

**BIOCHEMICAL EFFECT OF VIT C ON ZINC OXIDE NANOPARTICLES
INDUCED TOXICITY IN TESTIS AND EPIDIDYMIS OF MALE WISTAR
RATS**

BY

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**AN UNDERGRADUATE DISSERTATION SUBMITTED TO THE
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CERTIFICATION

This is to certify that this research titled “**BIOCHEMICAL EFFECT OF VIT C ON ZINC OXIDE NANOPARTICLES INDUCED TOXICITY IN TESTIS AND EPIDIDYMIS OF MALE WISTAR RATS**” was carried out by “**OHONSI MERCY REJOICE**” and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

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Date

DECLARATION

I “**Ohonsi Mercy Rejoice**” declare that “**BIOCHEMICAL EFFECT OF VIT C ON ZINC OXIDE NANOPARTICLES INDUCED TOXICITY IN TESTIS AND EPIDIDYMIS OF MALE WISTAR RATS**” is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

OHONSI MERCY REJOICE

.....

Date

DEDICATION

I dedicate this work to the Almighty God for his grace, kindness and guidance throughout the course of this study.

ACKNOWLEDGEMENT

I give thanks to God Almighty for His grace upon my life and for seeing me throughout this programme.

My profound gratitude goes to my supervisor **Dr. (Mrs). O.A. Edene** for her concern and supportive ideas which has aided this project work effectively. I specially thank the Head of Department Prof. A.A. Enuneku and all the lecturers in the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin.

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ABSTRACT

Zinc oxide nanoparticles are commonly used in many technologies. However, they are believed to have negative effects on the reproductive system. This research aims to explore how vitamin C can help protect the testis and epididymis of Wistar rats from the harmful effects of ZnO Nps. A total of 30 rats were separated into 5 groups of 6 and given different doses of ZnO Nps and Vitamin C for two weeks. In the ZnO group, malondialdehyde levels in the testis increased. On the other hand, the ZnO only group showed a decrease in gene expression and antioxidant enzyme activities compared to the control group. However, oxidative markers were significantly mitigated after combined treatment of ZnO Nps and Vit C compared to the rats given ZnO Nps ($p < 0.05$). This study demonstrate the ameliorating role of vitamin C on toxicity of ZnO Nps. In there epididymis, significant increase were observed in the SOD, MDA, CAT, and GPX of the ZnO NPS and combined treatment of ZnO Nps and vit c when compared to the control ($p > 0.05$). The study found that there was a noticeable drop in the number of live sperm cells when exposed to ZnO alone and ZnO with Vit C, compared to the control group. On the other hand, there was a notable increase in the number of dead sperm cells when exposed to ZnO Nps alone and ZnO Nps with Vit C. Additionally, there was a decrease in the movement of sperm cells when exposed to ZnO Nps alone and ZnO Nps with Vit C, in comparison to the control group. This research shows that ZnO Nps have a negative impact on sperm quality, as well as the functioning of the testis and epididymis.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Nanoparticles are tiny particles made to be less than 100 nanometers in size on purpose. These particles have special physical and chemical qualities like being good at conducting heat, light, and electricity, along with having biological properties. Because of this, nanoparticles are used in many different areas like healthcare, fuel, cosmetics, farming, medicine, food, bone health, and fighting germs. Although there are many good things about NPs, some downsides have been found that could be harmful to people and animals. NPs have been connected to health problems like lung damage, liver and kidney issues, immune system problems, brain damage, and permanent damage to the ability to have children. Scientists studying toxicology are starting to pay more attention to how NPs might affect the ability to have children. One type of nanoparticle called zinc oxide nanoparticles has become very popular. Zinc oxide nanoparticles are popular due to their affordability, ability to create various structures, and many biological uses like delivering medicine, imaging cells, treating cancer, fighting bacteria, and adjusting the immune system (Jiang et al., 2021). Research has shown that zinc oxide nanoparticles can harm cells in different ways, causing damage to cell membranes, inflammation, DNA damage, cell death, changes in how cells interact, and hormone level alterations in mammals through both lab and animal studies. ZnONPs can be harmful because they make reactive oxygen species (ROS) that can hurt cell membranes. Therefore, it is important to look into the dangers they might have on reproductive health, while also exploring how giving vit c along with them at various levels could help. The wistar rat is a popular type of rodent used in research labs now. It is recognized

by its big head, long ears, and a tail that is always shorter than its body, making it unique. Other strains like sprague dawley and long-evans come from the wistar rat family. Wistar rats are known to be more active than other breeds, such as Sprague Dawleys (Health Soote et al., 2022). This study focuses on the testis and epididymis, which are important organs for reproduction. The testis is very important for making babies and having sex, as it helps create sperm and hormones like testosterone. It does this by making androgens and producing sperm for moving around. Meanwhile, the epididymis, a tube-like part that connects the testis to another tube, helps sperm grow up and get ready for fertilizing eggs. It also helps keep sperm safe and store them until they are needed. It is made up of different areas where sperm go through various changes to be ready for fertilization.

1.2. Statement of Study

Zinc oxide nanoparticles are often found in things like makeup and food packaging because they have special qualities. But, some people are worried about how these nanoparticles might harm living things, like animals. Research shows that nanoparticles can get through a barrier in the body and harm the testicle. (Nasrin *et al.*, 2023). The male body has a special defense system called the BTB that stops harmful stuff from getting in. Scientists used to think tiny particles couldn't pass through this defense, but recent studies show they actually can and end up in the testes. The male reproductive system can be affected by things like toxins and tiny particles from the environment (Tang *et al.*, 2019). Some tiny particles have the ability to create reactive oxygen species, damage DNA, and trigger cell death. This makes them potentially useful in fighting bacteria and cancer. However, there are worries about how harmful they may be, how easily they can get into the body, and how they build up in living things. This raises questions about using them in medicine, especially when it comes to their effects on the male reproductive

system and fertility(Pinho *et al.*, 2020). While many studies have looked into the toxicity of ZnO nanoparticles in labs and animal tests, more research is needed to fully understand how they work in living creatures like rats. The intricate interactions of ZnO NPs with various tissues and organs need to be elucidated to assess the overall impact on health. This study aims to investigate the role of vit c in ameliorating Zno - Nps induced toxicity in the testis and epididymis of male wistar rats. It is hypothesized that exposure to ZnO NPs will induce organ-specific toxicity in wistar rats, leading to alterations in testicular and epididymal functions.

1.3. Justification of Study

Zinc oxide nanoparticles are commonly found in many everyday products and can get into the environment from wastewater. It's important to study how Vitamin C affects the harmful effects of these nanoparticles on the testis and epididymis.

Figuring out how different amounts of Vitamin C can help counteract the toxicity of zinc oxide nanoparticles is key. If Vitamin C can protect against the harmful effects of these nanoparticles, it could lead to new ways to treat reproductive damage in people exposed to nanoparticles at work or in the environment.

Male Wistar rats are often used in studies to look at how things might affect male reproductive health. One common thing they study is the effects of nanoparticles and how vitamin C could help protect against any harm they might cause. It's important to investigate how vitamin C could help with the negative effects of zinc oxide nanoparticles on the testis and epididymis of male Wistar rats. Thus, this can help us learn more about nanotoxicology and find ways to keep male reproductive health safe from nanoparticle exposure.

1.4. Aim and Objectives

This study is therefore aimed at assessing the biochemical effects of vitamin C on ZnO - Nps induced toxicity in the testis and epididymis of male wistar rats.

The specific objectives include:

1. To assess oxidative stress levels in testis and of male wistar rats exposed to ZnO - Nps and vitamin C.
2. To assess oxidative stress levels in epididymis of male wistar rats exposed ZnO - NPs and vitamin C.
3. To examine changed in sperm parameters (sperm count and sperm motility) in male wistar rats exposed to ZnO - NPs and vitamin C.

CHAPTER TWO

LITERATURE REVIEW

2.1. Toxicity of Zinc Oxide Nanoparticles

Many people disagree about whether ZnO NPs can be harmful, so it's important to find better ways to understand how they affect living things in different environments. Since ZnO NPs can enter the body in different ways like eating, breathing, or touching the skin, it's crucial to carefully check the methods used in studies about their toxicity. ZnO nanoparticles work in different ways to affect things. When nanoparticles reach important organs in the body, they can break through barriers like the blood-brain barrier or the blood-testis barrier, allowing them to get to organs like the testis and epididymis (Ziamajidi et al., 2023).

Once in the body, these nanoparticles can cause oxidative stress by creating reactive oxygen species. Too many reactive oxygen species can harm cell parts and cause inflammation by activating factors like the nuclear kappa B-factor. This can cause the body to release certain chemicals that can make inflammation worse (Ziamajidi et al., 2023).

Zinc oxide nanoparticles can change the way the testis and epididymis look under a microscope, upset the balance between harmful molecules and protective substances in the body, and lead to damage to germ cells and problems with sperm development. These nanoparticles might also

affect leydig cells and how testosterone is made, which could impact a guy's ability to have kids (Taha et al., 2023).

