INVITRO ANTIOXIDANT PROPERTIES OF METHANOLIC LEAF EXTRACT OF *Sphenocentrum jollyanum* AND ITS EFFECT ON SOME HORMONAL PARAMETERS AND LIVER AND KIDNEY FUNCTION IN MALE WISTAR RATS

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A THESIS WRITTEN IN THE DEPARTMENT OF BIOCHEMISTRY AND SUBMITTED TO SCHOOL OF POSTGRADUATE STUDIES, UNIVERSITY OF BENIN, BENIN CITY.

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CERTIFICATION OF THESIS

We the undersigned attest and declare that the thesis of Mr Osamudiamen Asemota ENEHIZENA titled Invitro Antioxidant Properties of Methanolic Leaf Extract of Sphenocentrum jollyanum and Its Effect on Some Hormonal Parameters and Liver and Kidney Function In Male Wistar Rats has successfully passed the anti-plagiarism test and does not violate any copy write regulations.

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External Examiner **Date**

DEDICATION

This work is dedicated to God Almighty for His Grace and Mercy. It is also dedicated to my parents and siblings.

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I will like to express my immense gratitude to my supervisor, Prof. N.P. Okolie for his understanding, patience, mentoring and immense contributions towards the success of this work. I will also like to express gratitude to the Head of Department, Prof. Eusebius Chukwu Onyeneke. I am also grateful to my lecturers, as well as the technical staff of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, thank you all for your help and support.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Procreation was an important moral issue, and to ensure that both men and women are potent enough to procreate, aphrodisiacs were used to correct sexual dysfunction. Sexual dysfunction is basically the inability to achieve a normal sexual intercourse, and this include premature or quick ejaculation, inhibited ejaculation, erectile dysfunction, reduced libido, and orgasmic disorder (Kotta *et al.*, 2013). In the 1990s, scientists carried out research work on aphrodisiacs and the first pharmacological remedy for impotence, Viagra (sildenafil) was approved. In a bid to look for an agent that arouses sexual desire (aphrodisiac) with fewer side effects, the hunt for natural supplement from medicinal plants was intensified. Aphrodisiac plants have since been discovered and they have been pharmacologically tested in animals.

Aphrodisiacs are classified into three types based on their mode of action, and they are: those that increase potency, those that increase sexual pleasure, and those that increase libido (Sandroni, 2001). The use of medicinal plants or plant-based products to stimulate sexual desire and to enhance performance is almost as old as the human race itself (Chauhan *et al*., 2014). The active crude extracts of these plants have been of help in handling sexual disorders and also improving sexual behavior and performance. The active crude extracts from these plants are also helpful in spermatogenesis (sperm formation) and reproduction. In Arab countries, Ambrein (a triterpene alcohol which is a major constituent of *Ambra grisea*) is used to increase libido by increasing the concentration of anterior pituitary hormones and serum testosterone (Kotta *et al.*, 2013). According to a study carried out by the World Health Organization (WHO), male

reproductive capacity was found to be deficient in nearly 50% of infertile couples (Chauhan *et al*., 2014). One of the plant scientists have carried research on in terms of sexual performance is *Sphenocentrum jollyanum*. *Sphenocentrum jollyanum* is a member of the plant family Menispermaceae. *Sphenocentrum jollyanum* is a shrub native to the tropical forest zones of West Africa and is widely distributed in Nigeria, Sierra Leone, Ghana, Cameroon and Ivory Coast (Nia *et al*., 2004).

Sphenocentrum jollyanum

Sphenocentrum jollyanum is commonly known as Aduro Kokko (red medicine), or Okramankote (dog's penis) in the Akan language of Ghana. The stem-bark, roots, leaves, fruits and other parts of the plant have been used extensively for the treatment of ailments in some West African countries. Root extract of *Sphenocentrum jollyanum* has been used for the treatment of constipation, rheumatism, sickle cell disease, and as cough medicine (Iwu, 1993; Moody *et al*., 2006). The fruits of *Sphenocentrum jollyanum* are used as an anti-fatigue snack (snack that reduce fatigue). Raji *et al*. (2006) have shown that methanolic extract of the root of *Sphenocentrum jollyanum* increased the level of testosterone hormone in a dose-dependent manner, and also reduced the count, motility and viability of spermatozoa in albino rats.

Viagra (Sildenafil)

Sildenafil is a highly selective inhibitor of the enzyme, phosphodiesterase type 5 (PDE5), an intracellular enzyme that degrades cyclic guanosine monophosphate (cGMP), thus converting cGMP to 5′-GMP. Sildenafil is widely used for the treatment of erectile dysfunction (Boolell *et al*., 1996; Benchkroun *et al*., 2003). PDES is present in penile tissue, platelets, skeletal muscles, vascular and visceral smooth muscles, and sildenafil acts via the release of nitric oxide (NO)

(Wellis *et al*., 1999). Sildenafil relaxes the muscles of the blood and also increases the flow of blood to particular areas of the body (mostly penis). Sildenafil at 50mg or 100mg has been shown to significantly improve erection quality, treatment satisfaction, anxiety levels, and the sexual experience as reported by Loran *et al*. (2009).

1.2 JUSTIFICATION OF THE STUDY

The use of *Sphenocentrum jollyanum* for medicinal purposes in the treatment or management of sexual dysfunction calls for proper scientific investigation. This study seeks to determine the effect of methanolic leaf extract of *Sphenocentrum jollyanum* on biochemical, hormonal, and histopathological parameters, and also to compare its effect with Viagra (sildenafil) on sexual performance. The study also seeks to determine the in vitro antioxidant properties of the methanolic leaf extract of *Sphenocentrum jollyanum*.

1.3 AIM AND OBJECTIVES OF THE STUDY

The aim of this study is to determine the in vitro antioxidant properties of the methanolic leaf extract of *Sphenocentrum jollyanum*, and its effect on some hormonal and biochemical parameters in male Wistar rats.

The objectives of this study are as follows;

i. To determine the in vitro antioxidant properties of the methanolic leaf extract of *Sphenocentrum jollyanum*.
ii. To determine the effect of the methanolic leaf extract of *Sphenocentrum jollyanum* on the following sex hormones; follicle stimulating hormone (FSH), Luteinizing Hormone (LH), and Testosterone.

iii. To determine the effect of methanolic leaf extract of *Sphenocentrum jollyanum* on the following biochemical parameters; Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Total Protein (TP), Total Bilirubin, Conjugated Bilirubin, Urea, Creatinine, Catalase, Superoxide Dismutase (SOD), Glutathione Peroxidase (GP_X), and Malondialdehyde (MDA).

iv. To determine the histopathological effect of the methanolic leaf extract of *Sphenocentrum jollyanum* on the testes and prostate.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Sphenocentrum jollyanum*

Sphenocentrum jollyanum is a member of a diverse family of plants which is known as Menispermaceae, and it is often employed in folkloric medicine as a cure for fever, cough, constipation, tumor in the breast, hypertension, and also as an aphrodisiac. It is also used to dress chronic wound. *Sphenocentrum jollyanum* is widely distributed in Nigeria and other West African countries. In South-Western Nigeria, *Sphenocentrum jollyanum* is traditionally known as Akerejupon or Ajo. In Benin Republic, it is known as Oban abe, while in Ivory Coast, it is known as Ouse-abe (Amidu, 2008). The plant is known as Orjinkoro in Izzi language of Ebonyi State (Ekpono *et al*., 2018).

2.1.1 Plant Description

Sphenocentrum jollyanum is a perennial plant which thrives in deep shade, from sea-level up to 400m altitude. *Sphenocentrum jollyanum* is mostly found in regions with mean minimum temperature of 20˚C and mean maximum of 29˚C. The plant grows to an average height of about 1.5m, with few scanty branches. The leaves of this plant are wedge-shaped and also smooth on both sides. The leaves have a small narrow apex and can grow up to about 20cm long. The roots are bright yellow in color and are characterized by a sour acidic taste which causes things eaten afterwards to taste sweet (Abbiw, 1990). The fruits are fleshy, edible and bright yellow or orange when ripe (Abbiw, 1990; Neuwinger, 1996). They (fruits) occur as clusters, ovoid-ellipsoid, with a single, large oval-shaped seed.

Plate 2.1: The tree of *Sphenocentrum jollyanum* (Olorunnisola *et al*., 2017).

Plate 2.2: The leaves of *Sphenocentrum jollyanum* (Olorunnisola *et al*., 2017).

Plate 2.3: Fruits of *Sphenocentrum jollyanum* (Olorunnisola *et al*., 2017).

Table 2.1: Taxonomical classification of *Sphenocentrum jollyanum*

Kingdom	Plantae
Division	Magnoliophyta (Cronquist)
Subdivision	Magnoliophytina (Frohne and Jensen)
Class	Ranunculopsida (Brongn)
Subclass	Ranunculidae (Takht)
Suborder	Ranunculanae (Takht)
Order	Menispermales (Bromhead)
Family	Menispermaceae (Juss.)
Genus	Sphenocentrum (Pierre)
Species	Jollyanum

2.1.2 Distribution of *Sphenocentrum jollyanum*

The presence of *Sphenocentrum jollyanum* has been recorded in West African countries (Ghana, Nigeria, Ivory Coast, Sierra Leone, Cameroun, and Benin Republic). Here in Nigeria, it is widely distributed in South-West, South-East and South-South.

2.1.3 Ethno-Medicinal Uses of *Sphenocentrum jollyanum*

Different parts of *Sphenocentrum jollyanum* has been employed traditionally in Africa for the treatment of different ailments. In Nigeria for instance, the roots of *Sphenocentrum jollyanum* are chewed to increase digestion, stimulate appetite and also relieve constipation. Interestingly, the morphological organs of *Sphenocentrum jollyanum* are used as important ingredients in the management of sickle cell anemia disease (Abbiw, 1990).

In Ghana, the roots of this plant are used by the men as an aphrodisiac. To achieve this, the roots are soaked in alcohol (ethanol) for some days so as to allow for the extraction of the active components in the roots. The alcoholic root extract is then drank as bitters to strengthen penile erection, and this effect is known to be long lasting (Irvine, 1961; Burkill, 1985).

Traditional healers in countries like Ghana, Ivory Coast have reported the roots of *Sphenocentrum jollyanum* to possess medicinal properties against breast tumor, hypertension, diabetes mellitus and irregular menstrual cycle (Iwu, 1993; Kayode *et al*., 2009).

2.1.3.1 Anti-Diabetic Activity

Plant extract of *Sphenocentrum jollyanum* has been shown to have blood glucose lowering potential. In a study carried out on alloxan-induced diabetic rats, the seed extract of *Sphenocentrum jollyanum* administered 30 min before a carbohydrate-rich diet considerably reduced blood glycemic level by 20% relative to the untreated group (Mbaka *et al*., 2010).

2.1.3.2 Antioxidant Activity

Antioxidants are known to prevent the oxidation of biomolecules in the body and by doing so, oxidative stress is averted. According to a study carried out by Olorunnisola & Afolayan (2013), *Sphenocentrum jollyanum* significantly ameliorated the oxidative stress related with *Plasmodium berghei* (a protozoan parasite that causes malaria) infection in mice, and this was evident in the reduced level of Malondialdehyde (MDA) in the liver.

2.1.3.3 Anti-Allergy Activity

The anti-allergic study of *Sphenocentrum jollyanum* was carried out on milk-induced leukocytosis and eosinophilia in mice. Olorunnisola *et al*., (2017) reported that the ethanolic fruit

extracts of *Sphenocentrum jollyanum* demonstrated a dose-dependent decrease in lymphocyte counts, as well as eosinophil counts in mice. This study suggested that the fruit extract of *Sphenocentrum jollyanum* has anti-allergic property, and the mode of action of the fruit extract may involve multiple mechanisms, particularly due to the interactions of different phytochemicals present in the fruit (Olorunnisola *et al*., 2017).

2.1.3.4 Anti-Bacterial Activity

The essential oil composition of the root extract of *Sphenocentrum jollyanum* was studied against *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus mirabilis* and *Pseudomonas aeruginosa* (Aboaba & Ekundayo, 2010). It was observed that the essential oil was effective against *Bacillus subtilis* and *Pseudomonas aeruginosa* strains (Aboaba & Ekundayo, 2010). Koleosho *et al*., (2013) showed that the plant extract of *Sphenocentrum jollyanum* is a potent inhibitor of *Salmonella typhi* and the moderate antimicrobial activity of the plant extract supports the use of the plant as a laxative which aids proper bowel movement and digestion.

2.1.3.5 Hematological Activity

The hematopoietic activities of the methanolic root and leaf extract of *Sphenocentrum jollyanum* were investigated on Wistar rats infected with chloroquine-resistant *Plasmodium berghei* NK67. The study showed that there was a significant increase in packed cell volume (PCV), mean corpuscular volume (MCV), and hemoglobin which was suggestive of the ability of the plant extract to stimulate hematopoietic stem cells (Olorunnisola *et al*., 2011; Mbaka *et al*., 2010).

2.2 Viagra (Sildenafil)

Description

Viagra is an oral therapy for erectile dysfunction. It is the citrate salt of sildenafil. The drug is a selective inhibitor of the intracellular enzyme, phosphodiesterase type 5 (PDE5), which catalyzes the degradation of cyclic guanosine monophosphate (cGMP). PDE5 is a key enzyme in the regulation of cGMP-specific signaling pathways in normal physiological processes, such as smooth muscle contraction and relaxation.

Sildenafil citrate is designated chemically as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine citrate (Batista *et al*., 2010) and has the following structural formula:

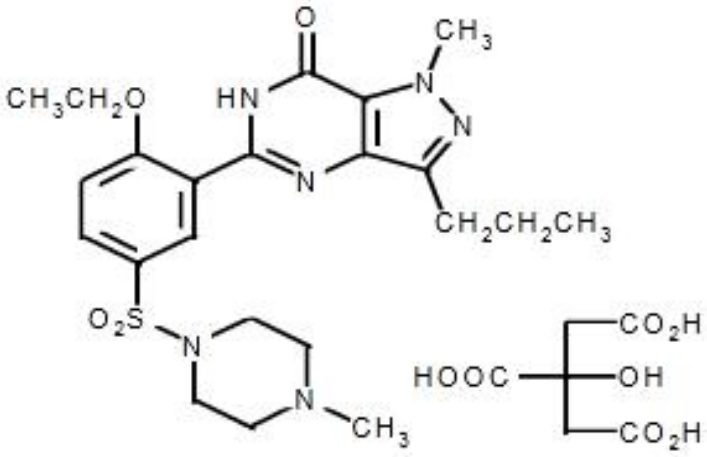


Figure 2.2: Chemical structure of sildenafil citrate (Batista *et al*., 2010)

As a white to off-white crystalline powder, sildenafil citrate has a solubility value of 3.5mg/mL in water and a molecular weight of 666.7. VIAGRA (sildenafil citrate) is formulated as a blue, film-coated rounded-diamond-shaped tablets equivalent to 25 mg, 50 mg and 100 mg of sildenafil for oral administration (Batista *et al*., 2010). Each tablet of sildenafil citrate contains the following inactive ingredients: magnesium stearate, titanium dioxide, anhydrous dibasic calcium phosphate, lactose, triacetin and microcrystalline cellulose.

2.2.1 Mechanism of Action of Sildenafil Citrate

The physiologic mechanism of erection of the penis involves the release of nitric oxide in the corpus cavernosum during sexual stimulation. The nitric oxide in turn activates the enzyme, guanylate cyclase (GC), which catalyzes the conversion of GTP to cGMP, thus increasing the level of the second messenger (cGMP) (Berzas *et al*., 2000). This effect produces smooth muscle contraction in the corpus cavernosum which allows the inflow of blood to the penis. Sildenafil citrate has no direct effect on isolated corpus cavernosum, what it does is to enhance the effect of nitric oxide by inhibiting PDE5, which is responsible for the degradation of cGMP in the corpus cavernosum. When sexual stimulation causes local release of NO, inhibition of PDE5 by sildenafil causes increased levels of cGMP in the corpus cavernosum, resulting in smooth muscle relaxation and inflow of blood to the corpus cavernosum.

2.2.2 Pharmacokinetics and Metabolism of Sildenafil Citrate

After oral administration, sildenafil citrate is rapidly absorbed with a mean absolute bioavailability of 41% (range 25-63%). The pharmacokinetics of sildenafil citrate is dose-proportional over the recommended dose range. The drug is eliminated predominantly by hepatic metabolism (mainly cytochrome P_{450} 3A4) and converted to an active metabolite with properties

similar to the parent compound (Berzas *et al*., 2000). Both sildenafil and the metabolite have terminal half-lives of about 4 hours.

2.2.3 Absorption and Distribution of Sildenafil Citrate

The maximum absorbed plasma concentration of sildenafil citrate is reached within 30 to 120 minutes (median 60 minutes) of oral dose in the fasted state. When this drug is taken with a fat meal, the rate of absorption is reduced (Badwan *et al*., 2001). The mean steady state volume of distribution (V_d) for sildenafil is 105L, indicating the distribution into the tissues. Sildenafil and its metabolite, N-desmethyl are both approximately 96% bound to plasma proteins.

2.2.4 Metabolism and Excretion of Sildenafil Citrate

Sildenafil citrate is cleared predominantly by the CYP3A4 (major route) and CYP2C9 (minor route) hepatic microsomal isoenzymes. The major circulating metabolite results from N-desmethylation of sildenafil, and is itself further metabolized. Plasma concentrations of this metabolite are approximately 40% of those seen for sildenafil, so that the metabolite accounts for about 20% of sildenafil's pharmacologic effects. After either oral or intravenous administration, sildenafil is excreted as metabolites predominantly in the feces (approximately 80% of administered oral dose) and to a lesser extent in the urine (approximately 13% of the administered oral dose) (Badwan *et al*., 2001).

