) levels in the BPA-treated flies in this research did not increase noticeably in comparison to the control group. The flies treated with BPA alone showed considerably lower levels of MDA than the ones that additionally received vitamin C ($P>0.05$).

The findings of Oluranti et al. (2021) and Churchill (2020), who discovered that MDA levels were significantly raised in rats and flies exposed to BPA and significantly lowered in BPA and antioxidant

co-treatment, are not consistent with our results. This occurred as a consequence of the experiment's brief length and the specimens' lack of extended exposure to the toxicant.

The detoxification of endogenous chemicals and exogenous molecules, such as medications and pollutants, is carried out by a set of enzymes called glutathione-S-transferases (GST) (Nerbert and Vasiliou, 2004). They accelerate the conjugation of GSH with toxic products of phase I detoxification and convert them into less toxic forms to lessen oxidative damage in tissues (Abolaji et al., 2015). Farombi et al. (2018) claim that GST play a crucial role in regulating the mechanisms that enable organisms to withstand oxidative stress. An essential enzyme involved in the conjugation process during xenobiotic metabolism is GST. It functions by quickening interactions between reduced glutathione and possibly dangerous electrophilic compounds, claims Lushcak (2012).

As previously noted in further research connected to causing toxicity in flies (Oyetayo BPA reduced the GST activities in flies, rats (Katyal et al., 1997), and mice (et al., 2020).
This drop-in GST activity indicates a potential limitation in the flies' ability to fully detoxify BPA and withstand oxidative stress. But in this study, vitamin C was able to boost GST activity in flies, indicating even more of its antioxidant power and ability to protect against ROS-induced oxidative damage. Vitamin C after BPA causes a rise in GST activity, as previously observed by Haroun et al. (2019). Vitamin C increased considerably in comparison to the control, but no other variables changed much. On the other hand, the levels of bisphenol groups and bisphenol-induced treatment with vitamin C showed a little decrease and rise, respectively.

Acetylcholinesterase (AchE) is an enzyme that breaks down acetylcholine, a neurotransmitter crucial for both motor and cognitive function. AChE inhibition raises acetylcholine levels, which improve motor and cognitive performance. The acetylcholinesterase activity was examined in this study, and it was found that while there were no significant changes in the vitamin C, co-treatment, and bisphenol-A toxicity induced groups when compared to control, there was a non-significant increase

in BPA treated files only. The levels of acetylcholinesterase were normal in every treatment group. This finding was in contrast to a 2010 research that was published in the journal Environmental Research and indicated that exposure to BPA changed the amounts of certain signaling molecules in the brain, increasing

AChE undertaking. They concluded that neurological and cognitive issues might be related to BPA exposure. The quantity of BPA administered and the duration of the flies' exposure to the toxin both had an impact on this. One feature of AD is negative geotaxis, an indication of decreased locomotion and a symptom of neurodegeneration (Oyetayo et al., 2020). The results of this investigation showed that flies treated with BPA exhibited a locomotor deficit (negative geotaxis and open field study) and decreased climbing activity. This study is consistent with previous findings in rats (Erazi et al., 2010; Nampoothiri et al. 2015) and flies (Oboh et al., 2020; Oyetayo et al., 2020). The flies treated with BPA showed lower motility, which may be explained by the earlier results of this research about impaired cholinergic transmission. Enhance the locomotor deficits seen in the fly performance (climbing activity) after BPA treatment. Previous research on vitamin C's anti-cholinesterase action has shown that it may facilitate movement. In this study, BPA significantly reduced the survival rate of flies. This data suggests that BPA has a hazardous effect and is in line with research done on flies by Churchill (2020) that shows how detrimental BPA is to flies' survival rates. Previous research has connected a decline in antioxidant enzymes and compromised cholinergic neurotransmission to the decreased survival rate of these flies after BPA exposure (Churchill, 2020). But when vitamin C was used in conjunction with BPA, it makes sense to believe that the vitamin C's presence helped to reduce

BPA-related death. It has been shown that vitamin C increases endogenous antioxidant enzymes and improves cholinergic transmission in flies.

5.1 CONCLUSION

This research has shown that vitamin C has anti-cholinesterase and antioxidant properties and may reduce BPA-induced toxicity when taken as directed

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