The harmful effects of zinc oxide nanoparticles happen because they can mess up how cells work, cause cells to die in a certain way called autophagy, set off inflammation, harm cells, and have properties that can damage genes. Zinc oxide nanoparticles can harm cells and tissues by damaging cell functions, breaking down lipids, and changing lipid structures (Taha et al., 2023).

2.2 Toxicity of Zinc Oxide Nanoparticles on Male wistar rat

Research has found that when adult male wistar rats were exposed to particles at levels higher than 50 mg/kg body weight, they experienced significant oxidative stress (Saptarshi *et al.*, 2015, Ziamajidi *et al.*, 2023 and Rajpota *et al.*, 2018). This oxidative stress is thought to be a potential reason for the toxicity of nanoparticles (Alkaladi, 2020). Zinc oxide nanoparticles, in particular, have been linked to increased lipid peroxidation and the onset of oxidative stress, which could contribute to their harmful effects (Sharma *et al.*, 2011). (John *et al.*, 2020), into how ZnO nanoparticles can cause oxidative stress. They checked levels of MDA, TAC, CAT, TOS, SOD, and GPX to do this. They found that MDA levels went up significantly in the blood of the test groups given ZnO nanoparticles at doses higher than 50 mg/kg compared to the control and nZnO50 groups. This matches with what (Srivastav *et al.*, 2016) that damage to DNA in skin cells could happen because of lipid peroxidation and oxidative stress. Researchers suggest that nanoparticles are able to interact with DNA due to their small size. In a study by (Wang et al., 2018), it was found that ZnO nanoparticles can cause DNA damage and inflammation in human nasal mucosa cells even at low levels when tested in a lab setting.

Zinc oxide nanoparticles can damage the testicular tissue, break through the blood-testis barrier, and impact the Sertoli cells in the testis. This can cause problems with the structure and function of the testis. These nanoparticles can disrupt the balance of the blood-testis barrier, damage the cytoskeleton, and mess with its normal functioning. When the blood-testis barrier is affected, the zinc oxide nanoparticles can enter the seminiferous epithelium and harm the spermatogenic cells, leading to cell death (Hong *et al.*, 2022). This causes more sperm cells to die and some sperm to become deformed (Osman *et al.*, 2023). Additionally, zinc oxide nanoparticles can harm interstitial cells, which affects testosterone production. As a result, Sertoli cells and seminiferous epithelium can't work well, leading to lower sperm count and movement, and affecting rats' ability to reproduce (Xie *et al.*, 2023).

2.3. Zoology of Wistar rat

The Wistar rat is a popular type of lab rat that scientists often use in their studies. These rats are medium-sized and usually weigh around 350-500 grams for adult males and 250-300 grams for adult females. They are known for being friendly and easy to care for, and they enjoy living in groups. Wistar rats usually live for 2-3 years, which can be affected by things like what they eat, where they live, and their genes. These rats become able to have babies at 5-6 weeks old, and female rats have a cycle where they are ready to have babies every 21-23 days, usually having 8-12 babies at a time. Scientists know a lot about the biology, genes, and actions of Wistar rats, so they are useful for studying sicknesses, actions, and body processes (Health Soothe *et al.*, 2022). You can easily get Wistar rats from stores and they are easy to take care of in labs because they are calm and can adapt well. Wistar rats have consistent genes and characteristics for research.

2.4. Oxidative stress

Oxidative stress happens when there is too many reactive oxygen species (ROS) and not enough antioxidants to balance them out. This can harm cells and maybe even destroy tissues (Sies *et al.*, 2020). But, ROS also help cells make energy in mitochondria. Increased oxidative stress has been linked to different situations like getting older, working out, and certain illnesses such as cancer, brain diseases, heart problems, diabetes, swelling, and being exposed to harmful substances. Although antioxidants are thought to protect against oxidative harm, they may not work very well.

New developments in nanotechnology have led to more nanoparticles being used in areas like healthcare and manufacturing (Khan and Saeed, 2017). This has caused some worry about how these tiny particles interact with our bodies and the possible harmful effects they may have on living things. One type of nanoparticle that is often used is zinc oxide nanoparticles, which are known for their special properties and ability to fight bacteria. As a result, these tiny particles have been added to many different things like food preservatives, sunscreen, fabric coatings, fertilizers, and medical tools. A few studies on animals have shown that ZnO NPs can harm different body parts, including the testes, by breaking through a protective barrier called the blood-testis barrier. This barrier is important for protecting the male reproductive system by keeping out harmful things. Although it is commonly believed that nanoparticles cannot pass through the blood-testis barrier, recent studies have shown that they can actually enter the testes and build up there. The exact way in which ZnO nanoparticles cause harm, especially in terms of male reproductive system problems, is not fully understood. But experts think that oxidative stress is a major factor in this. Research has shown that making too many reactive oxygen species (ROS) can hurt the process of making sperm and cause cell death (Jones *et al.*, 2019).

Reactive oxygen species (ROS) are important in sperm health, but too much can be harmful. The body has ways to fight off extra ROS using antioxidants. Enzymes like catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) can help lower oxidative stress. Research shows that taking vitamins by mouth can also help improve sperm health in men who struggle with infertility. Vitamin C helps strengthen the immune system by controlling important enzymes. Previous research has found that ZnO NPs can cause harmful effects on male reproductive health(Sies *et al.*, 2020).

Based on an earlier research, giving ZnO nanoparticles can cause stress in the testicles due to more oxidative stuff happening. This shows up as a bigger amount of oxidative stuff. Also, the enzymes GPx, SOD, and CAT, as well as the overall level of antioxidants, go down. The research shows that ZnO nanoparticles being harmful mostly comes from making too many reactive oxygen species (ROS), John *et al.* (2022), where they found that vitamins help reduce oxidative stress by working with detoxification enzymes like SOD, GPx, and CAT to prevent free radicals from forming. This is backed up by looking at the tissues under a microscope. The research found that ZnO nanoparticles can change how antioxidant enzymes work and raise MDA levels, which upsets the balance in the testicles. As a result, higher levels of oxidative stress from more damage could harm the sperm's membrane and DNA. Zinc oxide nanoparticles may have changed how certain genes related to oxidative stress are expressed in the testis.

2.5. Oxidative stress markers

2.5.1. Catalase: Catalase is a key antioxidant enzyme that can be found in most living things that need oxygen. It helps change hydrogen peroxide into oxygen and water (Miao *et al.*, 2020).

2.5.2. Superoxide dismutase: Superoxide dismutase is another antioxidant enzyme that fights against oxidative stress by getting rid of superoxide radicals. When the activity of SOD changes, it can show how much stress cells are under (Huang et al., 2021).

2.5.3. Gluthione peroxidase: Gluthione peroxidase is an important enzyme that helps cells stay safe from damage caused by oxidation by lowering hydrogen peroxide and lipid peroxides. If the activity of GPx changes, it can tell us if the cells' defenses against oxidation and stress have changed (Nandi et al., 2019).

2.5.4. Malondialdehyde: Malondialdehyde is a popular biomarker that is often used to measure how much oxidative stress is happening in the body. It is created when lipids are damaged during oxidative stress (Ayala *et al.*, 2022).

2.6. Toxicity of Zinc Oxide Nanoparticles on Testis and Epididymis

Zinc oxide nanoparticles have special qualities that can impact important organs like the Epididymis and testis by passing through barriers in the body (Abariku et al., 2020). This can cause changes in these organs due to issues with how oxygen is processed and antioxidants are disrupted. When cell parts are harmed by different oxygen molecules, harmful substances can be made (Ziamajidi et al.).

ZnO nanoparticles can cause oxidative stress that affects the epididymal tissue and disrupts the secretion process of the cells lining the epididymal duct. This disruption can lead to problems with sperm maturation. The breakdown of the epididymal cells may be connected to a toxic

substance causing damage through a process called phospholipidosis. Moreover, the research showed that using 600mg/kg of nano ZnO particles caused a drop in testosterone levels (Kamel *et al.*, 2022). This decline happened because of the creation of superoxide molecules and protein oxidation, which stopped testosterone from being made.

2.7. Histology of the Testis and Epididymis

Testis: In a Wistar rat, the testes are found in the scrotum. Each testis is oval-shaped and protected by a tough fibrous covering called the tunica albuginea. Inside the testis are lobules, which contain seminiferous tubules where sperm is made. These tubules are where spermatogenesis happens and where sperm cells are created. Sertoli cells and Leydig cells play a crucial role in the seminiferous tubules, which gather sperm and take them to the epididymis. The testis gets blood from the testicular artery and is drained by the testicular vein. It makes sperm and releases testosterone (Purnima and Ishwarlal, 2019).



Plate 2.1: Testis

Epididymis: The epididymis is a tightly coiled tube found at the back of each testis. It has three main parts: the head (caput), body (corpus), and tail (cauda). Sperm move from the testis to the epididymis where they mature and gain the ability to move. The epididymal duct connects the epididymis to the vas deferens, which carries sperm to the urethra during ejaculation. The epididymis is important for sperm maturation, storage, and transport. Sperm change in the epididymis to become able to move and fertilize an egg (Purnima and Ishwarlal, 2019).