2.3 INFERTILITY

Infertility is the inability to become pregnant or carry pregnancy. There are many causes of infertility, including some that can be treated by medical intervention (Makar & Toth, 2002). World Health Organization (WHO) defines infertility as a disease of the reproductive system

defined by the failure to achieve a clinical pregnancy after 12 months or more of regular sexual intercourse (as there is no other reason, such as breastfeeding or postpartum amenorrhea). Primary infertility is infertility in a couple who have never had a child. Secondary infertility is failure to conceive following a previous pregnancy. Infertility may be caused by infection in the man or woman, but often there is no obvious underlying cause. Male disorders account for 30-50% of the cases of infertility (Mustafa *et al*., 2019). In about 30-40% of infertility cases in males, the problem is usually associated with the testes (the glands which produce sperm), and testosterone (the male sex hormone). Hormonal deficiency can also cause infertility. The sex hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) stimulate the gonads (testes and ovaries) to produce primary sex hormones. FSH and LH are known as gonadotropins, and they are produced in the pituitary gland which is located in the brain. Low levels of FSH and LH can result to a condition known as hypogonadotropic hypogonadism, a condition which results from the failure of the gonads to produce sex hormones (testosterone and estrogen) due to abnormal pituitary gonadotropin levels (FSH and LH) (Basaria, 2014). Other causes of infertility include; low semen volume, testicular obstruction, endocrine disorder, congenital defects, ejaculatory dysfunction, sperm agglutination and irregular viscosity of sperm (Bayasgalan *et al*., 2004).

Maintaining a healthy life style, eating healthy diets, avoiding the use of recreational drugs are all recommended and helpful ways to increase the odds of successful infertility treatment.

2.3.1 Infertility and its Physiological and Social Impacts

The consequences of infertility are manifold and can include social repercussions and personal suffering. Advances in assisted reproductive technologies, such as IVF (In Vitro Fertilization), can offer hope to many couples where treatment is available. The medicalization of infertility has unwittingly led to a disregard for the emotional responses that couples experience, which include distress, loss of control, stigmatization, and a disruption in the development of trajectory of adulthood (Cousineau & Domar, 2007). Infertility may have profound-psychological effects: partners may become more anxious to conceive, thus increasing sexual dysfunction (Barratt & Cooke, 1993).

2.4 BIOCHEMICAL PARAMETERS

2.4.1 LIVER FUNCTION TESTS

As the liver is involved in excretory, synthetic and metabolic functions, Liver Function Test (LFT) is often ordered by clinicians as a standard order for non-specific symptoms (such as fever, nausea, fatigue, weight loss, joint pain, muscle pain and abdominal pains) (Clementine, 2010). Clinicians may also order LFT to confirm their pre-clinical suspicion of specific liver and non-liver diseases. Liver diseases may be grouped into four (4) groups: neoplastic, inflammatory, vascular and metabolic disorders (Clementine, 2010).

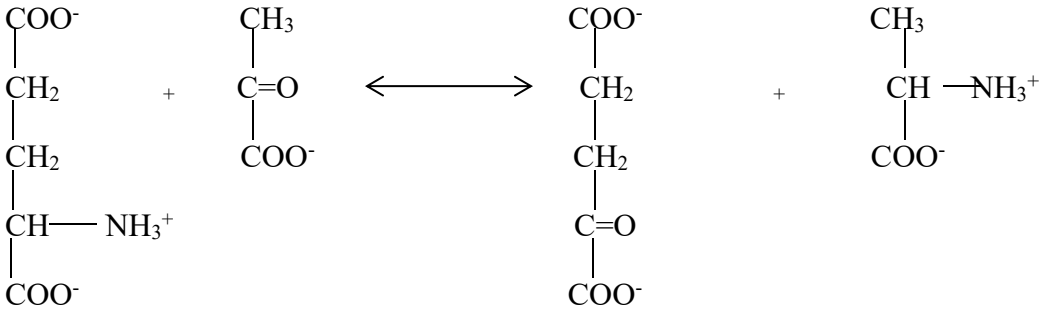
Enzymes are present in the liver and some of these enzymes are used as biomarkers to ascertain the functionality of the liver. These enzymes which act as biomarkers leak out from the liver and enter the blood when the liver is damaged or injured (hepatocellular injury). Some of these biomarkers are liver transaminases (alanine aminotransferase (ALT), aspartate aminotransferase

(AST)), alkaline phosphatase (ALP), and bilirubin which are all useful in the diagnosis of liver damage or injury (Singh *et al.,* 2011). ALT and AST are sensitive indicators of hepatocellular injury, but they lack specificity as they are also present in organs like the kidney, muscle (cardiac and skeletal muscle), and also in the erythrocytes (Clementine, 2010). In hepatocyte cytoplasm, AST is more abundant than ALT (Clementine, 2010).

2.4.1.1 Alanine Aminotransferase (ALT)

Alanine Aminotransferase (ALT) which is also called alanine transaminase was initially known as Serum Glutamate-Pyruvate Transaminase (SGPT). The enzyme was first characterized in the mid-1950 and it is present in the liver, heart, muscle, but greater proportion of the enzyme is found in the liver compared to other organs.

ALT catalyzes a reversible transamination reaction between glutamate and pyruvate to form L-alanine and α-ketoglutarate.



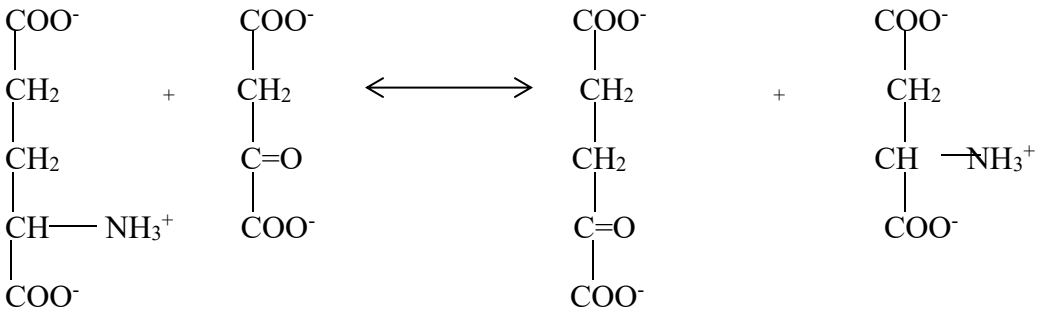
Glutamate Pyruvate α-Ketoglutarate Alanine

ALT levels in the blood are normally low, but when the liver is weakened, ALT leaks out of the liver and enters the blood, thus raising the level of the enzyme in the blood. The presence of liver disease is often indicated by significantly elevated ALT (SGPT) levels, to track or diagnose liver disease, ALT and aspartate transaminase (AST) are used together as part of a comprehensive

metabolic examination. The diagnosis for alcoholic hepatitis is supported by the finding of a ratio of AST to ALT of at least 2:1 and gamma- glutamyl-transpeptidase (GGT) that is twice the normal level (Pratt & Kaplan, 2000). The AST:ALT ratio may also be used to differentiate between different conditions: high ratio in hepatitis C with cirrhosis, liver metastases and HCV with cirrhosis versus low ratio in acute inflammation and cholestasis (Hughes & Jefferson, 2008). To access the type of liver disease present, ALT values are compared to other test results, such as alkaline phosphatase (ALP), AST, total protein, albumin to globulin ratio and bilirubin. The normal serum level of ALT is between 7-56 U/L and elevations above this level may indicate liver cell injury. Patients with hepatic disorders such as ischemic liver injury (shock liver), viral hepatitis, and other liver damage are more likely to have noticeable increase of ALT values greater than 500 U/L. (Gowda *et al.,* 2009).

2.4.1.2 Aspartate Aminotransferase (AST)

Aspartate Aminotransferase which is also called Serum Glutamate-Oxaloacetate Transaminase (SGOT) catalyzes a reversible transamination reaction between glutamate and oxaloacetate to form aspartate and α-ketoglutarate.



Glutamate Oxaloacetate α-Ketoglutarate Aspartate

AST is found primarily in striated muscle, the myocardium, and the liver. In human tissues, AST exists as two distinct isoenzymes, one of the isoform is present in the cytosol, while the other is located in the mitochondria. The measurement of AST isoenymes in human serum is helpful in determining the extent of damage to these organs. An injury to liver or muscle cells causes a release of AST into the blood. Standard serum AST levels ranges between 0 to 35U/L. The AST values may be amplified in the range of 10 to 20 times the normal values in cases of serious liver damage (Huang *et al.,* 2006).

2.4.1.3 Alkaline Phosphatase (ALP)

Alkaline Phosphatase is found in the liver, kidney, bones, placenta and intestine. In normal serum, ALP comprises of the liver and bone moieties: liver ALP is found in the canalicular surfaces and it is raised in any condition of biliary obstruction (intrahepatic and extra-hepatic), while the bone ALP is heat labile (Clementine, 2010). Serum ALP is thus a mixture of different ALP isoenzymes which can be fractionated by electrophoresis (Clementine, 2010). The standard range for serum ALP is 41 to 133U/L. In hepatocyte injury, ALP is often normal or marginally elevated. This feature is used as a guide to differentiate liver parenchymal disease from biliary dysfunction. Another canalicular enzyme, GGT corroborates the ALP increase in biliary disease. Moreover, GGT is normal in bone disease. Hepatic GGT production is increased with alcohol intake and has been used to indicate alcoholism or alcoholic liver disease. However, GGT is elevated with medications (anticonvulsants and histamine receptor blockers), prostate disease, obesity, diabetic nephropathy and hypertension. Hence, this mitigates against its use as a routine LFT. ALP is considered non-specific for determining liver damage, thus to differentiate liver diseases from bony disorders, GGT level is determined as it is increased only in cholestatic

disorders (a condition in which the flow of bile from the liver stops or slows and is caused by liver infection) and not in bone diseases (Gowda *et al.,* 2009).

2.4.1.4 Serum Bilirubin

Serum bilirubin is a mixture of α, β, γ and δ fragments which are unconjugated, singly conjugated, doubly conjugated and covalently bound to albumin, respectively. Although δ bilirubin measurement is available, it has not gained wider utility. In most cases a total bilirubin assay suffices for LFT, but fractionation may be required in isolated increases in bilirubin and neonatal jaundice. Direct bilirubin (DB) refers to the conjugated bilirubin that reacts directly with the diazo reagent, while indirect bilirubin is a derived value obtained from the difference of the total bilirubin and DB. DB assays measure only 70–90% of the conjugated and δ bilirubins, and may underestimate the severity of jaundice. In pre-hepatic jaundice due to haemolysis, unconjugated bilirubin is increased with little or no increase in conjugated bilirubin. In hepatic and post-hepatic jaundice, there is an increase in conjugated and δ bilirubins (Clementine, 2010). The range of total bilirubin (unconjugated and conjugated) for normal subject varies between 2 - 21μmol/L. The normal levels of the unconjugated bilirubin are <12μmol/L where as that of conjugated bilirubin is <8μmol/L. Serum bilirubin levels that are abnormally high could mean that the red blood cells are breaking down too quickly or that the liver is failing to properly break down waste. (Gowda *et al.,* 2009).

2.4.1.5 Albumin

Albumin is the major protein found in the blood stream. Albumin is synthesized in the liver and it is an indicator of liver function. Albumin performs several roles in the body; some of the functions include transportation of different substances such as hormones, vitamins, drugs

through the body, nourishment of tissues and prevention of fluid leakage from the blood vessels. Serum albumin levels change slowly due to the long half-life of albumin together with the capacity of the liver to synthesize albumin at twice the health basal rate to compensate for decreased synthetic capacity or albumin losses. Albumin level is decreased by trauma, inflammatory conditions and malnutrition. Also, reduction in Albumin levels (hypoalbuminaemia) in the blood may signify kidney damage, shock, liver damage, severe inflammation or malnourishment. Hyperalbuminemia (increase in albumin level) however have little diagnostic significance but may be an indication of dehydration.

2.4.1.6 Evaluation of Total protein

Total protein can be used as a diagnostic tool to distinguish between patients with hepatic damage and normal healthy people (Thapa & Walia, 2007). The total sum of two forms of proteins found in the blood (globulin and albumin) is referred to as total protein. A normal total protein value ranges from 6.0 to 8.3 g/dl. Hyperproteinaemia (increase in the level of protein in the blood) can be caused by dehydration, or as a result of increase in the concentration of specific proteins (immunoglobulins in chronic infection and myeloma) (Friedman & Young, 2001), while hypoproteinaemia (decrease in the level of protein in the blood) may be caused by low hematocrit (packed cell volume), resulting from increased plasma volume (hemodilution), severe malnutrition, chronic liver disease or by an excessive protein loss due to chronic kidney disease (Friedman & Young, 2001).

2.4.2 KIDNEY FUNCTION TESTS

The kidney is a bean-shaped organ and its main function is excretion of water-soluble waste products from the body. The kidney has filtration, secretory and excretory functions. The functional unit of the kidney is called the nephron which consists of the tubular system and the glomerulus. The glomerulus is composed of the Bowman's capsule and a leaky blood vessel which is encapsulated by the Bowman's capsule.

The functionality of the kidney can be checked by using indicators or markers such as urea, creatinine and electrolytes. Urea is produced by the cells of the liver (mainly in the cytosol and mitochondria of the liver) and then transported via the blood to the kidney for excretion. These indicators are expedient in assessing the function of the kidney and are useful for the indication of Glomerular Filtration Rate (GFR). A remarkable increase or decrease in the levels of these indicators or markers signifies kidney dysfunction (Gowda *et al.,* 2010).

2.4.2.1 Creatinine

Creatine is a small tripeptide found in the muscle. It stays in its phosphorylated form (as creatine phosphate) and releases energy in form of ATP for muscular activity. It is released from the muscles during regular wear and tear and is converted to creatinine (its internal anhydride). It is to be remembered that unlike urea, creatinine is not a toxic waste. It is simply used as a marker of renal function. Creatinine is freely filtered at the glomerulus and is also to a very small extent secreted into the tubules. The amount of creatinine in the blood is measured with a creatinine test. The estimation of serum creatinine levels has been established as the most popularly used screening test to determine kidney failure (Swedko *et al.,* 2003).

Normal serum creatinine level is 0.6 to 1.3 mg/dl (Lewis *et al.,* 2003). Serum creatinine is a better indicator of renal function and more specifically glomerular function than urea. For a

particular individual the creatinine level is dependent on the muscle mass and muscle wear and tear. There may be significant difference in creatinine level of individuals with vastly differing muscle mass. For example a body builder or athlete will have higher creatinine levels than a sedentary desk worker. Similarly creatinine level will also increase in case of any muscle trauma or excessive wear and tear as seems in athletes and people involved in hard physical labor. Chronic renal failure is characterized by an ultimate reduction in the removal of creatinine by both the tubules and glomeruli (Gowda *et al.,* 2010).

2.4.2.2 Blood Urea Nitrogen (BUN)

Sometimes the Serum urea level is expressed as blood urea nitrogen. BUN can be easily calculated from the serum urea level. The molecular weight of urea is 60 and it contains two nitrogen atoms of combined atomic weight of 28. Hence the contribution of nitrogen to the total weight of urea in serum is 28/60 that is equal to 0.47. Hence the serum urea levels can be easily converted to BUN by multiplying it by 0.47. A rise in blood nitrogen level is known as azotemia. A BUN test is carried out to ascertain if the kidney is functioning properly. When the kidneys are unable to extract urea from the blood, the blood urea level rises. Normal reference range of BUN in the blood is 2.1-7.1mmol/L or 6-20mg/dL (Lewis *et al.,* 2003).

Urease catalyses the hydrolysis of urea in the serum to produce ammonia. The ammonia concentration is then photometrically measured using Berthelot's reaction, which is a reaction between Berthelot's reagent (phenol and hypochlorite) and ammonia to yield a blue coloured compound known as indophenol.

$$\text{Urea} + H_2O \longrightarrow 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{Phenol} \longrightarrow \text{Indophenol (Blue compound)}$$

2.5 ANTIOXIDANT ENZYMES

Oxidative stress is a condition which occurs as a result of some physiological imbalance between the levels of antioxidants and oxidants in favour of oxidants. Antioxidants are substances or compound which prevents oxidation, while oxidants (free radicals or Reactive Oxygen species (ROS)) are substances or compounds that stimulate oxidation. ROS is a collective term used for a group of oxidants, which are either free radicals or molecular species capable of generating free radicals (Singh *et al*., 2018). These free radicals, which can be found as oxygen derived (ROS) or nitrogen derived (RNS) have rather high reactivity and short life. Generally, ROS/RNS are generated as by-products of cellular metabolism and ionizing radiation. Therefore, different reactive species are involved in cellular oxidative stress and oxidative damage. The common name for these reactive species is "free radicals". Free radicals are defined as "any chemical species capable of independent existence that contains one or more unpaired electrons".

Antioxidant enzymes are those enzymes that mop up the level of free radicals or reactive oxygen species in the body. ROS like hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{-\bullet}$), hydroxyl radical ($HO^{\bullet}$) are the three primary oxygen species which can react with organic substrate and lead to intermediate species which are able to produce other ROS. Superoxide anion is produced by the addition of a single electron to oxygen, and several mechanisms exist by which superoxide can be produced in vivo.

Antioxidant enzymes including superoxide dismutase, catalase, glutathione peroxidases, glutathione reductases and glutathione transferases have been widely investigated for the prevention and treatment of diseases resulting from oxidative damage. They highlight that the role and effectiveness of superoxide

dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) is important and indispensable in the entire defense strategy of antioxidants, especially in reference to superoxide anion radical ($\bullet O_2$) which is perpetually generated in normal body metabolism, particularly through the mitochondrial energy production pathway (MEPP).

2.5.1 Superoxide Dismutase (SOD)

SOD catalyzes the dismutation of super oxide anion. The metalloenzyme constitute an important antioxidant defense against oxidative stress. SOD acts as a good therapeutic agent against reactive oxygen species-mediated diseases (Younus, 2018).

$$2O_2^{-\bullet} + 2H_3O^+ \xrightarrow{SOD} H_2O_2 + 2H_2O + O_2$$

2.5.2 Catalase

Catalase is an antioxidant present in all aerobic organisms (organisms that use oxygen for cellular respiration). The enzyme catalyzes the conversion of hydrogen peroxide to water and oxygen in an energy-efficient manner in a cell exposed to oxidative stress (Sharma & Ahmad, 2014).

$$H_2O_2 \xrightarrow{catalase} H_2O + O_2$$

2.5.3 Glutathione Peroxidase (GP_X)

Glutathione Peroxidase is an antioxidant enzyme class with the capacity to scavenge free radicals. This in turn helps to prevent lipid peroxidation and maintain intracellular homeostasis, as well as redox balance (Mulgund *et al*., 2015). The cytosolic enzyme was first identified in 1957 as an activity that protects red blood cell against hydrogen peroxide. In the reaction, glutathione in the

reduced form (GSH) reacts with hydrogen peroxide to form water and oxidized glutathione (GSSG).

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

2.5.4 Malondialdehyde (MDA)

MDA is not an antioxidant, but it is a product of lipid peroxidation which occurs during oxidative stress. Therefore, MDA acts as a bio-indicator and the monitoring of MDA levels in different biological systems can be used as an important indicator of lipid peroxidation both in vitro and in vivo for various health disorders. The endogenous formation of MDA during intracellular oxidative stress and its reaction with DNA forms MDA-DNA adducts which makes it an important biomarker of endogenous DNA damage (Zhang *et al*., 2002).

2.6 SEX HORMONES

Hormones are chemical messengers (first messengers) which are secreted from ductless (endocrine) gland, released into the blood stream and carried to target organs were they exert their effects. Hormones that play an essential role in the development of sexual characteristics and reproduction are called sex hormones. These hormones are primarily involved in sexual development during puberty, growth, reproduction. An imbalance in sex hormones can lead to low sex drive, as well as health issues like infertility.

Gonadotropins like follicle-stimulating hormone (FSH) and luteinizing hormone (LH), together with testosterone are the prime regulators of germ cell development. The hypothalamus which secretes Gonadotropin Releasing Hormone (GnRH) elicits the release of these gonadotropins

(FSH & LH) from the pituitary gland. The FSH when released binds to receptors in the sertoli cells and stimulates spermatogenesis. The LH stimulates the production of testosterone hormone in the leydig cells, which in turn acts on the sertoli and peritubular cells of the seminiferous tubules and stimulates spermatogenesis (O'Donnel *et al*., 1994). When the pituitary gland fails to secret FSH and LH, it results in the disruption of testicular function which leads to infertility. The secretion of FSH and LH is controlled by testosterone and estradiol (Weinbauer & Nieschlag, 1995).

2.6.1 Testosterone

Testosterone is an important hormone which helps to maintain male sexual and fertility functions. Reduced level of testosterone leads to various sexual-related issues such as erectile dysfunction, decreased libido or impaired spermatogenesis (Matsumoto *et al*., 2016). Some symptoms and signs such as depressive mood or lack of physical performance are non-specific and can be found in the general population, regardless of testosterone levels. Hence, the measurement of testosterone levels to diagnose testosterone deficiency is an important medical parameter, particularly in patients complaining of only non-specific symptoms or signs (Bhasin *et al*., 2018). Increased testosterone levels have been linked to hypothalamic pituitary unit disorders, congenital adrenal hyperplasia testicular cancers, and prostate cancer in males. Decreased testosterone levels cause libido and erections in males to decrease, as well as more aches and pains in the bones and joints than normal. Low testosterone levels can be seen in patients suffering from hypopituitarism and testicular feminization. In general, the normal range in males is about 270ng/dL-1070ng/dL with an average of 679ng/dL.

2.6.2 Follicle-Stimulating Hormone

FSH is a glycoprotein which is synthesized by the anterior pituitary gland. The gonadotropin is active in the early stages of spermatogenesis and stimulates the development of the testicles and seminiferous tubules. FSH also promotes follicular development and ovarian follicles for LH action in females. Deficiency of FSH can cause infertility or subfertility in men and women. The level of FSH will rise in menopausal women and in women with early ovarian failure. FSH levels are higher in males with low sperm output, but in patients with testicular tumors, the level of FSH is low.

2.6.3 Luteinizing Hormone

LH is a 30,000-dalton glycoprotein which is synthesized by the anterior pituitary gland. It is one of the gonadotropins that play a role in the release of testosterone. LH promotes ovulation and synthesis of ovarian steroid, like estrogen. The hormone regulates the release of testosterone by leydig cells in men. LH levels are higher in the latter phase of the menstrual cycle, in women who are approaching menopause, and in people who have problems with their gonads.

CHAPTER THREE

MATERIALS AND METHOD

3.1 MATERIALS

3.1.1 Equipment

Micropipette (Microplux), Water bath (TT42D Multipurpose use, Techmel and Techmel, USA), Stirrer, Syringe**,** Weighing balance (Metler analytical balance), Burette (Pyrex, England), Conical Flask, beakers (Pyrex, England), UV spectrophotometer (Unico, China), Measuring cylinder (Pyrex, England), Centrifuge (Model 80-2, Harris, England), Glass jar, Muslin cloth, Microscope.

3.1.2 Chemicals and Reagents

During this study, analytical grade chemicals and reagents were used. These reagents and chemicals were obtained from standard suppliers. The chemicals and reagents include: 98% methanol, chloroform, distilled water, Alkaline Phosphatase Kit, Alanine Transaminase Kit, Total Protein Kit, Total Bilirubin Kit, Aspartate Transaminase Kit, Albumin Kit, Sex Hormones ELISA Kit, Urea Kit, Creatinine Kit, Superoxide Dismutase (SOD) Kit, Malondialdehyde (MDA) Kit, Glutathione Peroxidase Kit, Catalase Kit, Glacial Acetic acid, Ferric chloride, Sulphuric acid, Wagner's reagent, Sodium hydroxide, Hydrochloric acid, Lead Acetate solution, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Acetate buffer, TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) solution.

3.2 SAMPLE COLLECTION AND IDENTIFICATION

Fresh *Sphenocentrum jollyanum* leaves were collected from Capitol in Ovia North-East Local Government Area (LGA) of Edo State, Nigeria. The plant sections were identified and authenticated at the Department of Plant Biology and Biotechnology (PBB), University of Benin, Benin City, Edo State. A voucher specimen deposited in their reference with the registration number …….

3.2.1 Preparation of Plant Sample

The leaves of *Sphenocentrum jollyanum* were washed and removed from their stem. The leaves were air dried for some days and then crushed into powder at Uselu Market in Benin City, using a grinding machine. The ground leaves were kept in an airtight jar at room temperature until they were extracted.

3.2.2 Preparation of Methanolic Extract of *Sphenocentrum jollyanum*

3000g of the ground leaves of *Sphenocentrum jollyanum* were soaked for 72 hours in 100% methanol and stirred every day. A muslin cloth was used to filter the mixture. The filtrate was condensed using a rotary evaporator at 60˚C to remove the methanol. Prior to administration, the methanol extract was held in the refrigerator at 4°C. The methanolic extract of *Sphenocentrum jollyanum* was dissolved with Tween 80 and distilled water in a ratio of 1:9.

3.3 EXPERIMENTAL ANIMALS

Adult male Wistar rats were obtained from a local breeder in University of Benin, Benin City, Edo State and housed in the Animal house of the Department of Biochemistry, Faculty of Life Sciences, University of Benin.

3.3.1 Experimental Design

In this research work, a total of 25 male Wistar rats were used. The experimental animals weighed 125-172g and were grouped into five (5) groups. Five animals were placed per group. The control group was numbered one, and the test groups were numbered two through five. The animals were acclimatized and held in a clean, disinfected wooden cage for 14 days prior to extract administration. Pelletized feed was given to the animals. The feed and water were given *ad libitum*.

Group 1: The control group containing five (5) animals were administered the vehicle only, which is 1ml of distilled water.

Group 2: Sildenafil was given orally to the five (5) experimental animals in this group at 0.7mg/kg body weight. Calculated mass of sildenafil that was administered to each rat was dissolved in distilled water.

Group 3: The methanolic extract of *Sphenocentrum jollyanum* was given orally to the five (5) experimental animals in this group at 100mg/kg body weight. 1ml of distilled water containing the calculated gram of the extract based on body weight of each rat was administered.

Group 4: The methanolic extract of *Sphenocentrum jollyanum* was given orally to the six (6) experimental animals in this group at 250mg/kg body weight. 1ml of distilled water containing the calculated gram of the extract based on body weight of each rat was administered.

Group 5: The methanolic extract of *Sphenocentrum jollyanum* was given orally to the six (6) experimental animals in this group at 500mg/kg body weight. 1ml of distilled water containing the calculated gram of the extract based on body weight of each rat was administered.

Following the administration of the methanolic extract of *Sphenocentrum jollyanum* to different test groups, the rats were examined for 2 hours for changes in behavioral habits, eyes, tremors, skin, salivation, coma, diarrhea, and mortality, and then regularly for 28 days for changes in sleep, skin, fur, tremors, salivation, diarrhea, and coma. During the analysis, food intake and weight changes were also observed on a regular basis. Prior to sacrifice, the animals were fasted overnight (12 hours) at the conclusion of the experimental period. The rats were anesthetized with chloroform, and blood samples were taken from the abdominal aorta and heart. Blood samples were used to assay for these parameters (biochemical and sex hormones).

3.4 QUALITATIVE TESTS FOR PHYTOCHEMICAL SCREENING

Phytochemicals are bioactive compounds present in plants that have great antioxidant potential, and are of great interest due to their beneficial effects on the health of living organisms. Plant phytochemicals such as isoflavones, alylic sulfides, phenolic acids, saponins tannins carotenoids, curcumin, flavonoids, and carotenoids are present in high concentration in raw foods, but intensities are reduced during processing and handling (Monika & Renu, 2020). Phytochemicals can be separated from the plant by various extraction techniques. The most commonly used methods for extraction of phytochemicals from plants include percolation, infusion, digestion, hot continuous extraction, etc.

3.4.1 Qualitative Test for Cardiac Glycosides

Keller-Killani Test

Procedure: Add 1.5 mL of glacial acetic acid to 1 mL of plant extract. 1 drop of 5% Ferric chloride and concentrated sulphuric acid are added along the side of the test tube.

Observation: A blue coloured solution is formed. This indicates the presence of cardiac glycoside in the plant extract.

3.4.2 Qualitative Test for Tannins

Braymer's Test

Procedure: Add 3 mL of distilled water to 1 ml of plant extract. 3 drops of 10% Ferric chloride solution is also added to the test tube.

Observation: Formation of a blue-green colour indicates the presence of tannins in the plant extract.

3.4.3 Qualitative Test for Alkaloids

Wagner's Test: Add 1-2 drops of Wagner's reagent along the sides of the test tube to few ml of plant extract

Observation: Formation of a brown/reddish precipitate indicates the presence of alkaloids in the plant extract.

3.4.4 Qualitative Test for Saponins

Procedure: Add 10ml of distilled water to 1ml of the tepal extract. Shake in a graduated cylinder for 15 minutes.

Observation: Formation of one centimeter layer of foam indicates the presence of saponins in the plant extract.

3.4.5 Qualitative Test for Flavonoids

Alkaline Reagent Test

Procedure: Add 2ml of 2% NaOH solution and few drops of dilute HCl to 1ml of plant extract.

Observation: An intense yellow colour which becomes colourless on addition of dilute acid indicates the presence of flavonoids.

3.4.6 Qualitative Test for Phenolic Compounds

Lead Acetate Test

Procedure: Dissolve 1ml of plant extract in 5ml of distilled water. Then, add 3ml of 10% Lead Acetate solution.

Observation: Formation of a brown precipitate indicates the presence of phenolic compounds in the plant extract.

3.4.7 Qualitative Test for Triterpenoids

Salkowski's Test

Procedure: Add few drops of concentrated H_2SO_4 along the sides of the test tube to the plant extract.

Observation: Formation of a golden yellow layer at the bottom of the test tube indicates the presence of triterpenoids in the plant extract.

3.4.8 Qualitative Test for Anthocyanins

Procedure: Add 2ml of 2N HCl and few ml of ammonia solution to 2ml of plant extract.

Observation: Formation of a pink-red solution which turns to a blue-violet solution on addition of ammonia indicates the presence of anthocyanins in the plant extract.

3.5 INVITRO ANTIOXIDANT ASSAY

3.5.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay

The method of Chang *et al*. (2001) was used to determine DPPH concentration.

Principle:

2,2-diphenyl-1-picrylhydrazyl is a stable free radical with red colour, which turns yellow when scavenged. The scavenging reaction between DPPH and an antioxidant (H-A) can be written as:

DPPH + H-A $\longrightarrow$ DPPH-H + A

Antioxidants react with DPPH, and reduce it to DPPH-H by adding hydrogen atom to DPPH. This leads to a decrease in absorbance value. The degree of discoloration indicates that the scavenging potential of the antioxidant extract in terms of hydrogen donating ability.

Procedure:

The reagent required for this assay, 0.1 mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol. 2 - 20 µL of plant extracts were made up to 40 µL with DMSO (Dimethyl sulfoxide) and 2.96ml of 0.1mM DPPH solution was added. The reaction mixture was incubated in dark condition at 25˚C for 20 min. After the 20 minutes, the absorbance of the mixture was read at 517nm. The % radical scavenging activity of the plant extract that contains DPPH was calculated using the formula:

% Radical Scavenging Activity (RSA) = $\frac{Abs\ control - Abs\ sample}{Abs\ control}$ **x 100**

3.5.2 Ferric Reducing Antioxidant Power (FRAP) Analysis

FRAP assay is a widely used method that uses antioxidants as reductants in a redox-linked colorimetric reaction.

The method of Benzie & Strain (1996) was used to carryout FRAP assay.

Principle:

Ferric reducing ability of plasma FRAP assay is based on the reduction of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to ferrous tripyridyltriazine (Fe^{2+}-TPTZ). The end product (Fe^{2+}-TPTZ) has a blue colour with absorption maximum at 593nm, and the change in absorbance is related to the antioxidant capacity of the plasma.

Procedure:

The FRAP reagent was prepared by mixing the following:

1. 200ml acetate buffer
2. 20ml TPTZ solution
3. 20ml Ferric chloride, $FeCl_3$ solution
4. 24ml distilled water

The resultant solution was straw coloured and kept in a water bath at 37˚C. A set of blanks were run first. 30ml of distilled water was added to each cuvette, thereafter, 1ml of FRAP reagent was added to each test tubes so as to enable the contents to mix thoroughly. Absorbance values were read at 593nm.

The FRAP concentration (in μM) in the sample was determined by plotting a standard calibration curve of absorbance @593nm against concentration.

3.6 BIOCHEMICAL PARAMETERS

The blood samples for biochemical assays were collected in universal containers, and allowed to stand for 10 minutes. The blood samples were subjected to centrifugation at 6,000 rpm for 10 minutes to obtain serum which was used to determine the following biochemical parameters: Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Serum Albumin, Total Bilirubin, Direct Blirubin, Urea, Total Protein and Creatinine. The biochemical parameters were analyzed using Randox Diagnostic Kits.

The organ, testes was used for the following biochemical parameters: Superoxide Dismutase (SOD), Catalase, Malondialdehyde (MDA), Glutathione Peroxidase (GPx). The testes were homogenized with 0.9% Normal Saline, using a mortar and a pestle. The biochemical parameters were analyzed using Randox Diagnostic Kits.

3.6.1 LIVER FUNCTION TEST

3.6.1.1 Determination of Alanine Aminotransferase (ALT)

The method of Reitman and Frankel (1957) was used in the determination of ALT activity.

Principle

$$\alpha\text{-oxoglutarate} + \text{L-alanine} \xrightarrow{\text{ALT}} \text{L-glutamate} + \text{Pyruvate}$$

The total amount of Pyruvate Hydrazone formed by 2,4-dinitrophenylhydrazine is used to determine the level of Alanine Aminotransferase in the sample.

Procedure

In the test tube, 100 µL of each sample was added to 500 µL of ALT R1 (alanine transaminase reagent 1 solution containing phosphate buffer, L-alanine, and α-oxoglutarate). An aliquot of 0.1mL distilled water was added to 500 µL ALT R1 solution for the blank. The mixtures were incubated for 30 min at 37˚C. The mixtures were left to stand for 20 min at 25°C. Thereafter, an aliquot of 5.0 mL of sodium hydroxide was added to all the test tubes and left to stand for 5 min. The absorbance value of the sample was then compared to that of a reagent blank at 546nm. The test was done in triplicate. The ALT enzyme activity in the serum was then obtained using the formula below:

$$\textbf{Enzyme activity of ALT (U/L)} = \frac{\textbf{Absorbance of sample x concentration of standard}}{\textbf{Absorbance of standard}}$$

3.6.1.2 Determination of Alkaline Phosphatase (ALP)

The method developed by Tietz, (1976) was used in the evaluation of serum Alkaline Phosphate level.

Principle:

p-nitrophenylphosphate + H_2O $\xrightarrow{\text{ALP}}$ phosphate + p-nitrophenol

Procedure:

Dispense 500 µL of the substrate of Alkaline Phosphatase (p-nitrophenylphosphate) into test tubes labeled blank, standard and sample. The Alkaline Phosphatase substrate was incubated for 3 min at 37˚C. An aliquot of 50 µL of distilled water, standard reagent and sample was introduced into the blank, standard and sample test tubes respectively and then mixed thoroughly. The mixture was incubated at 37°C for another 10 minutes. Thereafter, 2500 µL of alkaline phosphatase color developer was placed into all test tubes and mixed. The absorbance values of the samples were read against blank at 590nm. The test was carried out in triplicates. The enzyme activity of ALP in the serum was then calculated using the formula:

$$\textbf{Enzyme activity of ALP (U/L)} = \frac{\textbf{Absorbance of sample x concentration of standard}}{\textbf{Absorbance of standard}}$$

3.6.1.3 Determination of Aspartate Aminotransferase (AST)

The method of Reitman and Frankel (1957) was used in the determination of AST.

Principle

α- oxoglutarate + L-aspartate $\xrightarrow{\text{AST}}$ L-glutamate + Oxaloacetate

The total amount of Oxaloacetate Hydrazone formed by 2,4-dinitrophenylhydrazine is used to determine aspartate aminotransferase.

Procedure

In the test tube, 100 µL of the sample was mixed with 500 µL of AST R1 solution (phosphate buffer, L-aspartate, and α-oxoglutarate). The blank was made up of 100 µL distilled water and 500 µL AST R1 solution. The mixtures were incubated at 37°C for 30 min. After incubation, 500 µL of AST solution R2 (containing 2,4-dinitrophenylhydrazine) was added to all the test tubes. The mixture was left to stand for 20 min at 20˚C. Thereafter, 500 µL sodium hydroxide was added to each test tube and allowed to sit for 5 min in order to stop the reaction. The absorbance values were measured at 546nm. The test was carried out in triplicates. The AST enzyme activity in serum was then obtained using the formula below:

$$\textbf{Enzyme activity of AST (U/L)} = \frac{\textbf{Absorbance of sample x concentration of standard}}{\textbf{Absorbance of standard}}$$

3.6.1.4 Determination of Serum Albumin

The method of Doumas *et al.,* (1971) was used in the determination of the protein, Albumin.