The testis makes sperm, while the epididymis helps sperm mature and be stored, so they can fertilize an egg.

2.8. Toxicity of zinc oxide nanoparticles on male reproductive hormones functions

Gonadotropins like Follicle stimulating hormones and leutenizing hormones, along with testosterone, play a big role in the development of germ cells. Changes in serum gonadotropins and testosterone levels are often linked to problems with sperm production. LH acts on Leydig cells in the seminiferous tubules to make testosterone, while FSH acts on sertoli cells in the same tubules to make a protein called androgen binding protein (ABP). ABP binds to the androgen

receptor and helps produce testosterone, as well as another hormone called Inhibin (Hussein et al., 2021). Testosterone helps create sperm, and FSH and LH work together to control the process. Testosterone helps with cell growth and repair, but if it's not there, it can affect how proteins work in the body (Mozaffari *et al.*, 2021).

Nanoparticles can cause inflammation, which can harm cells that make testosterone and lower its levels, possibly affecting the barrier that protects the testes. Making alterations to the blood-testis barrier (BTB) could be very risky for sperm production. Research has found that using ZnO nanoparticles (NPs) can lead to lower testosterone levels in the body, which can affect the growth and function of leydig cells.

Other studies, like the one done by Talebi et al. in 2020, have also found similar results. A study in the past found that exposure to tiny particles caused problems with semen and messed up the balance of reproductive hormones like Testosterone, FSH, and LH. This might be because the levels of harmful substances like free radicals and nitric oxide increased in the testes, while the levels of antioxidants decreased. Another study showed that low levels of antioxidants in semen were linked to poor sperm quality in infertile people. Damage to certain cells in the body also played a role in messing up the balance of hormones. They also mentioned that having lower levels of antioxidants in the fluid that carries sperm can harm the quality of semen.

2.9. Toxicity of Zinc Oxide Nanoparticles on Sperm Analysis

Research has found that ZnO NPs can cause problems in the male reproductive system by creating oxidative stress, which damages sperm cells. This damage can lead to changes in the shape of sperm, like their heads and tails, or the appearance of vacuoles. When sperm is checked

after exposure to ZnO NPs, there may be more abnormal sperm cells present, showing that sperm quality has been affected in a bad way.

2.9.1 Sperm cell

A sperm cell is a special male cell that helps in making babies. It is made in the testes through a process called spermatogenesis. Sperm cells are small, have a sleek shape, and a tail that helps them swim to the egg for making a baby.

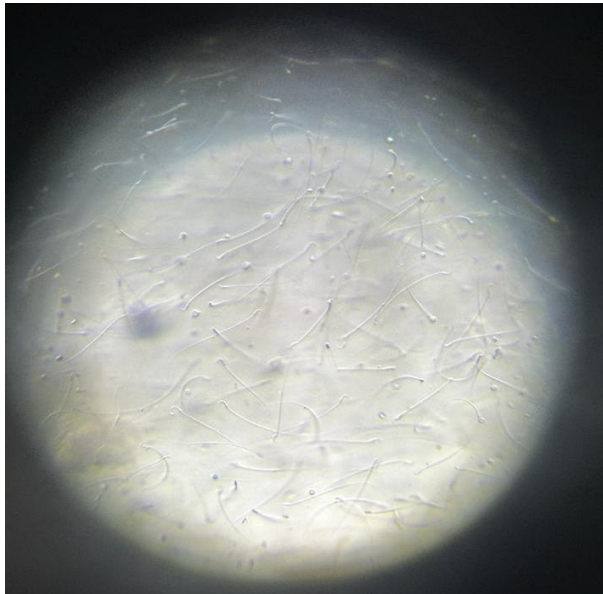


Plate 2.2: Sperm cell

2.9.2 Structure of a Sperm Cell

Head: The head of a sperm has the DNA needed for fertilization. It is covered by a cap-like structure called the acrosome, which helps the sperm penetrate the egg.

Midpiece: midpiece of the sperm contains mitochondria that give energy for the sperm to move.

Tail (Flagellum): Helps the sperm move towards the egg by swimming. ZnO NPs can change how sperm look and move. They can also make it hard for sperm to stay alive by causing stress and damaging their mitochondria. Researchers have found that ZnO NPs can cause harm to sperm cells by damaging their DNA, which could impact genes and cause reproductive problems.

These NPs might also mess up how mitochondria work in sperm cells, making it hard for them to make energy. This can create oxidative stress in sperm cells by making reactive oxygen species (ROS), which can lead to damage in fats and proteins (Mohamed *et al.*, 2019).

2.9.3. Sperm Count

Sperm count is the total number of sperm cells in a semen sample. It is an important factor in evaluating male fertility and reproductive health. Being exposed to ZnO NPs has been linked to a decrease in sperm count, which is called oligospermia. The harmful impact of ZnO NPs on the testes can disrupt the making of sperm, causing a drop in the overall amount of sperm cells. This can make it harder to have a baby and lower the likelihood of getting pregnant successfully (Halo *et al.*, 2021).

2.9.4. Sperm Motility

Sperm motility is how well sperm can swim and move around. ZnO NPs can make it hard for sperm to move properly, which is necessary for them to reach and fertilize an egg. When sperm are exposed to ZnO NPs, they may not be able to swim as well, making it tough for them to travel through the female reproductive system. This can make it harder for sperm to get to the egg and can greatly affect a person's ability to have a baby (Pinho et al., 2020).

2.10. Biological Effect of Vitamin C in wistar rat

Vitamin C, also called ascorbic acid, is a type of antioxidant that dissolves in water and collects in the brains of mammals more than in any other body parts. Wistar rats might face oxidative stress when they come into contact with ZnO oxide nanoparticles because these particles create harmful reactive oxygen species (ROS) in their bodies. Vitamin C works as a strong antioxidant that hunts down these ROS and keeps the balance in the cells (Ziamajidi et al., 2023). Vitamin C helps protect cells from damage caused by ZnO nanoparticles by stopping harmful substances called ROS. This can prevent problems like damaged lipids, proteins, and DNA. In wistar rats, vitamin C's protective power can lessen the bad effects of oxidative stress on different body parts. Vitamin C not only fights off bad stuff in our bodies, but it also helps other good stuff work even better. It makes antioxidants and enzymes like superoxide dismutase (SOD), catalase, and glutathione peroxidase more active, which helps protect our cells. Vitamin C also helps get rid of harmful substances made by ZnO oxide nanoparticles. Plus, it can reduce swelling and help fix tissues that have been hurt by oxidative stress. (Nourian et al., 2023) This can help Wistar rats feel better and recover when they are exposed to a specific amount of ZnO oxide nanoparticles.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Materials

3.1.1 Chemicals and reagents

The study employed reagents of analytical quality for all its experiments. The distilled water was obtained from the Department of Biochemistry laboratory, University of Benin, Benin City, Nigeria. Formaldehyde, normal saline, liquid soap, vitamin C, chloroform was procured at Pyrex-ig scientific company, shop 14, Gabano plaza, opp UBTH gate, Ugbowo Lagos road, Benin City, Nigeria, phosphate buffer was procured at Quality Analytical Laboratory in Benin City, Nigeria. ZnO nanoparticle was purchased from Sigma Aldrich, South Africa.

3.1.2 Apparatus/Equipment

Plastic cages were bought at the post graduate laboratory department of Pharmacy, University of Benin (Nigeria), latex surgical hand gloves, disposable nose masks, ceramics bowls, universal containers, 5ml syringes, 10l plastic keg, veterinary surgical kit, biros, permanent markers, masking tapes, pasture pipettes, filter paper, microscopic slides, plastic petri dish, gavage were all procured at Pyrex laboratory (Nigeria). Table top centrifuge was purchased from Harris (England) and ceramic mortar and pestle were obtained from Medax (England). Haematology

automated analyser, ACT Diff Analyzer (Beckman Coulter, USA). Burettes, beakers, (50, 100, 250 and 500 mL), EDTA bottles, heparinized sample bottles, incubator, micropipettes, plain sample bottles, refrigerator, round bottom-flask, spatula, stirrer, stop watch, test tubes and racks, water distiller and weighing balance (Methler, H8) were purchased from Pyrex (England).

3.1.3 Sample Collection and Preparation

The nanoparticles utilized in this research were sourced from Sigma Aldrich, South Africa. They were meticulously packaged to prevent any contamination, finely ground into powder using a mortar and pestle, and then a composite sample was generated for analysis in the laboratory.

3.2 Methods

3.2.1 Animal Protocol

A total of thirty (30) healthy adult male Wistar rats weighing between 80-120g were procured from the Animal house at the Department of Anatomy, University of Benin, Benin City, Nigeria for this research. Following the purchase, the rats underwent a one-week acclimatization period. They were housed in plastic cages with six (6) rats per cage, situated in a well-ventilated rat house. The rats were provided with rat pellets and water as needed. Throughout both the acclimatization phase and the administration of ZnO nanoparticles, the rats were subjected to a natural light-dark cycle lasting 12 hours each day.