Principle:

The evaluation of albumin is carried out on the basis of its quantitative binding to 3,3′,5,5′-tetrabromo m-cresol sulphonephthalein, which is also known as bromocresol green (BCG). At 578nm, the BCG-Albumin complex absorbs at a maximum capacity, and the amount of albumin present is proportional to the absorbance value read.

Procedure:

500 µL of distilled water was added to 3000 µL of bromocresol green (BCG) reagent in a test tube which is labeled blank. An aliquot of 10 µL of the albumin standard was added to 3000 µL BCG reagent which served as the standard. Thereafter, 10 µL of the serum from each sample was also added to 3000 µL of BCG reagent. The mixtures were incubated for 5 min at 25°C. At 630 nm, the absorbance value of the sample, and that of the standard were estimated against the reagent blank. The test was carried out in triplicates. The total amount of albumin in the serum was then calculated using the formula below:

$$\textbf{Albumin (g/L)} = \frac{\textbf{Absorbance of sample x concentration of standard}}{\textbf{Absorbance of standard}}$$

3.6.1.5 Determination of Bilirubin

The method of Jendrassik and Groff, (1938) was used in estimating bilirubin concentration.

Principle:

In an alkaline medium, conjugated bilirubin, also known as direct bilirubin, forms a blue-colored complex with diazotized sulphanilic acid.

Procedure:

Total Bilirubin

200 µL of reagent 1 (containing sulphanilic acid and hydrochloric acid), 1000 µL of reagent 3 (containing caffeine and sodium benzoate) and 0.2 mL of the sample were mixed. This served as sample blank. 0.2mL of reagent 1, 0.05mL of reagent 2 (containing sodium nitrite), 1mL of reagent 3 and 0.2mL of the sample were mixed. The solutions were incubated for 10 min at 25°C. An aliquot of 1mL of reagent 4 were placed after the incubation period to the solutions, thoroughly mixed and left to stand at 25°C for 5-30 min. The absorbance value of the sample was read at 578nm against the sample blank. The test was done in triplicates. The amount of total bilirubin in the serum was then calculated using the formula below:

Total Bilirubin (mg/dl) = 10.5 x A sample

Direct Bilirubin

200 µL of reagent 1 (containing sulphanilic acid and hydrochloric acid) and 0.2 mL of the sample were mixed. This served as sample blank. 0.2mL of reagent 1, 0.05mL of reagent 2 (containing sodium nitrite), 1000 µL of sodium chloride and 0.2mL of the sample were mixed. The solutions were mixed thoroughly and allowed to stand at 15-25˚C for 5 min. The absorbance value of the sample was read at 546nm against the sample blank. The test was done in triplicates. The total amount of total bilirubin in the serum was then calculated using the formula below:

Direct Bilirubin (mg/dl) = 14.0 x A sample

3.6.1.6 Determination of Total Protein

The total protein concentration was determined using the method by Tietz, (1995).

Principle:

Cupric ion (Cu^{2+}) interacts with the peptide bonds present in a protein under alkaline conditions to form a colored complex.

Procedure:

200 µL of distilled water was mixed with 1000 µL R1 (biuret reagent containing sodium hydroxide, Na-K tartrate, potassium iodide and cupric sulphate). This served as the blank solution. An aliquot of 0.02 mL of standard (containing protein and sodium azide) was mixed with 1.0 mL R1 to give the standard solution, while 20 µL of the sample was mixed with 1000 µL of R1. The solutions were thoroughly mixed and incubated at 20-25°C for 30 min. The absorbance value of the sample and that of the standard were read against blank @ 546nm. The test was carried out in triplicates. The amount of total protein in the serum was then calculated with the formula below:

$$\textbf{Total Protein concentration (g/L)} = \frac{Asample}{Astandard} \textbf{ x Standard concentration}$$

3.6.2 KIDNEY FUNCTION TEST

3.6.2.1 Determination of Creatinine

The method of Bartels and Bohmer (1972) was used to determine creatinine concentration.

Principle

Creatinine reacts with picric acid to form a colored complex in an alkaline solution. The accumulation of creatinine determines the intensity of the colored complex formed.

Procedure

1.0mL of the working reagent (containing equal volumes of Picric acid and Sodium hydroxid was mixed with 0.1 mL of creatinine (labelled as standard). This mixture constitutes the standard solution. 1.0 mL of the working reagent was also mixed with 0.1 mL of the sample. The absorbance was read at 492nm after 30 seconds, and read again after 2 minutes. The test was done in triplicate. The total amount of creatinine in the serum was then calculated using the formula below:

$$\text{Creatinine concentration (mg/dl)} = \frac{\Delta Asample}{\Delta Astandard} \times 2$$

ΔA_{sample} or $\Delta A_{standard}$ (change in absorbance of sample or standard) $= A_2 - A_1$

Where A_1 absorbance taken after 30 seconds,

A_2 is absorbance taken after 2 minutes

3.6.2.2 Determination of Urea

The method of Fawcett and Scott (1960) was used to estimate urea concentration.

Principle

The hydrolysis of urea to form ammonia and carbon (iv) oxide is catalyzed by the enzyme called urease. The ammonia formed is photometrically measured using Berthelot's reaction, which is a reaction between Berthelot's reagent (phenol and hypochlorite) and ammonia to yield a blue coloured compound known as indophenol.

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{Phenol} \longrightarrow \text{Indophenol (Blue compound)}$$

Procedure

0.01 mL of distilled water was mixed with 0.1mL of reagent 1 (EDTA, sodium nitropusside and urease). This constitutes the Blank solution. 0.01 mL of the standard was thoroughly mixed with 0.1 mL of reagent 1 (standard solution), while 0.01 mL of the sample was also thoroughly mixed with 0.1 mL of reagent 1. The contents were incubated at 37°C for 10 min. An aliquot of 2.5 mL of reagent 2 (containing phenol) and 2.5 mL of reagent 3 (containing hypochlorite) were added to the test tubes, thoroughly mixed and incubated for 15 min at 37°C. The sample absorbance and that of the standard were read against blank at 546nm. The test was carried out in triplicate. The amount of urea in the serum was then calculated using the formula below:

$$\textbf{Urea concentration (mg/dl)} = \frac{Asample}{Astandard} \textbf{ x Standard concentration}$$

3.6.3 INVIVO ANTIOXIDANT ASSAY

3.6.3.1 Determination of Glutathione Peroxidase (GPx)

Glutathione peroxidase acts as detoxifying peroxide in the cell by preventing lipid peroxidation caused by free radical damage in the cell. Glutathione peroxidase catalyzes the conversion of organic peroxides (R – O – O – H) to the corresponding stable alcohols (R – O – H), using glutathione as a source of reducing equivalent.

$$R\text{-}O\text{-}O\text{-}H + 2GSH \xrightarrow{GPx} R\text{-}O\text{-}H + GSSG + H_2O$$

Glutathione peroxidase was determined by the method of Paglia & Valentine, (1967).

Principle:

This assay is actually an indirect measure of the activity of glutathione peroxidase. Oxidized glutathione (GSSG) which is produced when glutathione peroxidase reduces organic peroxide to

alcohol is converted back to its reduced form (GSH) in a reaction catalyzed by glutathione reductase (GR).

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2GSH + NADP^+$$

NADPH is oxidized to $NADP^+$ with a concomitant decrease in absorbance at 340nm, thus providing a spectrophotometric means of monitoring the enzymatic activity of glutathione peroxidase. To determine the activity of glutathione peroxidase, a tissue homogenate is added to a solution which contains NADPH, glutathione and glutathione reductase. The enzymatic reaction is initiated by the addition of hydrogen peroxide, and the absorbance value is recorded. The rate of the decrease in absorbance at 340nm is directly proportional to the glutathione peroxidase activity in the sample.

Reagents:

1. Assay Buffer, pH 7.0
- 50mM phosphate buffer.
- 0.1% Triton X-100
2. NADPH Reagent

• Glutathione

• Glutathione reductase

• β - nicotinamide-adenine dinucleotide phosphate reduced

3. Substrate (Hydrogen peroxide)

Procedure:

Reagents	**Sample**

	Ml
Buffer (R1)	1.0
NADPH (R2)	0.1
Sample	0.01
H_2O_2 (R3)	0.1

Mix the mixture well and record the decrease of absorbance at 340nm/min over a period of 3 minutes.

The glutathione peroxidase activity was calculated using the formula:

Enzyme Activity (U/gT) = $\frac{A340/min}{0.00622}$ x 121

3.6.3.2 Determination of Superoxide Dismutase (SOD)

Superoxide Dismutase (SOD) is a metalloenzyme that catalyzes the dismutation of superoxide anions to molecular oxygen and hydrogen peroxide. SOD forms a crucial part of the cellular antioxidant defense mechanism.

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

The method of Nishikimi *et al*. (1972) was used to determine SOD concentration.

Principle:

This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye.

Reagents:

1. 50mM/L Phosphate Buffer, pH 8.5

2. 1mM/L Nitroblue tetrazolium (NBT)
3. 1mM/L NADH
4. 0.1mM/L Phenazine methosulphate (PMS)
5. Extraction Reagent

Procedure:

The Working Reagent was prepared by mixing R1, R2 and R3 in a ratio of 10:1:1 ml. 1.0ml of the Working Reagent was added to the control and sample test tubes. 0.1ml of the sample was added only to the sample test tube. 0.1ml of distilled water was added to the control test tube. The test tubes were mixed thoroughly and the reaction was initiated by adding 0.1ml of R4 (PMS). The absorbance values were read at 560nm for 5 minutes for control and for the sample at 25˚C.

The SOD activity was calculated as follows:

$$\% \text{ inhibition} = \frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}} \times 100$$

Where: $\Delta A_{control}$ = the change in absorbance at 560nm over 5 min following the addition of PMS to the reaction mixture in the absence of sample.

ΔA_{sample} = the change in absorbance at 560nm over 5 min following the addition of PMS to the reaction mixture in the presence of sample.

3.6.3.3 Determination of Catalase

Catalase is an antioxidant enzyme that is present in most aerobic cells. Catalase catalyzes the conversion of hydrogen peroxide to oxygen and water. Hydrogen peroxide is a strong oxidant that causes intracellular damage, and catalase is the body's defense system against hydrogen peroxide.

The method of Nishikimi *et al*. (1972) was used to determine SOD concentration.

Principle:

Catalase reacts with a known quantity of hydrogen peroxide. This reaction is brought to a halt after exactly 60 seconds with catalase inhibitor.

$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$

In the presence of peroxidase enzyme, the remaining hydrogen peroxide reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore, and the intensity of the colour is inversely proportional to the amount of catalase in the original sample.

$2H_2O_2 + \text{DHBS} + \text{AAP} \xrightarrow{\text{peroxidase}} \text{Quinoneimine Dye} + 4H_2O$

Reagents:

1. 100mM/L Phosphate Buffer, pH 7.0.
2. 500mM/L Hydrogen peroxide
3. Chromogen inhibitor
4. >2000/L Peroxidase
5. 2mM/L 4-Aminoantipyrine preservative

Procedure:

	Sample Blank (ml)	Sample (ml)	Standard Blank (ml)	Standard (ml)
Sample	0.05	0.05	-	-
Distilled water	0.05	-	0.10	0.05

R1	0.50	0.50	0.50	0.50
R2	-	0.10	-	0.10

The mixture is incubated for 1min at 25˚C. R3 and R4 are then added.

R3	0.20	0.20	0.20	0.20
R4	0.50	0.50	0.50	0.50

The mixture is incubated for 10min at 37 ˚C. The absorbance value of the sample and standard are read at 510nm.

The catalase activity was calculated using the formula:

$$\text{Catalase activity (U/L)} = \frac{A_{standard} - A_{sample}}{A_{standard}} \times 1000$$

3.6.3.4 Determination of Malondialdehyde (MDA)

MDA acts as a bio-indicator and the monitoring of MDA levels in different biological systems can be used as an important indicator of lipid peroxidation both in vitro and in vivo for various health disorders.

The method of Ohkawa *et al*. (1979) was used to determine MDA concentration.

Principle:

Thiobarbituric acid (TBA) reacts with malondialdehyde in an acidic medium at temperature of 95˚C for 30 minutes to form thiobarbituric acid reactive product. The absorbance of the resultant pink product can be measured at 534nm.

Reagents:

Standard

Chromogen

Thiobarbituric acid

Detergent

Stabilizer

Procedure:

	Sample Ml	Standard ml	Blank Ml
Sample	0.2	-	-
Standard	-	0.2	-
Chromogen	1.0	1.0	1.0

The mixture is heated in a boiling water bath for 30 minutes. The mixture is cooled, and the last reagent is added.

Sample	-	-	0.2

The absorbance of sample (A_{sample}) is read against that of the blank and standard at 534nm.

The MDA in the sample was calculated using the formula:

$$\boldsymbol{Tissue} = \frac{\textbf{Absorbance of sample x 10}}{\textbf{Absorbance of standard x g. tissue used}}$$

The value is expressed as nmol/g.tissue

3.7 HISTOPATHOLOGY

The following organs; testes and prostate were harvested, weighed and preserved in 10 % formalin. The organs were then removed, and tissue portions with a thickness of 5 μm were stained with haematoxylin and eosin (H&E) for histopathological study. The stained sections were subsequently mounted on a microscope, viewed and their photomicrographs taken (Magnification x100 and x400).

3.8 SEX HORMONE

A 5ml syringe was used to retrieve the blood samples, which were then placed in an EDTA tube.

3.8.1 Follicle stimulating hormone (FSH)

This was evaluated using the FSH Enzyme-Linked Immunosorbent Assay (ELISA) kit.

Principle:

The solid phase assay of the FSH ELISA has an immobilized streptavidin on each well. The samples are loaded in the streptavidin-coated wells, along with the anti-FSH/anti-Biotin conjugate. Thereafter, FSH is sandwiched between biotin-labeled antibodies and HRP in the patient's plasma. A wash buffer is introduced so as to remove all unbound proteins and HRP conjugates. The intensity of the colour formed is proportional to the concentration of FSH in the samples on addition of the substrate.

3.8.2 Testosterone

This was evaluated using the Testosterone ELISA kit

Principle:

The test sample, along with a working testosterone-horseradish peroxidase (HRP) conjugate and an anti-Testosterone-Biotin solution are all loaded in streptavidin-coated wells. The testosterone

present in the plasma competes for binding sites with the testosterone-HRP conjugate. A washing buffer is used to remove unbound testosterone and testosterone-enzyme conjugate. The intensity of the colour formed is inversely proportional to the concentration of testosterone in the samples on addition of the substrates.

Procedure:

An aliquot of 0.05mL of the samples, standards and control were all pipetted into the designated wells, after which 0.1mL of working testosterone -enzyme conjugate reagent were then added to all wells and immediately followed by 0005mL biotin reagent. The microplate wells were then swirled for 20-30 seconds so the reagent can mix properly, after which they were covered and incubated for 60 min at 25˚C. The liquid was removed from all wells after the incubation period and then washed three times with 300µL of 1X wash buffer. The wells were blotted on absorbent paper towels to remove any of the remaining solution. 100µL of 3,3',5,5'- tetramethylbenzidine (TMB) substrate was then added to all wells and incubated at 25˚C for 30 min. An aliquot of 50µL of stop solution was added to all well at the end of the incubation period. The wells were gently swirled for 15-20seconds to mix the solution and absorbance read on an ELISA reader @ 450nm within 15 min after adding the stop solution.

3.8.3 Luteinizing hormone

The LH ELISA kit was used to assess this.

Principle:

The LH ELISA package is a solid-phase assay that uses streptavidin and biotin to detect Luteinizing Hormone. The interaction of the streptavidin coated well and the exogenously bound biotinylated monoclonal anti-Luteinizing Hormone antibody causes immobilization at the

surface of a microplate well during the assay without any competition. The complex is simultaneously stored in the well using a high affinity reaction with streptavidin and biotinylated antibody. Unbound proteins and conjugated proteins are washed away with a wash buffer after a one-hour incubation cycle. The enzyme activity in the enzyme-bound fraction is proportional to the concentration of luteinizing hormone in the sample after adding the substrate.

Procedure:

An aliquot of 25µL of control, FSH standards, and samples were placed into selected wells. 100µL of 1X biotin/enzyme conjugates were added to all wells. The microplate wells were covered and allowed to stand at 25˚C for 60 min. The wells were emptied and washed three times using 300µL of 1X wash buffer at the end of the one-hour incubation period. Absorbent paper towels were then used to blot out excess liquid from the wells. An aliquot of 100µL of 3,3',5,5'- tetramethylbenzidine (TMB) substrate was added to all the wells and left to stand for 15 min at 25˚C. After the incubation period, 50 µL of stop solution was applied to each well. 15 minutes after adding the stop solution, the resulting mixture was gently shaken. Absorbance was measured @ 450nm on an ELISA reader.

3.9 Semen Analysis

Semen analysis is also known as sperm count test and it is carried out to ascertain the health and viability of a man's semen. The analysis shows the number of sperm cells, shape of the sperm and sperm motility which are all indicative of reproductive health in males.

Procedure

The semen of the male Wistar rats was released by cutting the epididymis of the rat after sacrifice with a pair of fine-pointed scissors (Kempinas & Lamano-Carvalho, 1988). The sperm suspension was drawn into a pipette and was diluted to 1:100 with the enzyme, trypsin. The

sperm suspension was dropped on a microscope slide and viewed under the microscope with a magnification of x500.

3.9.0 DATA ANALYSIS

All measurements were done in triplicates, and the results were represented as Mean ± Standard Error of Mean (SEM). Data were analyzed using One-Way Analysis of Variance (ANOVA), and the differences between means were determined using the Least Significant Difference (LSD) on Statistical Package for Social Sciences (SPSS) version 20. The significance level was set at $P < 0.05$.

CHAPTER FOUR

RESULTS

4.1 QUALITATIVE PHYTOCHEMICAL ANALYSIS OF METHANOLIC LEAF EXTRACT of *Sphenocentrum jollyanum*

Table 4.1: Phytochemical Screening of Methanolic Leaf Extract *Sphenocentrum jollyanum*

TEST	OBSERVATION
Cardiac Glycoside	+
Tannins	–
Alkaloids	–
Triterpenes	+
Saponins	+
Flavonoids	+
Anthraquinone	–

(+) sign indicates present, while (–) sign indicates not present.

4.2 EFFECT OF METHANOLIC LEAF EXTRACT OF *Sphenocentrum jollyanum* ON BODY WEIGHT OF MALE WISTAR RATS

The effect of methanolic leaf extract of *Sphenocentrum jollyanum* on body weight of animal is shown in Table 4.2. There was a non-significant increase ($p > 0.05$) in body weight of test rats compared with the control.

4.3 EFFECT OF METHANOLIC LEAF EXTRACT OF *Sphenocentrum jollyanum* ON RELATIVE ORGAN WEIGHTS OF MALE WISTAR RATS

The oral administration of methanolic leaf extract of *Sphenocentrum jollyanum* for 28 days did not show any significant change ($p > 0.05$) in the relative weight of the organs (kidney, testes, prostate and liver) compared with the animals in the control group. The effect of methanolic leaf extract of *Sphenocentrum jollyanum* on relative organ weight of animal is shown in Table 4.3.

Table 4.2: Effect of methanolic leaf extract of *Sphenocentrum jollyanum* on mean body-weight of male Wistar rats

Tests groups	Mean Initial Body Weight (g)	Mean Final Body Weight (g)	Change in Body Weight (g)
Control	128.95±2.77	172.55±3.61	43.60±3.18
Standard Drug	154.94±4.12	175.28±1.82	20.35±3.65
100mg/kg B.Wt	150.68±4.11	194.16±2.50	43.48±4.14
200mg/kg B.Wt	154.05±3.52	186.10±4.15	32.06±4.17
500mg/kg B.Wt	148.55±7.84	192.55±8.40	44.00±7.40

Data reported as Mean ± Standard Error of Mean, n=5. Values with superscripts are significantly different ($P < 0.05$) from the control group.

4.3: **Effect of Methanolic leaf extract of *Sphenocentrum jollyanum* on relative organ weight (%) in male Wistar rats.**

The oral administration of aqueous-methanol extract of *Sphenocentrum jollyanum* for 28 days did not show any significant change ($p > 0.05$) in the relative weight of the organs (kidney, testes, prostate and liver) compared with the animals in the control group (Table 2).

Test groups	Kidney (g)	Liver (g)	Testes (g)	Prostate (g)
Control	0.50±0.02	5.46±0.26	0.58±0.74	0.10±0.02
Standard Drug	0.50±0.02	5.17±0.27	0.69±0.03	0.09±0.01
100mg/kg B.Wt	0.51±0.01	5.65±0.09	0.50±0.02	0.09±0.02
200mg/kg B.Wt	0.54±0.02	5.14±0.21	0.64±0.03	0.11±0.04
500mg/kg B.Wt	0.53±0.03	5.65±0.49	0.73±0.08	0.13±0.01

Data reported as Mean ± Standard Error of Mean, n=5. Values with superscripts are significantly different ($P < 0.05$) from the control group.

4.4 EFFECT OF METHANOLIC EXTRACT OF *Sphenocentrum jollyanum* ON BIOCHEMICAL PARAMETERS IN MALE WISTAR RATS

4.4.1 LIVER FUNCTION TEST

Table 4.4 and 4.5 show the effect of methanolic leaf extract of *Sphenocentrum jollyanum* on liver function parameters (Total Protein, Albumin, Total Bilirubin, Direct Bilirubin, AST, ALT, ALP) of rats. There was a significant difference ($P < 0.05$) in ALT level in the group administered 100mg/kg of extract, and in the group which received standard drug when compared to the control group. There was a significant difference ($P < 0.05$) in ALP level in the group administered 100mg/kg and 500mg/kg of extract, and in the group which received standard drug when compared to the control group. There was also a significant difference ($P < 0.05$) in Total Protein in the group which received standard drug and 500mg/kg of extract when compared to the control group. There was a significant difference ($P < 0.05$) in Albumin level in the group administered 100mg/kg of extract when compared to the control group, and the group that received standard drug. However, there was no significant difference ($P > 0.05$) in AST, Direct Bilirubin and Total Bilirubin in the test groups when compared to the control group.

Table 4.4: Effect of Methanolic leaf extract of *Sphenocentrum jollyanum* on Total Bilirubin, Total Protein, Albumin and Direct Bilirubin in male Wistar rats

Test groups	Total Protein (g/dL)	Albumin (g/dL)	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)
Control	4.80±0.38	3.03±0.15	3.98±1.29	1.35±0.47
Standard Drug	4.37±0.19[a,b]	2.93±0.11[a,b]	4.72±0.40	0.98±0.22
100mg/kg B.Wt	5.24±0.54	2.82±0.14	3.31±1.53	0.97±0.23
200mg/kg B.Wt	4.38±0.25	3.24±0.13	3.34±0.11	0.75±0.24
500mg/kg B.Wt	5.51±0.30[a,c]	3.48±0.05[a,c]	3.31±0.80	0.60±0.24

Data reported as Mean ± Standard Error of Mean, n=5. Values with superscripts are significantly different ($P < 0.05$) from the control group.

Table 4.5: Effect of Methanolic leaf extract of *Sphenocentrum jollyanum* on AST, ALT and ALP activity in male Wistar rats

Test groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	80.00±7.51	19.75±2.75[a,b]	21.60±1.72[a,b]
Standard Drug	61.50±5.62	17.75±2.14[a,b]	16.00±1.93[a,c]
100mg/kg B.Wt	78.00±12.37	31.75±3.04[a,c]	16.15±1.99[a,d]
200mg/kg B.Wt	69.50±11.84	24.00±2.52	19.71±1.58
500mg/kg B.Wt	50.50±10.07	21.00±2.83	23.52±0.81[a,c,e]

Data reported as Mean ± Standard Error of Mean, n=5. Values with superscripts are significantly different ($P < 0.05$) from the control group.

4.4.2 KIDNEY FUNCTION TEST

Table 4.6 shows the effect of methanolic leaf extract of *Sphenocentrum jollyanum* on creatinine and urea levels of rats. The creatinine levels of rats reduced in all three test groups that received the extract (100mg/kg, 200mg/kg, 500mg/kg), and the group that received the standard drug. However, the groups which received 200mg/kg and 500mg/kg and the standard drug were significantly different ($P < 0.05$) from the control group. There was no significant difference ($P < 0.05$) in the level of urea when compared to the control.

Table 4.6: Effect of Methanolic leaf extract of *Sphenocentrum jollyanum* on Urea and Creatinine Levels in male Wistar rats

Test groups	UREA (mg/dL)	CREATININE (mg/dL)
Control	55.86±24.66	1.43±0.32[a,b]
Standard Drug	26.55±7.04	0.87±0.05[a,c]
100mg/kg B.Wt	36.82±3.82	0.97±0.06
200mg/kg B.Wt	30.06±4.75	0.62±0.23[a,d]
500mg/kg B.Wt	23.55±3.78	0.75±0.05[a,e]

Data reported as Mean ± Standard Error of Mean, n=5. Values with superscripts are significantly different ($P < 0.05$) from the control group.

4.4.3: ANTIOXIDANT ASSAY

Table 4.7 shows the effect of methanolic leaf extract of *Sphenocentrum jollyanum* on SOD, Catalase, Glutathione Peroxidase, MDA. There was no significant difference ($P > 0.05$) in SOD, Catalase, Glutathione Peroxidase and MDA in the test groups when compared to the control group.

Table 4.7: Effect of Methanolic leaf extract of *Sphenocentrum jollyanum* on SOD, Catalase, Glutathione Peroxidase and MDA in male Wistar rats

Test groups	SOD (U/g. Prost)	CATALASE (U/g. Prost)	GP_X (U/g. Prost)	MDA (U/g. Prost)
Control	1.36±0.10	0.22±0.02	1.16±0.11	0.45±0.08
Standard Drug	1.35±0.10	0.21±0.02	1.13±0.08	0.33±0.04
100mg/kg B.Wt	1.42±0.06	0.22±0.01	1.25±0.09	0.44±0.77
200mg/kg B.Wt	1.47±0.06	0.23±0.02	1.21±0.08	0.35±0.04
500mg/kg B.Wt	1.42±0.10	0.21±0.01	1.18±0.05	0.47±0.15

Data reported as Mean ± Standard Error of Mean, n=5. Values with superscripts are significantly different ($P < 0.05$) from the control group.

4.4.4: HORMONAL ASSAY

Table 4.8 shows the effect of methanolic leaf extract of *Sphenocentrum jollyanum* on Testosterone, Follicle Stimulating Hormone, and Luteinizing Hormone. There was a significant difference ($P < 0.05$) in Luteinizing Hormone (LH) level in the group that received 100mg/kg body weight and 500mg/kg body weight of extract compared to the control group. The study also showed a significant difference ($P < 0.05$) in the level of Follicle Stimulating Hormone (FSH) in the group that received 100mg/kg body weight of extract when compared with the control group. There was no significant difference ($P > 0.05$) in testosterone level in the test groups when compared with the control group, and the group that received standard drug.

Table 4.8: Effect of Methanolic leaf extract of *Sphenocentrum jollyanum* on LH, FSH and Testosterone Levels of male Wistar rats

Test groups	LH (mIU/mL)	FSH (mIU/mL)	TESTOSTERONE (ng/mL)
Control	3.10±0.06[a,b]	295.00±37.53[a,b]	4.90±0.29
Standard Drug	3.30±0.12[c]	324.50±37.24	4.75±0.20
100mg/kg B.Wt	3.60±0.12[a,c,d]	392.00±31.18[a,c]	5.30±0.12
200mg/kg B.Wt	3.25±0.09	307.00±11.55	5.05±0.14
500mg/kg B.Wt	2.75±0.03[a,d,e]	287.00±19.63	4.35±0.03

Data reported as Mean ± Standard Error of Mean, n=5. Values with superscripts are significantly different ($P < 0.05$) from the control group.

4.5: SEMEN ANALYSIS

Table 4.9 shows the effect of methanolic leaf extract of *Sphenocentrum jollyanum* on the semen of male Wistar rats. There was no significant difference ($P > 0.05$) in total motility, progressive motility and normal forms of the semen when compared to the control group and the group that received standard drug. There was a significant difference ($P < 0.05$) in total sperm cell count in the test group that received 500mg/kg body weight of the extract, when compared to the test group that received standard drug.

Table 4.9: Effect of METHANOLIC LEAF extract of *Sphenocentrum jollyanum* on the semen of male Wistar rats

Test groups	Progressive Motility (%)	Sluggish (%)	Total Motility (%)	Normal Forms (%)	Count ($x10^6$Cells/mL)
Control	0.00±0.00	0.00±0.00	0.00±0.00	25.00±5.00	91.67±74.18
Standard Drug	6.67±4.41	6.67±3.33	13.33±7.26	40.00±10.41	20.00±5.00[a,b]
100mg/kg B.Wt	5.00±2.89	8.33±4.41	13.33±7.26	33.33±10.93	30.00±15.00
200mg/kg B.Wt	3.33±1.67	5.00±2.89	8.33±4.41	43.33±12.02	50.00±35.00
500mg/kg B.Wt	1.67±1.67	1.67±1.67	3.33±3.33	41.67±1.67	228.33±101.67[a,c]

Data reported as Mean ± Standard Error of Mean, n=5. Values with superscripts are significantly different ($P < 0.05$) from the control group.

4.6: INVITRO ANTIOXIDANT ASSAY

4.6.1: FRAP ASSAY

Table 4.9.1 shows the FRAP scavenging activity of methanolic leaf extract of *Sphenocentrum jollyanum.* The methanolic leaf extract of *Sphenocentrum jollyanum* exhibited FRAP radical scavenging activity when compared to Ascorbic acid.

Table 4.9.1: FRAP scavenging activity of methanolic leaf extract of *Sphenocentrum jollyanum*

Concentration of Ascorbic Acid (Standard)	2,507 µM
Concentration of FRAP	486.5 µM

The mean concentration of FRAP (in µM) in the sample was calculated using the formula in appendix and it was compared with that of the standard (Ascorbic acid).

4.6.2: DPPH ASSAY

Table 4.9.2 and 4.9.3 show the percentage inhibition of DPPH and Half Inhibitory Concentration (IC50) value of DPPH respectively. The methanolic leaf extract of *Sphenocentrum jollyanum* exhibited DPPH radical scavenging activity when compared to Ascorbic acid.

Table: 4.9.2: Table showing the %DPPH inhibition of methanolic leaf extract of *Sphenocentrum jollyanum*.

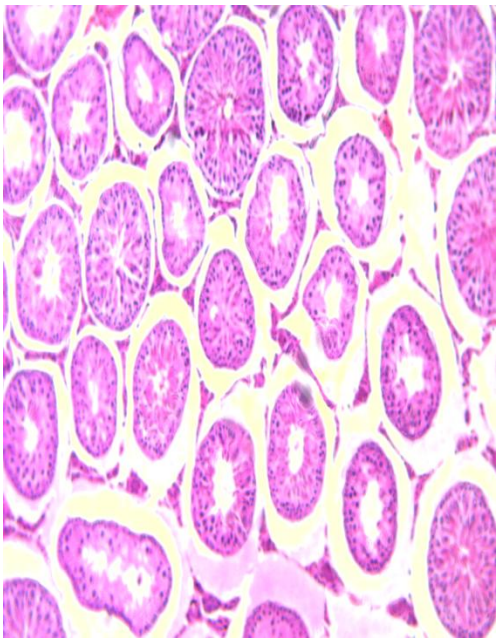
SAMPLE	% DPPH
A	20.46263345
B	43.00118624
C	52.21431396
D	70.16607355
E	69.55318308
A (STANDARD ASCORBIC ACID)	95.67022539
B	96.22380388
C	94.91894029
D	95.74930803
E	97.37050217

Table 4.9.3: Table showing the Half Inhibitory Concentration (IC50) value of DPPH in *Sphenocentrum jollyanum* As Compared To the IC50 Value of Standard (Ascorbic Acid)

ASCORBIC ACID	**0.1368µg/mL**
DPPH	**0.0464µg/mL**

4.7: EFFECT OF METHANOLIC LEAF EXTRACT OF *Sphenocentrum jollyanum* ON THE HISTOLOGY OF THE TESTES AND PROSTATE OF MALE WISTAR RATS

4.7.1: TESTES



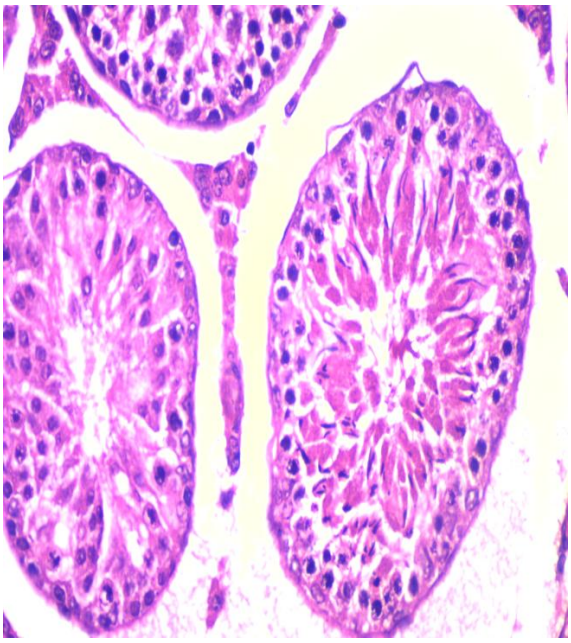


Plate 4.1: Photomicrograph of the testes of control rat (H+E x100)

Plate 4.2: Photomicrograph of the testes of control rat (H+E x400)

Section of the testis shows oval shaped seminiferous tubules containing sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with presence of Leydig cells in the interstitium. Features are in keeping with NORMAL SPERMATOGENESIS.

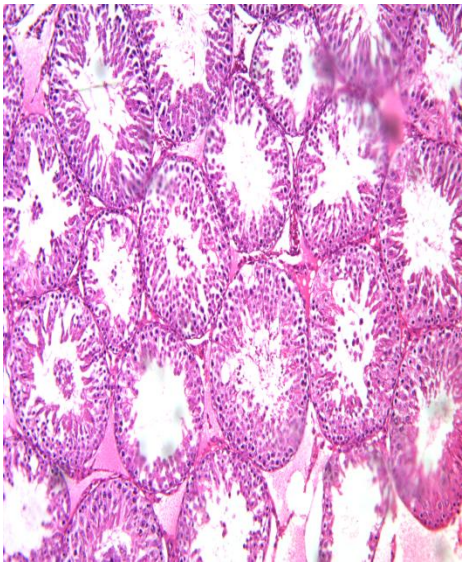


Plate 4.3: Photomicrograph of the testes of rat administered sildenafil citrate (H+E x100)

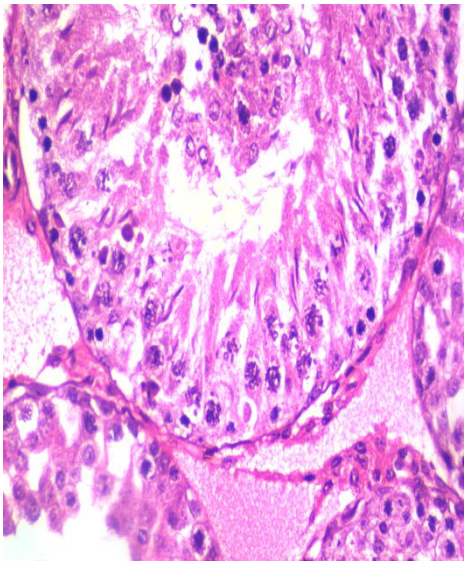


Plate 4.4: Photomicrograph of the testes of rat administered sildenafil citrate (H+E X400)

Section of the testis shows oval shaped seminiferous tubules containing sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with presence of Leydig cells in the interstitium. Features are in keeping with NORMAL SPERMATOGENESIS.