3.2.2 Grouping and treatment of experimental animals (Experimental Design)

A total of 30 male wistar rats were divided into 5 groups consisting of control (distilled water) and four (4) treatment groups, administered doses of 250, 500, 1000 and 1500 mg/kg bw of zinc oxide nanoparticles and vitamin c as shown in figure 3.1

After the acclimation period, the rats received the nanoparticle treatment for seven days. The nanoparticles were administered to the rats through intraperitoneal injection, a method where drugs are directly injected into the peritoneal cavity, located within the abdomen and housing the abdominal organs. Furthermore, the rats were provided water through gavage, a method involving the forced delivery of fluids or substances, typically to animals, by introducing a tube down the throat and into the stomach.

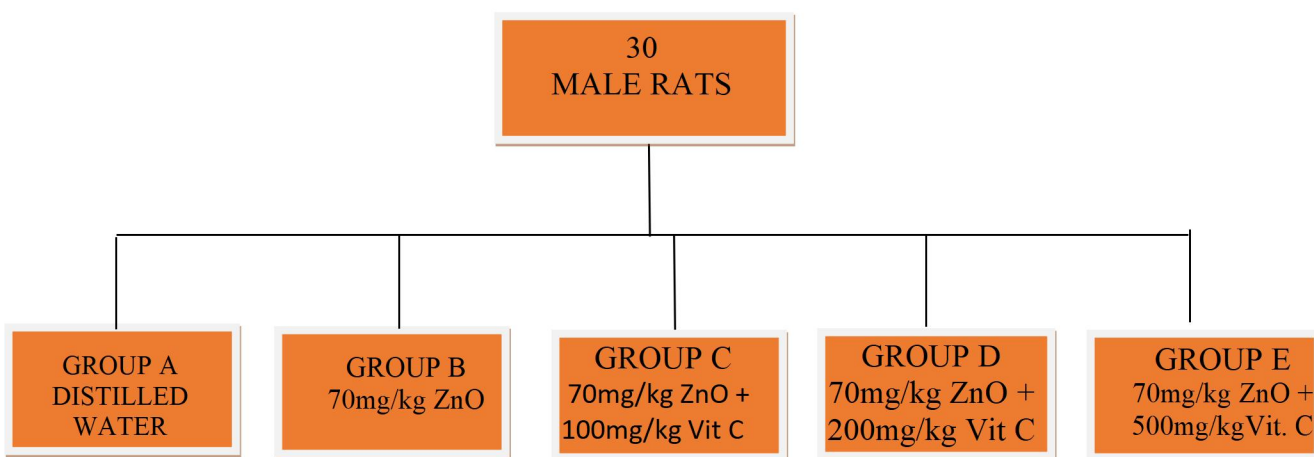


Figure 3.1: Schematic representation of the experimental design

The volume administered to each rat was calculated using the formula:

$$\text{Volume} = \text{dose} \times \text{weight} / \text{Conc. of stock}$$

Stock concentration: 1.0 of ZnO nanoparticles in 10ml of distilled water

Stock concentration : 4.0 g of vitamin in 20ml of distilled water. Shaken vigorously to ensure that the nanoparticles were completely dissolved in water.

Procedure: To dissolve the nanoparticles, 1.0g of ZnO - NPs was dissolved in 10ml of distilled water and shaken vigorously to ensure that it completely in water.

Group A: 1ml of distilled water

Group B: 0.1ml of ZnO nanoparticles

Group C: 0.1ml of ZnO nanoparticles + 0.08 vitamin C

Group D: 0.1ml of ZnO nanoparticles + 0.15 vitamin C

Group E: 0.1ml of ZnO nanoparticles + 0.4ml of vitamin C

3.2.3 Sacrifice of experimental animals

The experimental rats were exposed to chloroform, an inhalable or skin-absorbable volatile organic compound.

Blood was withdrawn from the rats' beating hearts with 5ml syringes and collected in heparinized tubes. Plasma isolation was done by centrifuging at 3000rpm for 10 minutes using a table centrifuge. The testes and epididymis tissues were excised with dissecting tools, stored at 4oC in a refrigerator, and representative samples were preserved in formalin for histopathological examination. The organs were cleaned in normal saline, dried with filter paper, weighed, and homogenized in four volumes of a homogenizing buffer (0.1 M phosphate buffer, pH 7.4) using a mortar and pestle. The homogenates were centrifuged at 10,000 g for 10 minutes

in a cold centrifuge, and the resulting supernatant was utilized for biochemical analysis. Furthermore, the testes and epididymis were macerated with a razor blade to extract mature sperm onto a slide with normal saline for sperm analysis, including motility assessment such as random or circular movements.

3.2.4 Oxidative stress assays

3.2.4.1 Determination of Catalase (CAT)

Catalase (CAT) activity was estimated by the method described by Cohen et al., (1970).

Reagents

Hydrogen peroxidase (H_2O_2)

Suphuric acid (6M) H_2SO_4

Preparation of reagents

0.01M KMnO_4 was prepared by distilling 0.158g of KMnO_4 in 100ml of distilled water.

Phosphate buffer (pH 7.4) 0.426g of NaHPO_4 and 0.240g of NaH_2PO_4 was weighed and dissolved in 100ml of distilled water. 6M H_2SO_4 and 32.3ml of conc. H_2SO_4 was added to 66.7ml of distilled water.

Procedure

To an unknown volume of plasma (0.5ml), 5.0ml of H₂O₂ was added. This was mixed by inversion and allowed to stand for 30min. The reaction was stopped by adding 1.5ml of 6M H₂SO₄ and 7ml of 0.01M KMnO₄. These were mixed by inversion and allowed to stand for 10min. The absorbance was read at 480nm within 30-60 seconds against distilled water. The enzyme blank was run simultaneously with 1.0ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as μmoles of H₂O₂ decomposed/min/mg/protein.

Calculation

$$\text{Activity} = \frac{\text{OD/min} \times V}{M \times V \times L \times Y}$$

Where OD = Absorbance

L= Light path

V= Total volume of reaction sample

M= Molar coefficient of H₂O₂ (40/m/cm)

V= Volume of sample

Y= mg protein in the sample

3.2.4.2 Estimation of Superoxide Dismutase Activity (SOD)

This was determined according to the methods of Masra and Fridorich (1972)

Principle

Adrenaline undergoes auto oxidation rapidly to adrenochrome whose concentration can be determined at 420nm with the aid of a spectrophotometer. The auto oxidation of adrenaline depends on the presence of superanions.

Superoxide dismutase inhibits the auto-oxidation of adrenaline by catalysing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 420nm.

Reagent and preparation

Carbonate buffer (0.05M) pH 10.2: This was prepared by dissolving 0.2014g of Na_2CO_3 , 0.2604g NaHCO_3 and 0.0372g of EDTA in 100ml of distilled water. The pH was adjusted to 10.2 using Sodium hydroxide.

Hydrochloric acid (0.005M): This was prepared by adding 0.044 concentration of HCL to 99.96mls of distilled water.

Adrenaline solution (0.3mM): This was prepared by dissolving 0.01098g of adrenaline in 100mls of 0.005M HCL solution.

Plasma volume of 100ml was mixed with 125ml of carbonate buffer and 150ml of adrenaline solution. 100ml of distilled water was mixed with 1.25ml of carbonate buffer as reference sample. These were mixed and absorbance read at 420nm.

These were mixed and read at 420nm

$$\% \text{inhibition} = \frac{(\text{O.D test} - \text{ODref}) \times 100}{\text{OD test}}$$

Enzyme concentration can thus be calculated

$$\text{Unit/mg protein} = \frac{\% \text{ inhibition}}{50 \times Y}$$

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

3.2.4.4 Estimation of glutathione peroxidase (GPx)

This was determined according to Nyman (1959)

Principle

This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a deep brown color disposition, read at 420nm.

Reagent and preparation

Pyrogallol (20mM): 0.2552g of pyrogallol was dissolved in 100mls of distilled water.

Procedures

To an aliquot of plasma (0.2ml), 2.5ml of phosphate buffer, 2.5ml of H₂O₂, 1.5ml of distilled water and 2.5ml of pyrogallol was added.

The reaction was allowed to stand for 30mins at room temperature. A deep brown color was formed which was read at 480nm.

Calculations

$$\text{Activity} = \frac{\text{OD/min} \times v_t \times D_f}{E \times V_s \times Y}$$

OD= Absorbance of test

V_t= Total volume of reaction mixture

D_f= Diution factor = 1

E= Molar extinction co-efficient (12/m/cm)

V_s= Volume of sample

Y= mg of protein used

3.2.4.5 Determiration of Malondialdehyde (MDA)

Malonaldehyde was determined using the thiobarbituric acid assay (Buege and Aust, 1978)

Principle

Malonaldehyde which is a product of lipid peroxidation react with thiobabituric acid (TBA) to give a red species.