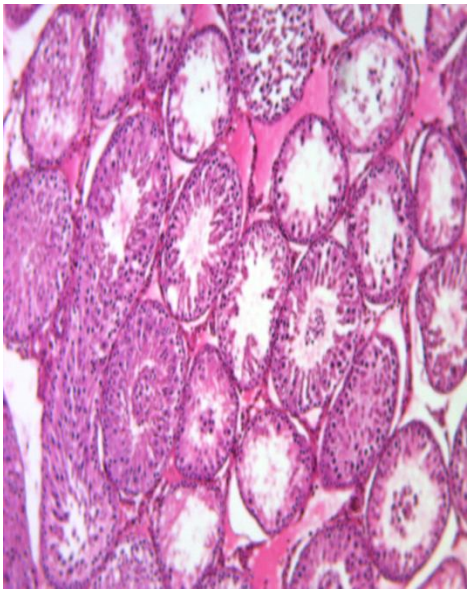


Plate 4.5: Photomicrograph of the testes of rat administered 100mg/kg b.wt extract (H+E x100)

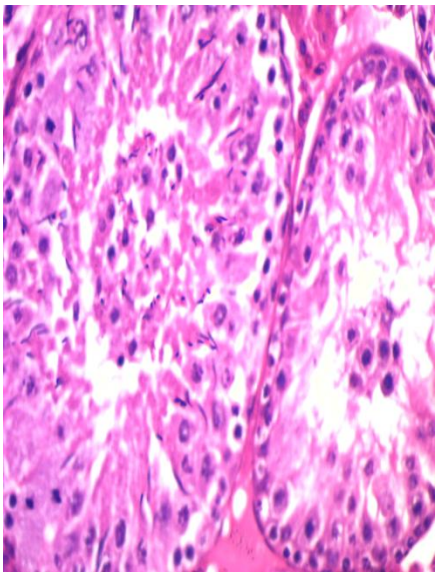


Plate 4.6: Photomicrograph of the testes of rat administered 100mg/kg b.wt extract (H+E x400)

Section of the testis shows oval shaped seminiferous tubules containing sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with presence of Leydig cells in the interstitium. Features are in keeping with NORMAL SPERMATOGENESIS

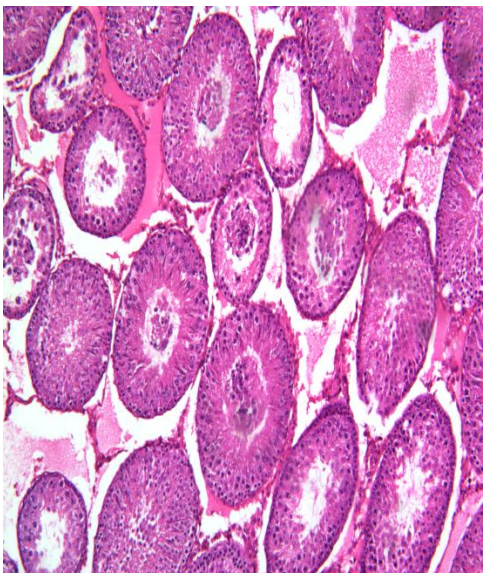


Plate 4.7: Photomicrograph of the testes of rat administered 200mg/kg b.wt extract (H+E x100)

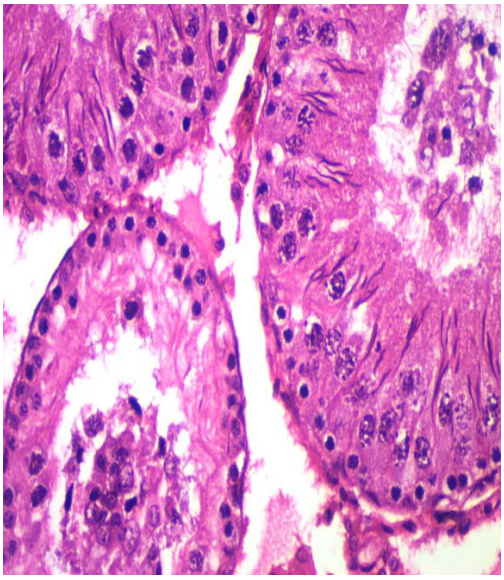


Plate 4.8: Photomicrograph of the testes of rat administered 200mg/kg b.wt extract (H+E x200)

Section of the testis shows oval shaped seminiferous tubules containing sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with presence of Leydig cells in the interstitium. Features are in keeping with NORMAL SPERMATOGENESIS

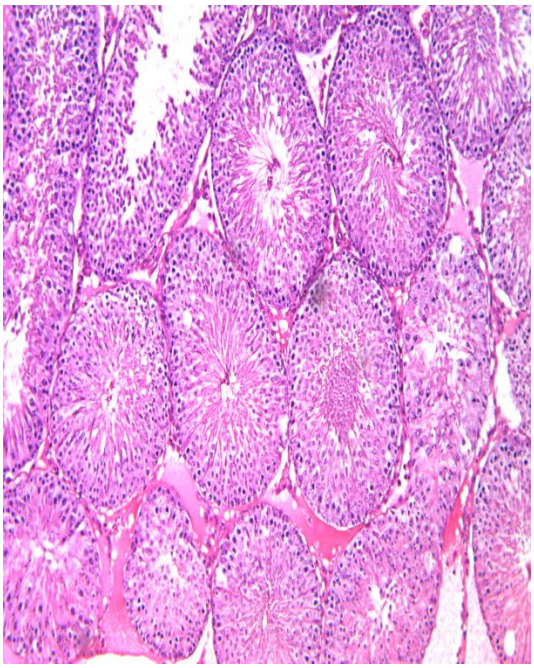


Plate 4.9: Photomicrograph of the testes of rat administered 500mg/kg b.wt extract (H+E x100)

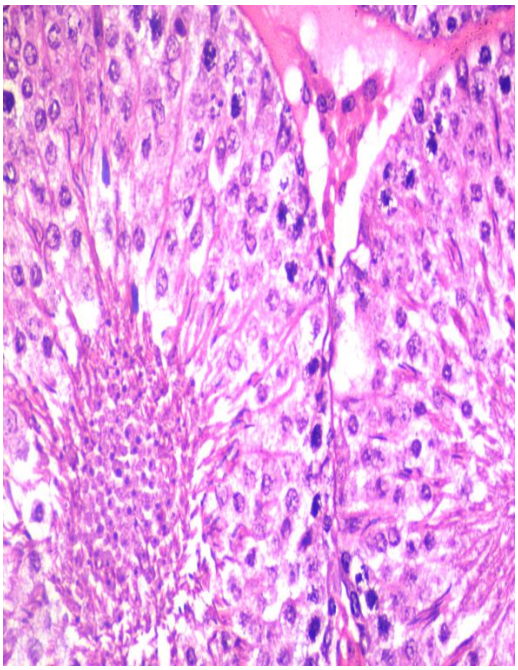
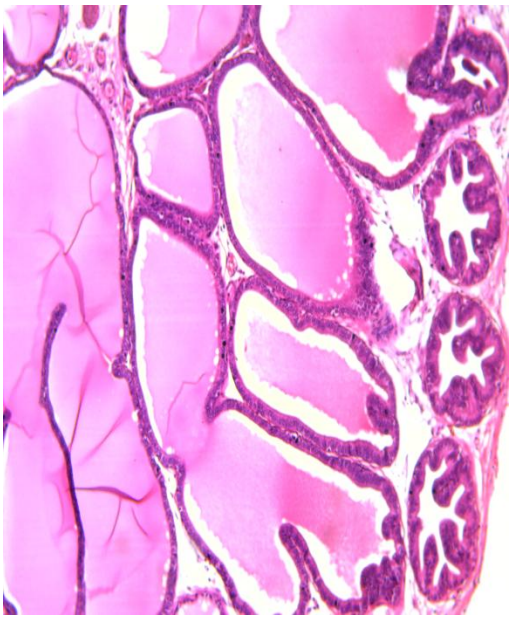


Plate 4.9.0: Photomicrograph of the testes of rat administered 500mg/kg b.wt extract (H+E x400)

Section of the testis shows oval shaped seminiferous tubules containing sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with presence of Leydig cells in the interstitium. Features are in keeping with NORMAL SPERMATOGENESIS.

4.7.2: PROSTATE



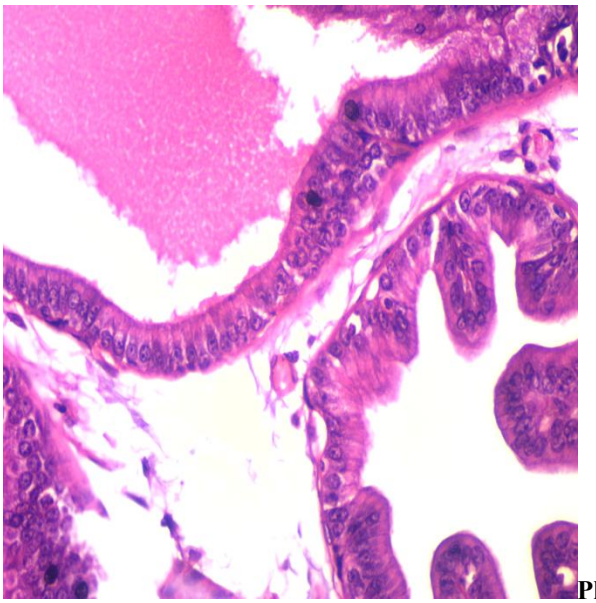


Plate 4.9.1: Photomicrograph of the prostate of control rat (H+E x100)

Plate 4.9.2: Photomicrograph of the prostat of control rat (H+E x400)

Section of the prostate shows acini and ducts of various sizes with some cystically dilated with homogenous pale fluid. The acini are lined by a double layer of epithelium (luminal columnar cells and basal cells) that are thrown into folds, within a fibromuscular stroma.

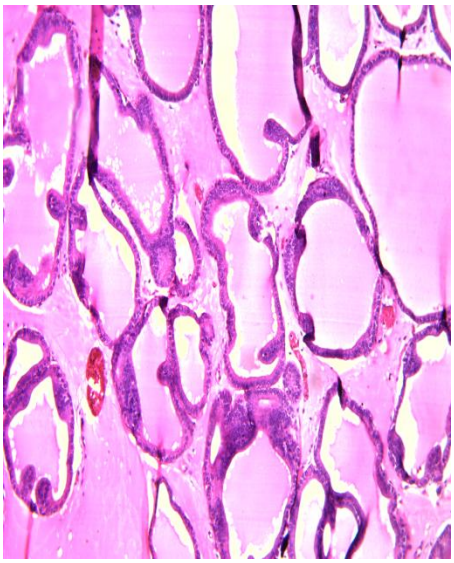


Plate 4.9.3: Photomicrograph of the prostate of rat administered sildenafil citrate (H+E x100)

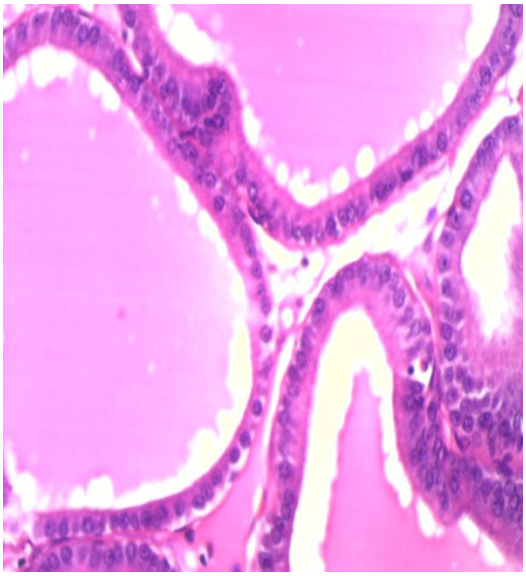


Plate 4.9.4: Photomicrograph of the prostate of rat administered sildenafil citrate (H+E x400)

Section of the prostate shows acini and ducts of various sizes with some cystically dilated with homogenous pale fluid. The acini are lined by a double layer of epithelium (luminal columnar cells and basal cells) that are thrown into folds, within a fibromuscular stroma.

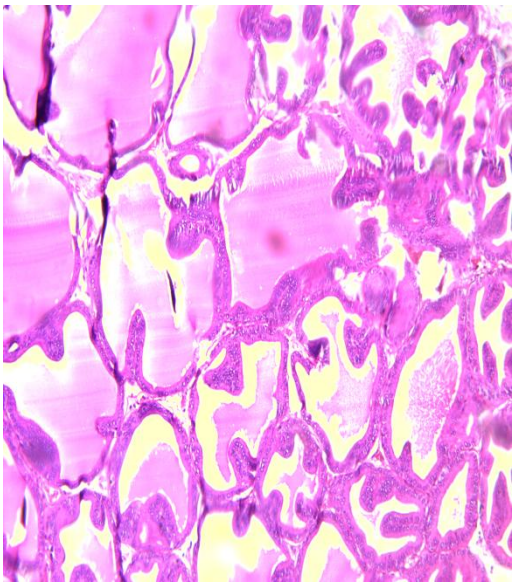


Plate 4.9.5: Photomicrograph of the prostate of rat administered 100mg/kg b.wt extract (H+E x100)

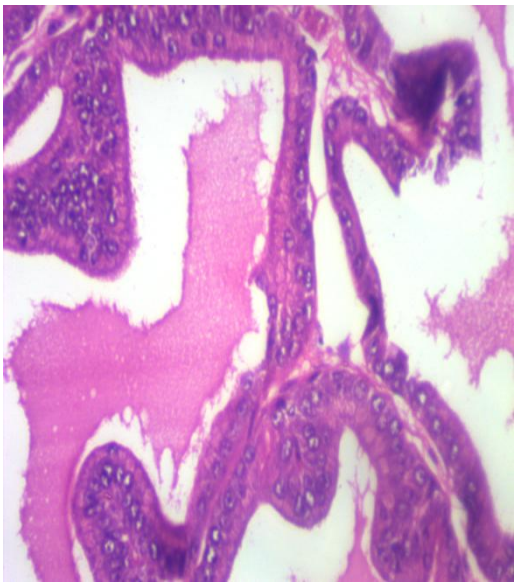


Plate 4.9.6: Photomicrograph of the prostate of rat administered 100mg/kg b.wt extract (H+E x400)

Section of the prostate shows acini and ducts of various sizes with some cystically dilated with homogenous pale fluid. The acini are lined by a double layer of epithelium (luminal columnar cells and basal cells) that are thrown into folds, within a fibromuscular stroma.

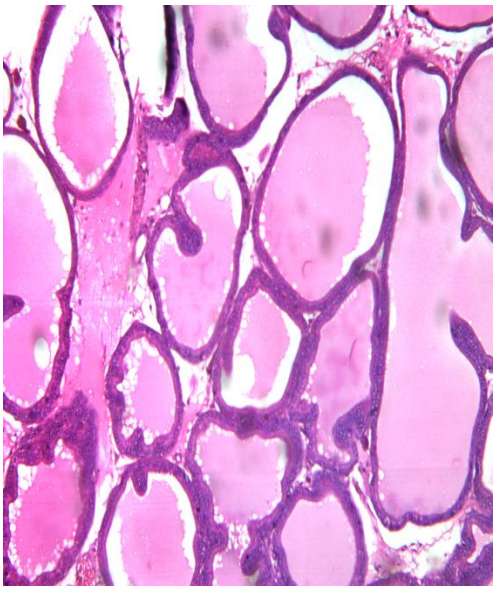


Plate 4.9.7: Photomicrograph of the prostate of rat administered 200mg/kg b.wt extract (H+E x100)

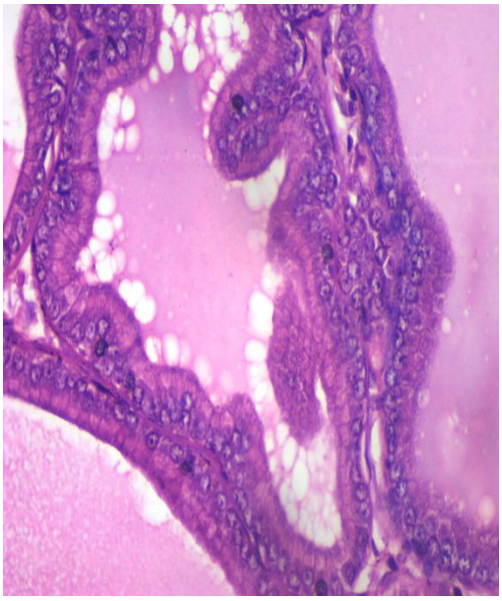


Plate 4.9.8: Photomicrograph of the prostate of rat administered 200mg/kg b.wt extract (H+E x400)

Section of the prostate shows acini and ducts of various sizes with some cystically dilated with homogenous pale fluid. The acini are lined by a double layer of epithelium (luminal columnar cells and basal cells) that are thrown into folds, within a fibromuscular stroma.

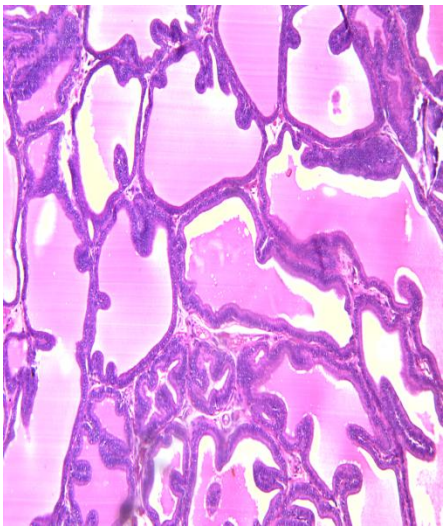


Plate 4.9.9: Photomicrograph of the prostate of rat administered 500mg/kg b.wt extract (H+E x100)

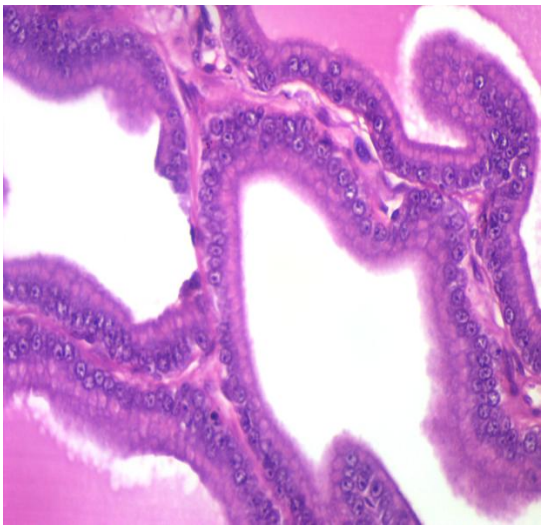


Plate 4.9.9.0: Photomicrograph of the prostate of rat administered 500mg/kg b.wt extract (x400)

Section of the prostate shows acini and ducts of various sizes with some cystically dilated with homogenous pale fluid. The acini are lined by a double layer of epithelium (luminal columnar cells and basal cells) that are thrown into folds, within a fibromuscular stroma.

CHAPTER FIVE

5.1: DISCUSSION

Sphenocentrum jollyanum is a perennial plant with increasing interest in its use in the enhancement of sexual behavior. This study was carried out to examine the in-vitro antioxidant properties of methanolic leaf extract of *Sphenocentrum jollyanum* on biochemical parameters in male Wistar rats. Its effect on sex hormones was also evaluated to make findings on why the plant is seen to enhance sexual behavior in males.

5.1.1: Biochemical Parameters

5.1.1.1: Liver Function

5.1.1.1.1: Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP)

These enzymes are liver marker enzymes which are useful in the detection of liver damage. These enzymes are found in the cells of the liver, but when there is hepatocellular injury, these enzymes leak out from the liver and enter the blood, thus elevating their level in the blood (Viriyavejakul *et al*., 2014). An increase in the plasma level of these enzymes is an indicator of liver damage or hepatocellular injury. The result obtained from this study showed no significant difference ($P > 0.05$) AST level in the test groups when compared to the control group and the group that received standard drug. There was a significant increase ($P < 0.05$) in ALT level in the group that received 100mg/kg body weight of the extract when compared with the control group and the group that received standard drug. There was an increase in ALT level in the other groups that received higher doses of the extract, but this was not significantly different ($P > 0.05$) from the control group and the group that received standard drug. There was a significant reduction ($P < 0.05$) in ALP level in the group that received 100mg/kg body weight of the extract when compared with the control. The group that received 500mg/kg body weight of the extract showed a significant increase in ALP level when compared to the group that received standard drug. The non-significant difference in AST level correlates with the findings of Mbaka *et al*., (2010). The elevation in ALT level in the group that received 100mg/kg body weight of the extract is consistent with the findings of Iheayichukwu *et al*., (2019). The significant increase in ALP level in the group that received 500mg/kg body weight of the extract as compared to the group that received the standard drug is consistent with the findings of Onengiyeofori *et al*., (2021), which showed that sildenafil citrated lowered serum ALP level.

5.1.1.1.2: Total Protein and Albumin

Total protein refers to the combined amount of albumin and globulin in the blood. These two proteins are produced by the liver and a decreased level of albumin or globulin is used in the detection of liver function. Results from this study showed a significant increase in total protein ($P < 0.05$) in the group that received 500mg/kg body weight of the extract when compared with the group that received standard drug. There was also a significant increase in albumin level ($P < 0.05$) in the group that received 500mg/kg body weight of the extract, when compared to the group that received standard drug. This significant increase in total protein and albumin level correlates with the findings of Arinzechukwu *et al*., (2020).

5.1.1.1.3: Total and Direct Bilirubin

Bilirubin is a product of heme breakdown in the liver and after its formation, it mixes with bile and it is ultimately excreted as bile pigment. When the liver is functioning well, the bilirubin is excreted, but when the level of total bilirubin increases, this may indicate a failure in the ability of the liver to excrete bile, or an obstruction in the passage route (bile duct) of bile (Veena *et al*., 2012). The result obtained from this study showed no significant difference ($P > 0.05$) in total and direct bilirubin level in the test groups when compared to the control group and the group that received standard drug. The decrease in total and direct bilirubin level in the test groups which was not significantly different from the control group suggests that the methanolic leaf extract of Sphenocentrum jollyanum may be hepatoprotective in function, as this correlates with the findings of Olorunnisola *et al*., (2020) which showed a significant decrease ($P < 0.05$) in total bilirubin when *Sphenocentrum jollyanum* was administered to CCl_4 - induced rats.

5.1.1.2: Kidney Function

The kidney is a homeostatic organ that is chiefly responsible for the removal of excess urea produced by the liver from the body. The kidney also removes creatinine (a metabolic waste product produced from the breakdown of creatine in skeletal muscles). It also regulates plasma electrolyte concentration in the body. The rate at which creatinine is cleared from the body (creatinine clearance rate), and the removal of excess urea from the body is indicative of the functionality of the kidney. The result obtained from this study showed a decrease in urea level in the test groups and in the group which received the standard drug, but this decrease was not significantly different ($P > 0.05$) from the control group. The result also showed a significant decrease ($P < 0.05$) in creatinine level in the groups which were given 200mg/kg body weight and 500mg/kg body weight of the extract, and in the group which received the standard drug when compared to the control group. The non-significant decrease in urea level is consistent with the findings of Wopara *et al*., (2018). The result of creatinine level disagrees with the findings of Wopara *et al*., (2018) who reported an increase in creatinine level in the test groups which received *Sphenocentrum jollyanum* when compared to the control.

5.1.1.3: Antioxidants Enzymes

5.1.1.3.1: Superoxide Dismutase (SOD), Catalase, Glutathione Peroxidase (GP_x)

Antioxidant enzymes are involved in preventing the accumulation of free radicals in the cell by converting the free radicals to a form that is not toxic. Malondialdehyde (MDA) which is one of the final products of lipid peroxidation in the cell acts as a bio-marker of oxidative stress (Gawel *et al*., 2004). The results obtained from this study showed no significant difference ($P > 0.05$) in the level of SOD, Catalase, GP_x and MDA in the test groups when compared to the control group and the group that received the standard drug. The non-significant difference in MDA, SOD, GP_x

and Catalase level disagrees with the finds of Chijioke & Ngozi, (2020) who reported that methanolic leaf extract of *Sphenocentrum jollyanum* significantly increased the activity of SOD, GP_x and catalase, and also significantly reduced MDA level in Wistar rats treated with Rifampicin to induce liver damage.

5.1.1.4: In Vitro Antioxidant

5.1.1.4.1: 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP)

The DPPH radical scavenging activity of *Sphenocentrum jollyanum* methanolic leaf extract showed a decrease in percentage inhibition of free radical in comparison to a standard antioxidant (ascorbic acid). The results also revealed that the concentration of *Sphenocentrum jollyanum* methanolic leaf extract required to scavenge the initial DPPH radical concentration by 50% (IC_{50} value) was 0.0464µg/mL, whereas the IC50 value of the standard compound, ascorbic acid was 0.1368µg/mL.

The ferric reducing antioxidant power assay of *Sphenocentrum jollyanum* methanolic leaf extract showed that the leaf extract had a reducing power in comparison to ascorbic acid. The results also revealed that the concentration *of Sphenocentrum jollyanum* methanolic leaf extract required to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) was 486.5 µM, whereas the value of ascorbic acid was 2,507 µM. These results correlate with the findings of Uka *et al*., (2020) who reported that the leaf extract of *Sphenocentrum jollyanum* exhibited DPPH and FRAP radical scavenging activity.

5.1.1.5: Sex Hormones

This study showed a significant difference ($P < 0.05$) in Luteinizing Hormone (LH) level in the group that received 100mg/kg body weight and 500mg/kg body weight of extract. There was a significant increase in LH level in the group that received 100mg/kg body weight of extract, and a significant decrease in LH level in the group that received 500mg/kg body weight of extract when compared with the control group and the group that received standard drug. The study also showed a significant increase ($P < 0.05$) in the level of Follicle Stimulating Hormone (FSH) in the group that received 100mg/kg body weight of extract when compared with the control group. The test group that received the standard drug showed an increase in FSH level when compared with the control group, but this was not significantly different ($P > 0.05$). There was no significant difference ($P > 0.05$) in testosterone level in the test groups when compared with the control group, and the group that received standard drug. There was also a progressive decrease in the level of FSH, LH and testosterone at graded doses of the extract (100mg/kg, 200mg/kg and 500mg/kg body weight). The increase in FSH level in the group that received 100mg/kg body weight of extract is consistent with the findings of Owiredu *et al*., (2007) who determined FSH level at weekly intervals for three weeks, and reported that there was a 30% increase in FSH level by the second week which dropped by the third week. The non-significant change in testosterone level disagrees with the findings of Owiredu *et al*., (2007) who reported that there was as a significant increase in testosterone level in rodents which received ethanolic root extract of *Sphenocentrum jollyanum*. This may suggest that the root extract of *Sphenocentrum jollyanum* is more potent in increasing testosterone level than the leaf extract of the plant. The significant decrease in LH in the group that received 500mg/kg body weight of extract is consistent with the findings of Owiredu *et al*., (2007) who determines the level of LH at weekly intervals for three weeks, and reported a significant decrease in LH level by the second week. The result also

showed that the plant extract at a lower dose (100mg/kg body weight) increased Testosterone, LH and FSH levels more than the standard drug (Sildenafil citrate) did.

5.1.1.6: Semen Analysis

Semen analysis is also known as sperm count test and it is carried out to ascertain the health and viability of a man's semen. The analysis shows the number of sperm cells, shape of the sperm and sperm motility which are all indicative of reproductive health in males. The results obtained from this study showed no significant difference ($P > 0.05$) in total motility, progressive motility and normal forms of the semen when compared to the control group and the group that received standard drug. There was a significant increase ($P < 0.05$) in total sperm cell count in the test group that received 500mg/kg body weight of the extract, when compared to the test group that received standard drug, but no significant difference ($P > 0.05$) in total sperm cell count in the group that received standard drug when compared to the control group. This study disagrees with the findings of Raji *et al*., (2006) who reported that the extract caused a dose-dependent significant reduction in progressive motility of semen, viability and total sperm counts. The non-significant difference in sperm parameters in the test group that received sildenafil citrate whwn compared to the control group is consistent with the findings Mokhtari *et al*., (2022) who reported that sildenafil had no positive effect on semen parameters in male participants with idiopathic infertility.

5.1.1.7: Histopathological Findings

The histopathological findings from this study showed a normal testes and prostate gland. The photomicrographs of the testes and prostate did not actually show an increased effect of the plant extract and the standard drug (Sildenafil citrate) in terms of boosting sperm production as it is

believed that the test animals may be fertile, and some may also exhibit hyper-fertility without the plant extract and Sildenafil citrate. The effect of the plant extract and sildenafil citrate may be carefully understood by first inducing Benign Prostatic Hyperplasia (BPH) using testosterone and estradiol, so as to ascertain whether the plant extract and sildenafil citrate are potent enough in handling infertility in males. This suggestion correlates with the findings of Mbaka *et al*., (2019) who reported a significant decrease in stromal hyperplasia in adult Wistar rats with BPH upon administration of the plant extract.

5.2: Conclusion

The results of this study show that the methanolic leaf extract of *Sphenocentrum jollyanum* exhibits free radical scavenging activity. The methanolic leaf extract of *Sphenocentrum jollyanum* also has an effect on some sex hormones (LH and FSH). Although there was a significant increase in ALT level in the group that received 100mg/kg body weight of extract, and a significant increase in ALP in the group that received 500mg/kg body weight of extract, the significant increase in total protein and albumin level may provide evidence to rule out liver damage since the liver is still carrying out its normal function in terms of protein production. The non-significant difference in total and direct bilirubin level and the significant increase in total protein suggest that the methanolic leaf extract of *Sphenocentrum jollyanum* has no negative impact on the liver. The significant decrease in creatinine level and the non-significant decrease in urea level show that the methanolic leaf extract of *Sphenocentrum jollyanum* has no negative effect on the kidney.

5.3: Contribution to Knowledge

The study has contributed to knowledge in the following ways:

i. This study established that the methanolic leaf extract of *Sphenocentrum jollyanum* has no adverse effect on the liver and kidney in male Wistar rats.

ii. This study also revealed that the methanolic leaf extract of *Sphenocentrum jollyanum* exhibits radical scavenging activity in male Wistar rats.

iii. This study established that the methanolic leaf extract of *Sphenocentrum jollyanum* increases the level of some sex hormones (LH and FSH) in male Wistar rats.

5.4: Further Findings

From this study, the further findings that should be carried out are:

i. If the methanolic leaf extract of *Sphenocentrum jollyanum* is effective in handling infertility in BPH-induced rats.

ii. If sildenafil citrate is effective in the enhancement of fertility in BPH-induced rats.

iii. If the methanolic leaf extract of *Sphenocentrum jollyanum* is effective in the management of hepatocellular injury.

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APPENDIX

Mean ± S.E.M Value of Total Protein

Report

Sample

Group	Mean	N	Std. Error of Mean
First	4.8025	4	.38082
Second	4.3650	4	.19333
Third	5.2425	4	.53548
Fourth	4.3750	4	.24747
5	5.5125	4	.30341
Total	4.8595	20	.17541

Statistical Analysis of Total Protein

Multiple Comparisons

Dependent Variable: Sample

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	First	Second	.43750	.49897	.901	-1.1033	1.9783
		Third	-.44000	.49897	.899	-1.9808	1.1008
		Fourth	.42750	.49897	.908	-1.1133	1.9683
		5	-.71000	.49897	.623	-2.2508	.8308
	Second	First	-.43750	.49897	.901	-1.9783	1.1033
		Third	-.87750	.49897	.431	-2.4183	.6633
		Fourth	-.01000	.49897	1.000	-1.5508	1.5308
		5	-1.14750	.49897	.198	-2.6883	.3933
	Third	First	.44000	.49897	.899	-1.1008	1.9808
		Second	.87750	.49897	.431	-.6633	2.4183
		Fourth	.86750	.49897	.442	-.6733	2.4083
		5	-.27000	.49897	.981	-1.8108	1.2708
	Fourth	First	-.42750	.49897	.908	-1.9683	1.1133
		Second	.01000	.49897	1.000	-1.5308	1.5508
		Third	-.86750	.49897	.442	-2.4083	.6733
		5	-1.13750	.49897	.205	-2.6783	.4033
	5	First	.71000	.49897	.623	-.8308	2.2508
		Second	1.14750	.49897	.198	-.3933	2.6883
		Third	.27000	.49897	.981	-1.2708	1.8108
		Fourth	1.13750	.49897	.205	-.4033	2.6783

LSD	First	Second	.43750	.49897	.394	-.6260	1.5010
		Third	-.44000	.49897	.392	-1.5035	.6235
		Fourth	.42750	.49897	.405	-.6360	1.4910
		5	-.71000	.49897	.175	-1.7735	.3535
	Second	First	-.43750	.49897	.394	-1.5010	.6260
		Third	-.87750	.49897	.099	-1.9410	.1860
		Fourth	-.01000	.49897	.984	-1.0735	1.0535
		5	-1.14750*	.49897	.036	-2.2110	-.0840
	Third	First	.44000	.49897	.392	-.6235	1.5035
		Second	.87750	.49897	.099	-.1860	1.9410
		Fourth	.86750	.49897	.103	-.1960	1.9310
		5	-.27000	.49897	.596	-1.3335	.7935
	Fourth	First	-.42750	.49897	.405	-1.4910	.6360
		Second	.01000	.49897	.984	-1.0535	1.0735
		Third	-.86750	.49897	.103	-1.9310	.1960
		5	-1.13750*	.49897	.038	-2.2010	-.0740
	5	First	.71000	.49897	.175	-.3535	1.7735
		Second	1.14750*	.49897	.036	.0840	2.2110
		Third	.27000	.49897	.596	-.7935	1.3335
		Fourth	1.13750*	.49897	.038	.0740	2.2010

*. The mean difference is significant at the 0.05 level.

Mean ± S.E.M Value of Albumin

Report

Sample

Group	Mean	N	Std. Error of Mean
First	3.0325	4	.15140
Second	2.9250	4	.11244
Third	2.8150	4	.13543
Fourth	3.2400	4	.12517
5	3.4800	4	.05083
Total	3.0985	20	.07232

Statistical Analysis of Albumin

Multiple Comparisons

Dependent Variable: Sample

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	First	Second	.43750	.49897	.901	-1.1033	1.9783
		Third	-.44000	.49897	.899	-1.9808	1.1008
		Fourth	.42750	.49897	.908	-1.1133	1.9683
		5	-.71000	.49897	.623	-2.2508	.8308
	Second	First	-.43750	.49897	.901	-1.9783	1.1033
		Third	-.87750	.49897	.431	-2.4183	.6633
		Fourth	-.01000	.49897	1.000	-1.5508	1.5308
		5	-1.14750	.49897	.198	-2.6883	.3933
	Third	First	.44000	.49897	.899	-1.1008	1.9808
		Second	.87750	.49897	.431	-.6633	2.4183
		Fourth	.86750	.49897	.442	-.6733	2.4083
		5	-.27000	.49897	.981	-1.8108	1.2708
	Fourth	First	-.42750	.49897	.908	-1.9683	1.1133
		Second	.01000	.49897	1.000	-1.5308	1.5508
		Third	-.86750	.49897	.442	-2.4083	.6733
		5	-1.13750	.49897	.205	-2.6783	.4033
	5	First	.71000	.49897	.623	-.8308	2.2508
		Second	1.14750	.49897	.198	-.3933	2.6883
		Third	.27000	.49897	.981	-1.2708	1.8108
		Fourth	1.13750	.49897	.205	-.4033	2.6783

LSD	First	Second	.43750	.49897	.394	-.6260	1.5010
		Third	-.44000	.49897	.392	-1.5035	.6235
		Fourth	.42750	.49897	.405	-.6360	1.4910
		5	-.71000	.49897	.175	-1.7735	.3535
	Second	First	-.43750	.49897	.394	-1.5010	.6260
		Third	-.87750	.49897	.099	-1.9410	.1860
		Fourth	-.01000	.49897	.984	-1.0735	1.0535
		5	-1.14750*	.49897	.036	-2.2110	-.0840
	Third	First	.44000	.49897	.392	-.6235	1.5035
		Second	.87750	.49897	.099	-.1860	1.9410
		Fourth	.86750	.49897	.103	-.1960	1.9310
		5	-.27000	.49897	.596	-1.3335	.7935
	Fourth	First	-.42750	.49897	.405	-1.4910	.6360
		Second	.01000	.49897	.984	-1.0535	1.0735
		Third	-.86750	.49897	.103	-1.9310	.1960
		5	-1.13750*	.49897	.038	-2.2010	-.0740
	5	First	.71000	.49897	.175	-.3535	1.7735
		Second	1.14750*	.49897	.036	.0840	2.2110
		Third	.27000	.49897	.596	-.7935	1.3335
		Fourth	1.13750*	.49897	.038	.0740	2.2010

*. The mean difference is significant at the 0.05 level.