Procedure

A volume of plasma (1.0ml) was added to 2.0ml of TCA-TBA-HCL and mixed thoroughly. The solution was heated for 15mins in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuged at 1000g for 10min. The absorbance was determined using the formula;

$$\text{MDA (mol/mg protein)} = \frac{A \times V \times 100}{M \times V \times Y}$$

A= Absorbance

V= Total volume of reaction mixture

M= Molar extinction coefficient

V= volume of the sample

Y= mg protein

CHAPTER FOUR

RESULTS

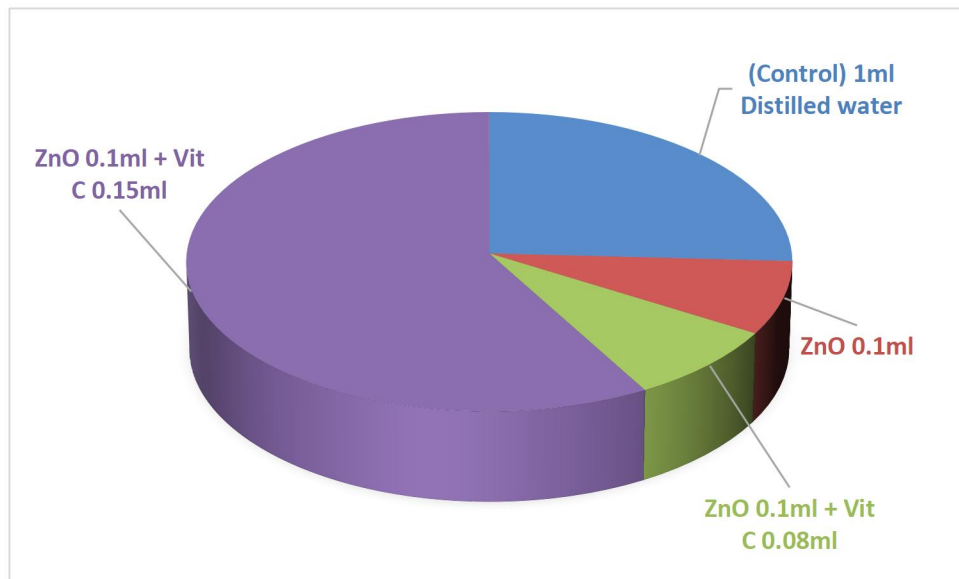


Figure 4.1: Change in weight of wistar rat

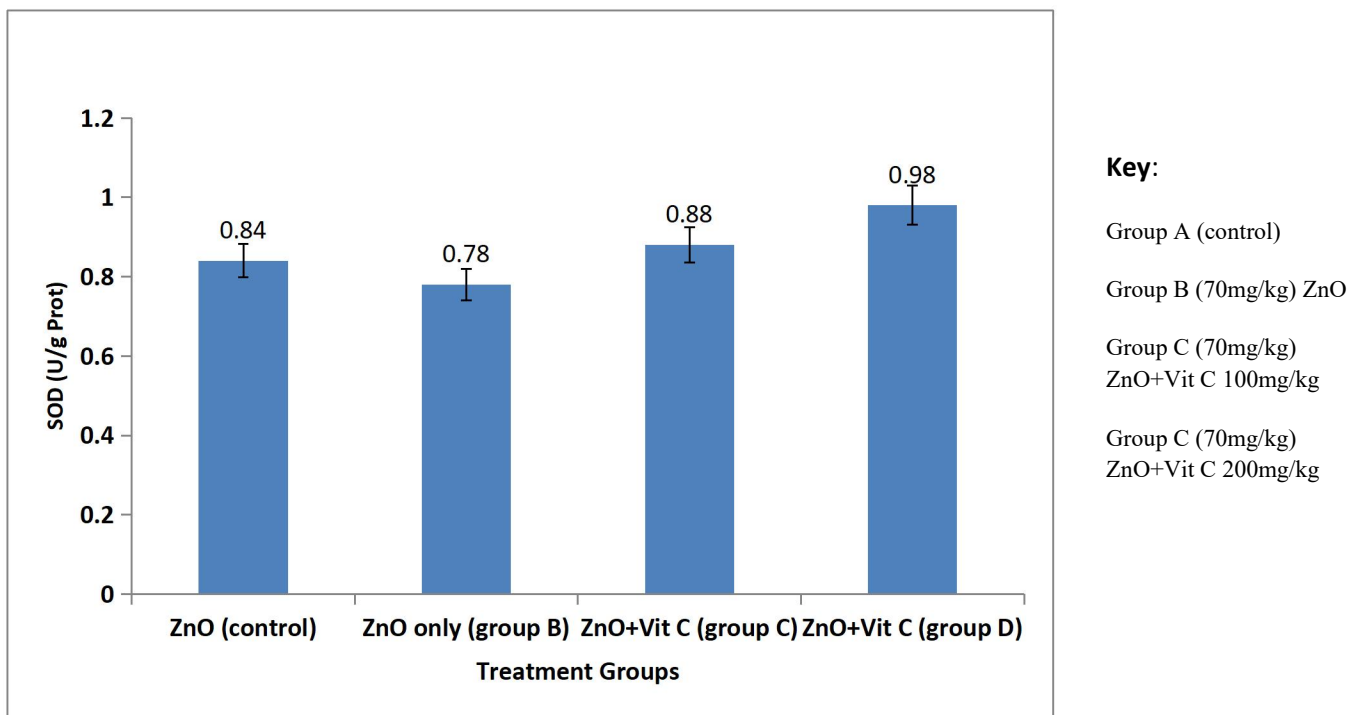


Figure 4.2: Effect of ZnO + vit C on SOD enzyme activity in the epididymis of male wistar rats.

Values are expressed as mean \pm SEM (n=5)

Significant as compared with control at $p < 0.05$

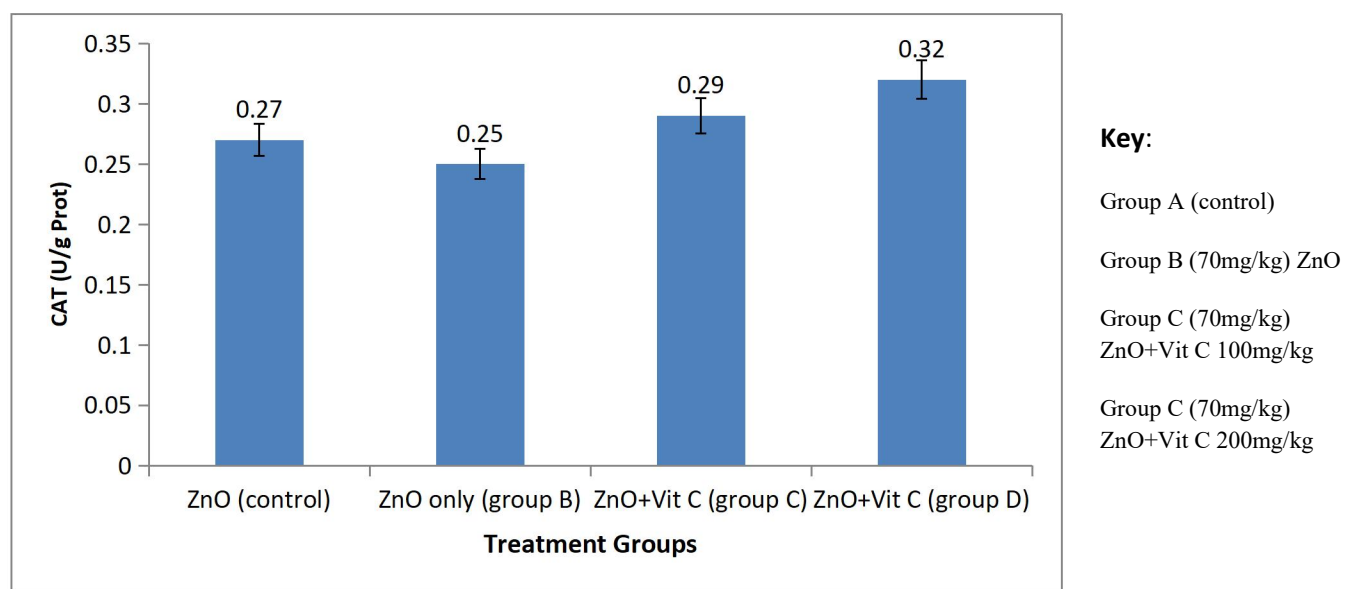


Figure 4.3: Effect of ZnO + vit C on CAT enzyme activity in the epididymis of male wistar rats.

Values are expressed as mean \pm SEM (n=5)

Significant as compared with control at $p < 0.05$

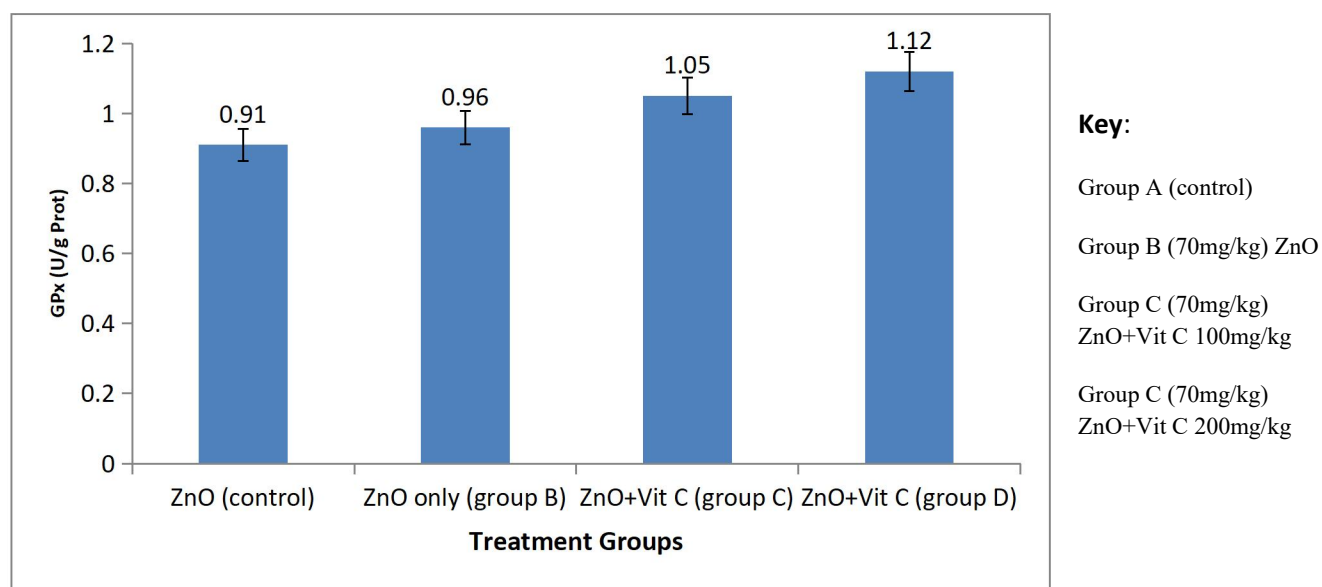


Figure 4.4: Effect of ZnO + Vit C on GPX enzyme activity in the epididymis of male wistar rats.

Values are expressed as mean \pm SEM (n=5)

Significant as compared with control at $p < 0.05$

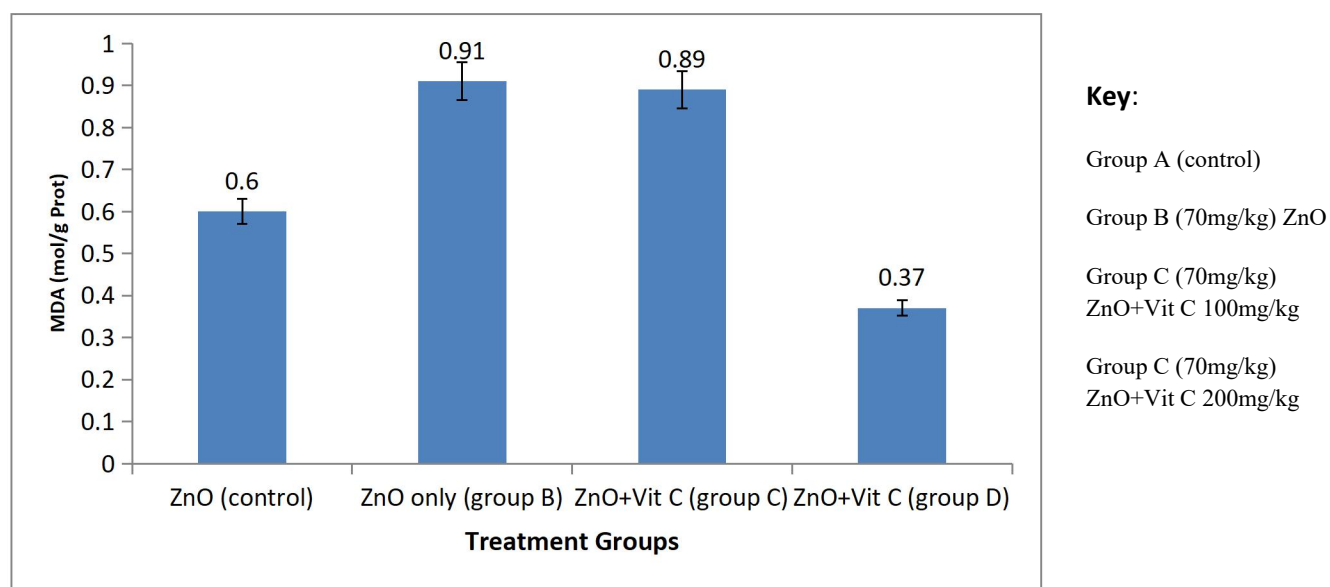


Figure 4.5: Effect of ZnO + vit C on MDA enzyme activity in the epididymis of male wistar rats.

Values are expressed as mean \pm SEM (n=5)

Significant as compared with control at $p < 0.05$

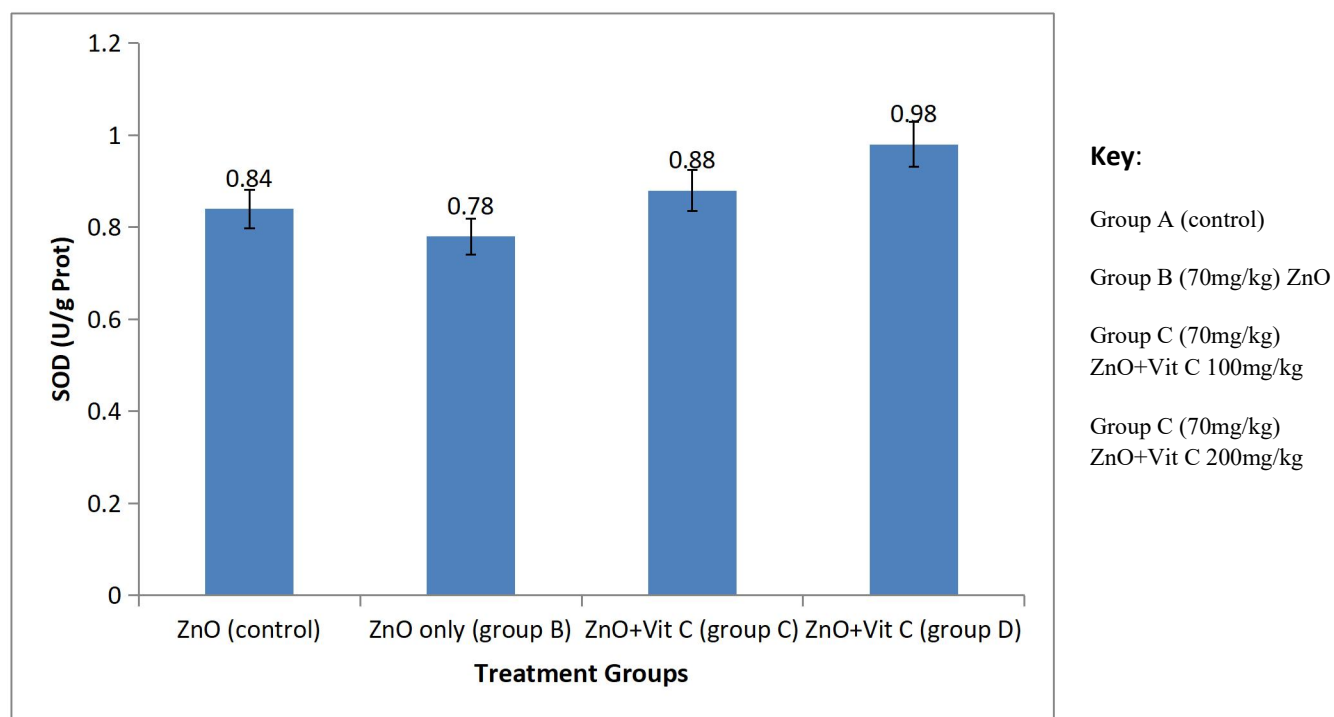


Figure 4.6: Effect of ZnO + vit C on SOD enzyme activity in the testis of male wistar rats.

Values are expressed as mean \pm SEM (n=5)

Significant as compared with control at $p < 0.05$

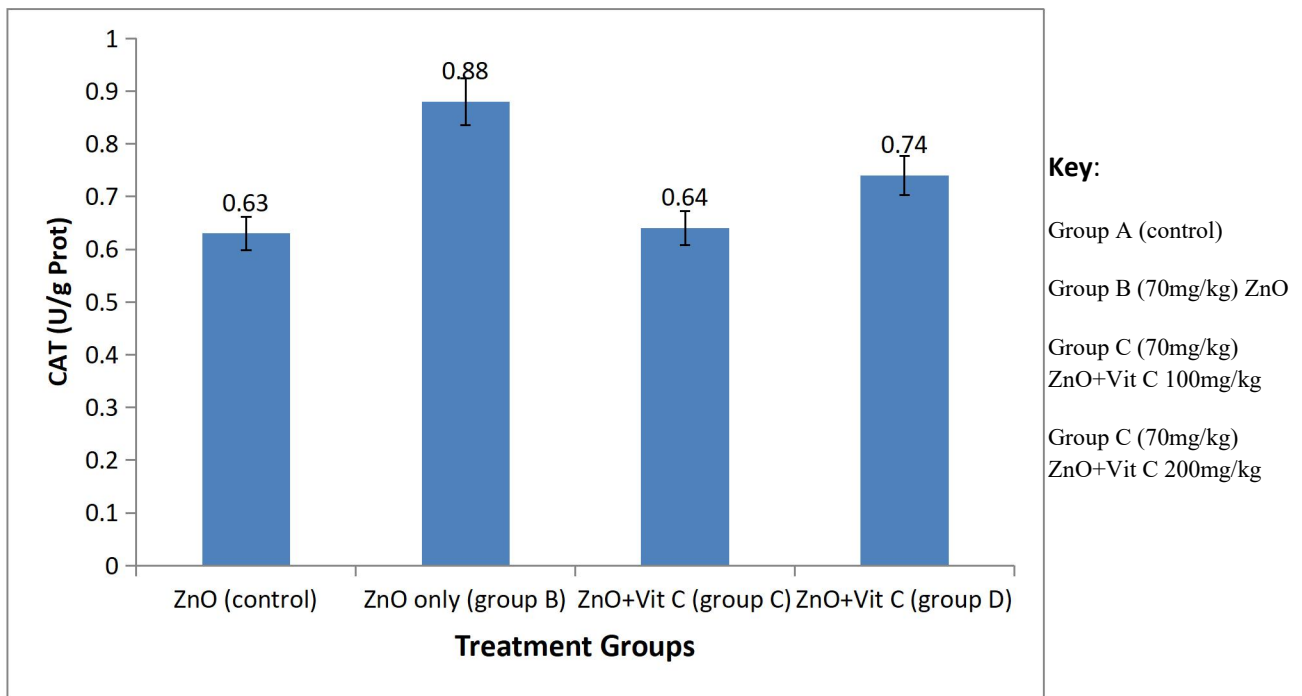


Figure 4.7: Effect of ZnO + vit C on CAT enzyme activity in the testis of male wistar rats.

Values are expressed as mean \pm SEM (n=5)

Significant as compared with control at $p < 0.05$

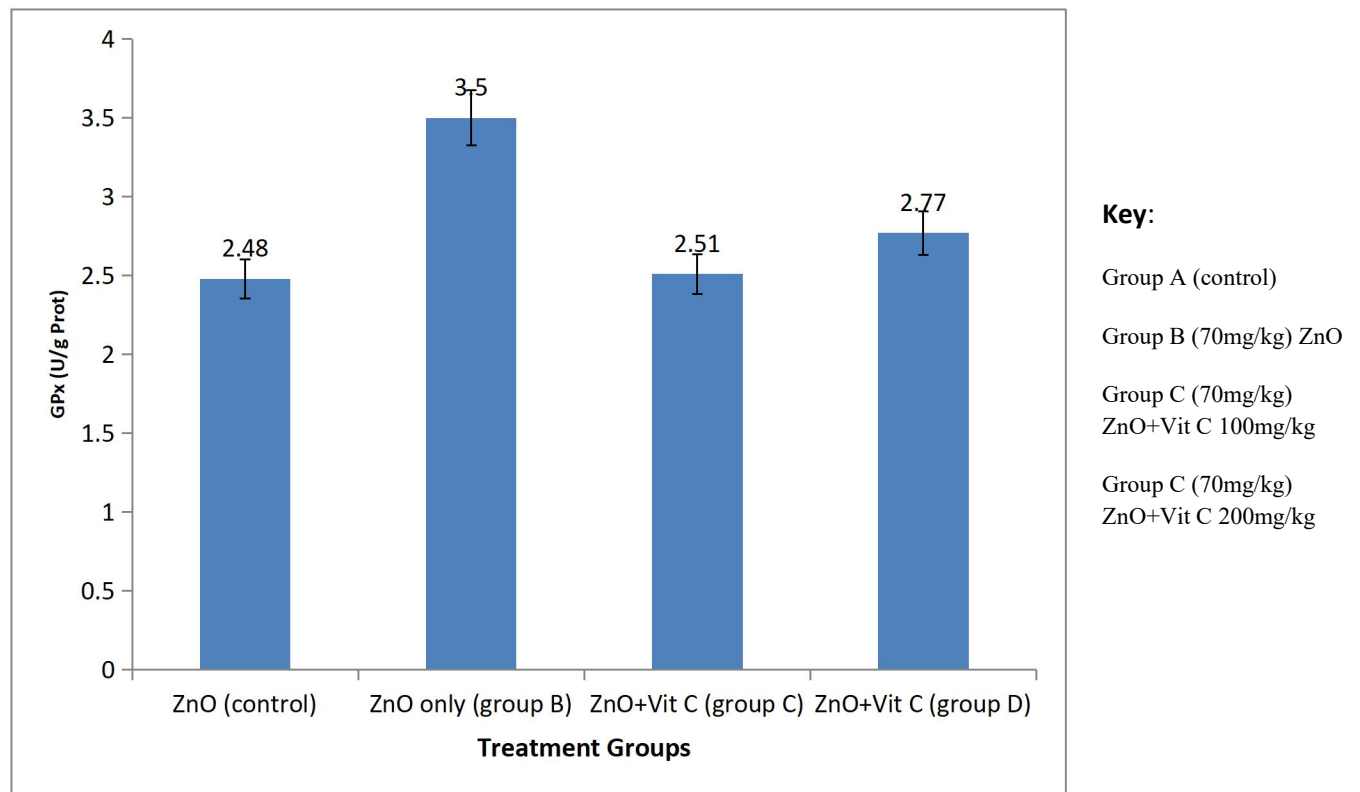


Figure 4.8: Effect of ZnO + vit C on GPX enzyme activity in the testis of male wistar rats.

Values are expressed as mean \pm SEM (n=5)

Significant as compared with control at $p < 0.05$

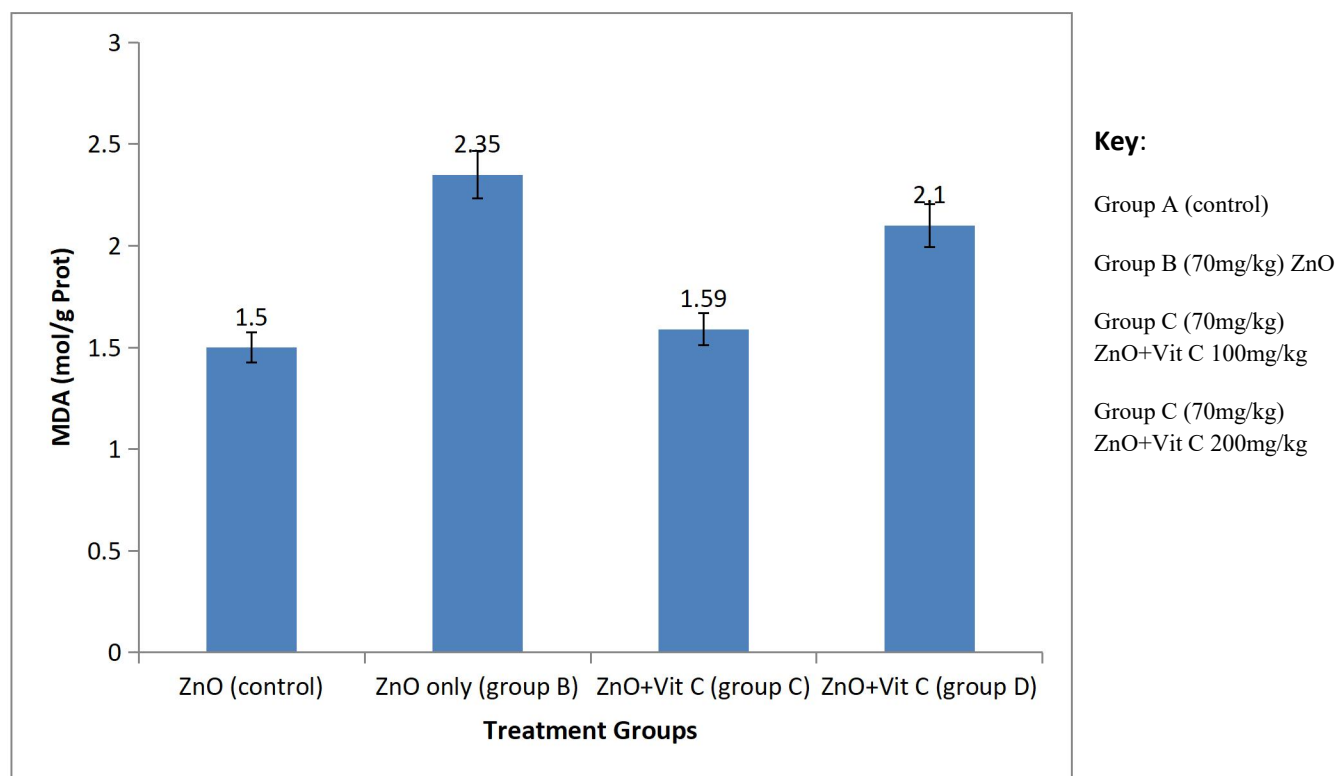


Figure 4.9: Effect of ZnO + vit C on MDA enzyme activity in the testis of male wistar rats.

Values are expressed as mean \pm SEM (n=5)

Significant as compared with control at $p < 0.05$

Table 4.1: Effect of ZnO + vit C on sperm parameters in male wistar rats.

	Control	ZnO only	ZnO 70mg/kg + Vit C 100mg/kg	ZnO 70mg/kg + Vit C 200mg/kg
Live (100%)	83.0±8.51	1.67±0.33	60.5±7.32	38.3±2.89
Dead (100%)	17.0±8.51	98.3±0.33	39.3±7.45	65.0±8.66
Progressive movement	66.7±8.82	1.00±0.00	54.5±7.60	21.7±18.9
Circular movement	0.33±0.33	0.00±0.00	3.00±0.71	1.00±1.73

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1. Discussion

Researchers wanted to see how vitamin C affects the harmful effects of ZnO-Nps on the testis and epididymis of male wistar rats. Vitamin C is a strong antioxidant that can protect against damage caused by oxidative stress. Wistar rats were chosen for the study because they are easy to get and take care of in labs, thanks to their friendly nature and ability to adapt easily. Wistar rats have the same genetic backgrounds and traits for research. Group D in the study had animals that lost weight, while the other groups did not show significant weight differences. Another finding was that all animals in group E (ZnO-Np 70mg/kg + Vit 500mg/kg) died, showing a high mortality rate. The decrease in weight seen in the test subjects suggests a possible negative impact on their health. Losing weight could indicate different health issues, such as toxicity or overall stress on the body. The study indicates that being around zinc oxide nanoparticles, by

themselves or with other things, may have hurt the animals' health. This finding matched what Yousef et al. (2019) found in their research on how zinc oxide nanoparticles and vitamin C together can be harmful.

SOD is like a shield that protects our body from harmful substances called reactive oxygen species (ROS). It is an important antioxidant enzyme that helps to fight against oxidative stress by getting rid of superoxide radicals. In a study, researchers found that the SOD enzyme was activated when the epididymis was exposed to a combination of zinc oxide and vitamin C. They noticed a big increase in the groups that received 100mg/kg and 200mg/kg of "ZnO NPs+ Vit C" treatment. A big drop was seen in the group that only had ZnO, but it wasn't meaningful ($p > 0.05$) compared to the control. However, in the testis, there were big drops and rises in the groups that got "ZnO NPs+ Vit C" at 100mg/kg and 200mg/kg. There was also a big rise in the group that only had ZnO, which was meaningful ($p < 0.05$) compared to the control. This outcome is like what O. Ighodaro and O. Akinloye found in 2018. They saw a big increase in the ZnO NPs+ Vit C groups and a decrease in the group that only had ZnO.

Catalase (CAT) enzyme is an important antioxidant that is found in almost all aerobic organisms, breaking down hydrogen peroxide into oxygen and water. This result shows that there were significant increase in the epididymis of the treatment groups "ZnO NPs+ Vit C" at (100mg/kg and 200mg/kg). For ZnO only group, significant decrease was observed, which was insignificant ($p > 0.05$) when compared to the control. In the testicles, there was a big increase seen in the groups that had "ZnO NPs+ Vit C" at doses of 100mg/kg and 200mg/kg. There was also a noticeable increase in the group that only had ZnO. This increase was significant compared to the control group, as shown by the results ($p < 0.05$). This matches what Tang et al. (2019) found in their research.

Glutathione peroxidase is an important enzyme that helps cells stay healthy by fighting off damage caused by oxygen and fats. Changes in how well this enzyme works can show if a cell's defenses against damage are changing. In this study, it was found that the groups treated with "ZnO NPs+ Vit C" at 100mg/kg and 200mg/kg had a big increase in glutathione peroxidase levels in the epididymis. The group treated with just ZnO also had an increase, but it wasn't as big and didn't make a significant difference compared to the control group.

But, in the testis, it was noticed that there were big increases in the groups that were treated with "ZnO NPs+ Vit C" at (100mg/kg and 200mg/kg) and the group that only had ZnO, which was important ($p < 0.05$) when compared to the control. This outcome is like what Ogunsuyi et al. (2020) found.

Malondialdehyde: is a commonly used biomarker to evaluate oxidative stress levels, formed as a byproduct of lipid peroxidation during oxidative stress. The result in this study shows that there were significant increase and decrease in the epididymis of the treatment groups " ZnO NPs+ Vit C " at (100mg/kg and 200mg/kg), while it was observed that there was significant increase in the znO only group, which was insignificant ($p > 0.05$) when compared to the control. However, in the testis it was observed that there were significant increases in the treatment groups " ZnO NPs+ Vit C " and ZnO only group, but which was insignificant ($p > 0.05$) when compared to the control. This outcome aligns with the research by Mumtaz and colleagues from 2020. Their study indicates that ZnO NPs can have harmful effects on the tissue structure of the epididymis and testicles. Previous studies reported that oxidative biomarkers (malondialdehyde) was increased in the ZnO only group. By contrast, the gene expression and activities of antioxidant enzymes (SOD, GPx, and CAT) noted a significant decrease in the ZnO group regarding control ($p < 0.05$). However, oxidative markers were remarkably mitigated after combined treatment of ZnO

NPs and Vit C compared to the rats given ZnO NPs ($p < 0.05$). Additionally, compared to the ZnO NP group, the rats receiving Vit C + ZnO NPs exhibit increased antioxidant enzyme activity ($p < 0.05$). The study demonstrates the role of Vit C ameliorative effects on toxicity incurred by ZnO NPs Ziamajidi et al. (2023).

The sperm test results indicated that there were fewer live sperm cells in Wistar rats treated with ZnO alone and ZnO+Vitamin C compared to the control group. This finding supports the study by Mokhimar et al. (2020) which also observed a decrease in live sperm cells in Wistar rats exposed to ZnO alone and ZnO+Vitamin C. Researchers noticed that there were more live sperm cells in male rats treated with ZnO or ZnO+Vit C compared to those not treated. This finding supports a previous study by Khodarahmian et al. (2021). Additionally, the movement of sperm cells in treated rats was slower than in the control group. Researchers found that there was a big decrease in the number of sperm cells moving in circles in Wistar rats treated with only ZnO or ZnO combined with Vitamin C. This matches what Zaki and colleagues discovered in 2024, where they also saw a notable drop in sperm count in Wistar rats exposed to ZnO compared to those not exposed. This study's results support what Agarwal and Shanmugam found in 2019. Earlier research showed that ZnO can cause cells to die through apoptosis, leading to lower sperm motility. This study also observed disturbances in spermatogenesis and primary and secondary spermatocytes caused by ZnO. Ahmadi (2016) found that ZnO can cause harm to testicular cells by creating oxidative damage.

5.2 Conclusion

In summary, our research shows that ZnO NPs can harm the testes of Wistar rats, but taking Vit C might help lessen the damage caused by ZnO NPs. Vit C intake could protect the male

reproductive system by reducing oxidative stress. Our study found that rats given 100mg/kg of Vit C had significant protective effects.

5.3 Recommendation

The result of this study suggest that more research be done on the possible application of vitamin C supplementation as a defense against the toxic effect of ZnO - NPs on testis and epididymis function, oxidative stress, and sperm health in wistar rat

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APPENDIX

Table 1: Weight of rats before administration (initial) and before sacrifice (final)

Groups	Initial weight	Final weight	Change in weight
A (Control) 1ml Distilled water	126.5g	137.6g	11.1
B ZnO 0.1ml	144.2g	140.8g	3.4
	147.3g	143.8g	3.5

C ZnO 0.1ml + Vit C 0.08ml			
D ZnO 0.1ml + Vit C 0.15ml	145g	120g	25

Table 2: Oxidative stress responses in epididymis of male wistar rats administered ZnO + Vit C.

	ZnO (control)	ZnO only (group B)	ZnO+Vit C (group C)	ZnO+Vit C (group C)	P-value
SOD (U/g Prot)	0.84 ± 0.08	0.78 ± 0.06	0.88 ± 0.17	0.98 ± 0.17S	0.769
CAT (U/g Prot)	0.27 ± 0.03	0.25 ± 0.02	0.29 ± 0.06	0.32 ± 0.06	0.729
GPx (U/g Prot)	0.91 ± 0.06	0.96 ± 0.08	1.05 ± 0.22	1.12 ± 0.22	0.801
MDA (mol/g Prot)	0.60 ± 0.04	0.91 ± 0.14	0.89 ± 0.18	0.37 ± 0.08	0.876

Table 3: Oxidative stress responses in testis of male wistar rats administered ZnO + vit C.

	ZnO (control)	ZnO only (group B)	ZnO+Vit C (group C)	ZnO+Vit C (group C)	P-value
SOD (U/g Prot)	1.99 ± 0.04	2.56 ± 0.13	1.97 ± 0.15	2.22 ± 0.21	0.032
CAT (U/g Prot)	0.63 ± 0.03	0.88 ± 0.06	0.64 ± 0.05	0.74 ± 0.07	0.016
GPx (U/g Prot)	2.48 ± 0.08	3.50 ± 0.31	2.51 ± 0.21	2.77 ± 0.28	0.027
MDA (mol/g Prot)	1.50 ± 0.32	2.35 ± 0.23	1.59 ± 0.17	2.10 ± 0.37	0.123