Mean ± S.E.M Value of Total Bilirubin

Report

Sample

Group	Mean	N	Std. Error of Mean
First	3.9750	4	1.29057

Second	4.7200	4	.39684
Third	3.3050	4	1.52574
Fourth	3.3350	4	.11478
5	3.3075	4	.80282
Total	3.7285	20	.41015

Statistical Analysis of Total Bilirubin

Multiple Comparisons

Dependent Variable: Sample

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	First	Second	-.74500	1.38688	.982	-5.0276	3.5376
		Third	.67000	1.38688	.988	-3.6126	4.9526
		Fourth	.64000	1.38688	.990	-3.6426	4.9226
		5	.66750	1.38688	.988	-3.6151	4.9501
	Second	First	.74500	1.38688	.982	-3.5376	5.0276
		Third	1.41500	1.38688	.842	-2.8676	5.6976
		Fourth	1.38500	1.38688	.852	-2.8976	5.6676
		5	1.41250	1.38688	.843	-2.8701	5.6951
	Third	First	-.67000	1.38688	.988	-4.9526	3.6126
		Second	-1.41500	1.38688	.842	-5.6976	2.8676
		Fourth	-.03000	1.38688	1.000	-4.3126	4.2526
		5	-.00250	1.38688	1.000	-4.2851	4.2801
	Fourth	First	-.64000	1.38688	.990	-4.9226	3.6426
		Second	-1.38500	1.38688	.852	-5.6676	2.8976
		Third	.03000	1.38688	1.000	-4.2526	4.3126
		5	.02750	1.38688	1.000	-4.2551	4.3101
	5	First	-.66750	1.38688	.988	-4.9501	3.6151
		Second	-1.41250	1.38688	.843	-5.6951	2.8701
		Third	.00250	1.38688	1.000	-4.2801	4.2851
		Fourth	-.02750	1.38688	1.000	-4.3101	4.2551
LSD	First	Second	-.74500	1.38688	.599	-3.7011	2.2111
		Third	.67000	1.38688	.636	-2.2861	3.6261
		Fourth	.64000	1.38688	.651	-2.3161	3.5961

		5	.66750	1.38688	.637	-2.2886	3.6236
	Second	First	.74500	1.38688	.599	-2.2111	3.7011
		Third	1.41500	1.38688	.324	-1.5411	4.3711
		Fourth	1.38500	1.38688	.334	-1.5711	4.3411
		5	1.41250	1.38688	.325	-1.5436	4.3686
	Third	First	-.67000	1.38688	.636	-3.6261	2.2861
		Second	-1.41500	1.38688	.324	-4.3711	1.5411
		Fourth	-.03000	1.38688	.983	-2.9861	2.9261
		5	-.00250	1.38688	.999	-2.9586	2.9536
	Fourth	First	-.64000	1.38688	.651	-3.5961	2.3161
		Second	-1.38500	1.38688	.334	-4.3411	1.5711
		Third	.03000	1.38688	.983	-2.9261	2.9861
		5	.02750	1.38688	.984	-2.9286	2.9836
	5	First	-.66750	1.38688	.637	-3.6236	2.2886
		Second	-1.41250	1.38688	.325	-4.3686	1.5436
		Third	.00250	1.38688	.999	-2.9536	2.9586
		Fourth	-.02750	1.38688	.984	-2.9836	2.9286

Mean ± S.E.M Value of Direct Bilirubin

Report

Sample

Group	Mean	N	Std. Error of Mean

First	1.3525	4	.47384
Second	.9750	4	.22235
Third	.9725	4	.23167
Fourth	.7500	4	.23759
5	.5950	4	.23680
Total	.9290	20	.13169

Statistical Analysis of Direct Bilirubin

Multiple Comparisons

Dependent Variable: Sample

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	First	Second	.37750	.41960	.893	-.9182	1.6732
		Third	.38000	.41960	.890	-.9157	1.6757
		Fourth	.60250	.41960	.616	-.6932	1.8982
		5	.75750	.41960	.406	-.5382	2.0532
	Second	First	-.37750	.41960	.893	-1.6732	.9182
		Third	.00250	.41960	1.000	-1.2932	1.2982
		Fourth	.22500	.41960	.982	-1.0707	1.5207
		5	.38000	.41960	.890	-.9157	1.6757
	Third	First	-.38000	.41960	.890	-1.6757	.9157
		Second	-.00250	.41960	1.000	-1.2982	1.2932
		Fourth	.22250	.41960	.983	-1.0732	1.5182
		5	.37750	.41960	.893	-.9182	1.6732
	Fourth	First	-.60250	.41960	.616	-1.8982	.6932
		Second	-.22500	.41960	.982	-1.5207	1.0707
		Third	-.22250	.41960	.983	-1.5182	1.0732
		5	.15500	.41960	.996	-1.1407	1.4507
	5	First	-.75750	.41960	.406	-2.0532	.5382
		Second	-.38000	.41960	.890	-1.6757	.9157
		Third	-.37750	.41960	.893	-1.6732	.9182
		Fourth	-.15500	.41960	.996	-1.4507	1.1407
LSD	First	Second	.37750	.41960	.383	-.5169	1.2719
		Third	.38000	.41960	.379	-.5144	1.2744

		Fourth	.60250	.41960	.172	-.2919	1.4969
		5	.75750	.41960	.091	-.1369	1.6519
	Second	First	-.37750	.41960	.383	-1.2719	.5169
		Third	.00250	.41960	.995	-.8919	.8969
		Fourth	.22500	.41960	.600	-.6694	1.1194
		5	.38000	.41960	.379	-.5144	1.2744
	Third	First	-.38000	.41960	.379	-1.2744	.5144
		Second	-.00250	.41960	.995	-.8969	.8919
		Fourth	.22250	.41960	.604	-.6719	1.1169
		5	.37750	.41960	.383	-.5169	1.2719
	Fourth	First	-.60250	.41960	.172	-1.4969	.2919
		Second	-.22500	.41960	.600	-1.1194	.6694
		Third	-.22250	.41960	.604	-1.1169	.6719
		5	.15500	.41960	.717	-.7394	1.0494
	5	First	-.75750	.41960	.091	-1.6519	.1369
		Second	-.38000	.41960	.379	-1.2744	.5144
		Third	-.37750	.41960	.383	-1.2719	.5169
		Fourth	-.15500	.41960	.717	-1.0494	.7394

Sample

Group	Mean	N	Std. Error of Mean
First	80.0000	4	7.50555
Second	61.5000	4	5.61991
Third	78.0000	4	12.36932

AST

Fourth	69.5000	4	11.84272
5	50.5000	4	10.07058
Total	67.9000	20	4.63675

Multiple Comparisons

Dependent Variable: Sample

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
First	Second	18.50000	13.89364	.203	-11.1136	48.1136
	Third	2.00000	13.89364	.887	-27.6136	31.6136
	Fourth	10.50000	13.89364	.462	-19.1136	40.1136
	5	29.50000	13.89364	.051	-.1136	59.1136
Second	First	-18.50000	13.89364	.203	-48.1136	11.1136
	Third	-16.50000	13.89364	.253	-46.1136	13.1136
	Fourth	-8.00000	13.89364	.573	-37.6136	21.6136
	5	11.00000	13.89364	.441	-18.6136	40.6136
Third	First	-2.00000	13.89364	.887	-31.6136	27.6136
	Second	16.50000	13.89364	.253	-13.1136	46.1136
	Fourth	8.50000	13.89364	.550	-21.1136	38.1136
	5	27.50000	13.89364	.066	-2.1136	57.1136
Fourth	First	-10.50000	13.89364	.462	-40.1136	19.1136
	Second	8.00000	13.89364	.573	-21.6136	37.6136
	Third	-8.50000	13.89364	.550	-38.1136	21.1136
	5	19.00000	13.89364	.192	-10.6136	48.6136
5	First	-29.50000	13.89364	.051	-59.1136	.1136
	Second	-11.00000	13.89364	.441	-40.6136	18.6136
	Third	-27.50000	13.89364	.066	-57.1136	2.1136
	Fourth	-19.00000	13.89364	.192	-48.6136	10.6136

Report

Sample

Group	Mean	N	Std. Error of Mean
First	19.7500	4	2.75000

ALT

Second	17.7500	4	2.13600
Third	31.7500	4	3.03795
Fourth	24.0000	4	2.51661
5	21.0000	4	2.82843
Total	22.8500	20	1.54456

Multiple Comparisons

Dependent Variable: Sample

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
First	Second	2.00000	3.77823	.604	-6.0531	10.0531
	Third	-12.00000*	3.77823	.006	-20.0531	-3.9469
	Fourth	-4.25000	3.77823	.278	-12.3031	3.8031
	5	-1.25000	3.77823	.745	-9.3031	6.8031
Second	First	-2.00000	3.77823	.604	-10.0531	6.0531
	Third	-14.00000*	3.77823	.002	-22.0531	-5.9469
	Fourth	-6.25000	3.77823	.119	-14.3031	1.8031
	5	-3.25000	3.77823	.403	-11.3031	4.8031
Third	First	12.00000*	3.77823	.006	3.9469	20.0531
	Second	14.00000*	3.77823	.002	5.9469	22.0531
	Fourth	7.75000	3.77823	.058	-.3031	15.8031
	5	10.75000*	3.77823	.012	2.6969	18.8031
Fourth	First	4.25000	3.77823	.278	-3.8031	12.3031
	Second	6.25000	3.77823	.119	-1.8031	14.3031
	Third	-7.75000	3.77823	.058	-15.8031	.3031
	5	3.00000	3.77823	.440	-5.0531	11.0531
5	First	1.25000	3.77823	.745	-6.8031	9.3031
	Second	3.25000	3.77823	.403	-4.8031	11.3031
	Third	-10.75000*	3.77823	.012	-18.8031	-2.6969
	Fourth	-3.00000	3.77823	.440	-11.0531	5.0531

*. The mean difference is significant at the 0.05 level.

Report

Sample

Group	Mean	N	Std. Error of Mean
First	21.5975	4	1.71794
Second	15.9675	4	1.92561
Third	16.1500	4	1.99372
Fourth	19.7075	4	1.58497

ALP

5	23.5150	4	.80557
Total	19.3875	20	.94916

Multiple Comparisons

Dependent Variable: Sample

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
First	Second	5.63000*	2.34907	.030	.6231	10.6369
	Third	5.44750*	2.34907	.035	.4406	10.4544
	Fourth	1.89000	2.34907	.434	-3.1169	6.8969
	5	-1.91750	2.34907	.427	-6.9244	3.0894
Second	First	-5.63000*	2.34907	.030	-10.6369	-.6231
	Third	-.18250	2.34907	.939	-5.1894	4.8244
	Fourth	-3.74000	2.34907	.132	-8.7469	1.2669
	5	-7.54750*	2.34907	.006	-12.5544	-2.5406
Third	First	-5.44750*	2.34907	.035	-10.4544	-.4406
	Second	.18250	2.34907	.939	-4.8244	5.1894
	Fourth	-3.55750	2.34907	.151	-8.5644	1.4494
	5	-7.36500*	2.34907	.007	-12.3719	-2.3581
Fourth	First	-1.89000	2.34907	.434	-6.8969	3.1169
	Second	3.74000	2.34907	.132	-1.2669	8.7469
	Third	3.55750	2.34907	.151	-1.4494	8.5644
	5	-3.80750	2.34907	.126	-8.8144	1.1994
5	First	1.91750	2.34907	.427	-3.0894	6.9244
	Second	7.54750*	2.34907	.006	2.5406	12.5544
	Third	7.36500*	2.34907	.007	2.3581	12.3719
	Fourth	3.80750	2.34907	.126	-1.1994	8.8144

*. The mean difference is significant at the 0.05 level.

UREA

Report

Sample

Group	Mean	N	Std. Error of Mean
First	55.8600	4	24.65863
Second	26.5525	4	7.04339
Third	36.8225	4	3.82356
Fourth	30.0600	4	4.75413
5	23.5450	4	3.78132
Total	34.5680	20	5.42125

Multiple Comparisons

Dependent Variable: Sample

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
First	Second	29.30750	16.84253	.102	-6.5915	65.2065
	Third	19.03750	16.84253	.276	-16.8615	54.9365
	Fourth	25.80000	16.84253	.146	-10.0990	61.6990
	5	32.31500	16.84253	.074	-3.5840	68.2140
Second	First	-29.30750	16.84253	.102	-65.2065	6.5915
	Third	-10.27000	16.84253	.551	-46.1690	25.6290
	Fourth	-3.50750	16.84253	.838	-39.4065	32.3915
	5	3.00750	16.84253	.861	-32.8915	38.9065
Third	First	-19.03750	16.84253	.276	-54.9365	16.8615
	Second	10.27000	16.84253	.551	-25.6290	46.1690
	Fourth	6.76250	16.84253	.694	-29.1365	42.6615
	5	13.27750	16.84253	.443	-22.6215	49.1765
Fourth	First	-25.80000	16.84253	.146	-61.6990	10.0990
	Second	3.50750	16.84253	.838	-32.3915	39.4065
	Third	-6.76250	16.84253	.694	-42.6615	29.1365

	5	6.51500	16.84253	.704	-29.3840	42.4140
	First	-32.31500	16.84253	.074	-68.2140	3.5840
	Second	-3.00750	16.84253	.861	-38.9065	32.8915
5	Third	-13.27750	16.84253	.443	-49.1765	22.6215
	Fourth	-6.51500	16.84253	.704	-42.4140	29.3840

Report

Sample

CREATININE

Group	Mean	N	Std. Error of Mean
First	1.4250	4	.32001
Second	.8700	4	.04813
Third	.9650	4	.06007
Fourth	.6225	4	.22618
5	.7475	4	.04871
Total	.9260	20	.09532

Multiple Comparisons

Dependent Variable: Sample

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
First	Second	.55500*	.25445	.045	.0127	1.0973
	Third	.46000	.25445	.091	-.0823	1.0023
	Fourth	.80250*	.25445	.007	.2602	1.3448
	5	.67750*	.25445	.018	.1352	1.2198
Second	First	-.55500*	.25445	.045	-1.0973	-.0127
	Third	-.09500	.25445	.714	-.6373	.4473
	Fourth	.24750	.25445	.346	-.2948	.7898
	5	.12250	.25445	.637	-.4198	.6648
Third	First	-.46000	.25445	.091	-1.0023	.0823
	Second	.09500	.25445	.714	-.4473	.6373
	Fourth	.34250	.25445	.198	-.1998	.8848
	5	.21750	.25445	.406	-.3248	.7598
Fourth	First	-.80250*	.25445	.007	-1.3448	-.2602
	Second	-.24750	.25445	.346	-.7898	.2948
	Third	-.34250	.25445	.198	-.8848	.1998
	5	-.12500	.25445	.630	-.6673	.4173
5	First	-.67750*	.25445	.018	-1.2198	-.1352
	Second	-.12250	.25445	.637	-.6648	.4198
	Third	-.21750	.25445	.406	-.7598	.3248
	Fourth	.12500	.25445	.630	-.4173	.6673

*. The mean difference is significant at the 0.05 level.

SOD

Report

Sample

Group	Mean	N	Std. Error of Mean
First	1.3600	4	.09806
Second	1.3475	4	.09877
Third	1.4150	4	.06076
Fourth	1.4650	4	.05852
5	1.4225	4	.09733
Total	1.4020	20	.03512

Multiple Comparisons

Dependent Variable: Sample

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
First	Second	.01250	.11994	.918	-.2431	.2681
	Third	-.05500	.11994	.653	-.3106	.2006
	Fourth	-.10500	.11994	.395	-.3606	.1506
	5	-.06250	.11994	.610	-.3181	.1931
Second	First	-.01250	.11994	.918	-.2681	.2431
	Third	-.06750	.11994	.582	-.3231	.1881
	Fourth	-.11750	.11994	.343	-.3731	.1381
	5	-.07500	.11994	.541	-.3306	.1806
Third	First	.05500	.11994	.653	-.2006	.3106
	Second	.06750	.11994	.582	-.1881	.3231
	Fourth	-.05000	.11994	.683	-.3056	.2056
	5	-.00750	.11994	.951	-.2631	.2481
Fourth	First	.10500	.11994	.395	-.1506	.3606
	Second	.11750	.11994	.343	-.1381	.3731
	Third	.05000	.11994	.683	-.2056	.3056
	5	.04250	.11994	.728	-.2131	.2981
5	First	.06250	.11994	.610	-.1931	.3181
	Second	.07500	.11994	.541	-.1806	.3306
	Third	.00750	.11994	.951	-.2481	.2631
	Fourth	-.04250	.11994	.728	-.2981	.2131

Report

Sample

CATALASE

Group	Mean	N	Std. Error of Mean
First	.2175	4	.02097
Second	.2100	4	.01958
Third	.2200	4	.01225
Fourth	.2325	4	.01652
5	.2100	4	.01225
Total	.2180	20	.00691

Multiple Comparisons

Dependent Variable: Sample

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
First	Second	.00750	.02363	.755	-.0429	.0579
	Third	-.00250	.02363	.917	-.0529	.0479
	Fourth	-.01500	.02363	.535	-.0654	.0354
	5	.00750	.02363	.755	-.0429	.0579
Second	First	-.00750	.02363	.755	-.0579	.0429
	Third	-.01000	.02363	.678	-.0604	.0404
	Fourth	-.02250	.02363	.356	-.0729	.0279
	5	.00000	.02363	1.000	-.0504	.0504
Third	First	.00250	.02363	.917	-.0479	.0529
	Second	.01000	.02363	.678	-.0404	.0604
	Fourth	-.01250	.02363	.605	-.0629	.0379
	5	.01000	.02363	.678	-.0404	.0604
Fourth	First	.01500	.02363	.535	-.0354	.0654
	Second	.02250	.02363	.356	-.0279	.0729
	Third	.01250	.02363	.605	-.0379	.0629
	5	.02250	.02363	.356	-.0279	.0729
5	First	-.00750	.02363	.755	-.0579	.0429
	Second	.00000	.02363	1.000	-.0504	.0504
	Third	-.01000	.02363	.678	-.0604	.0404
	Fourth	-.02250	.02363	.356	-.0729	.0279

Report

Sample

GPx

Group	Mean	N	Std. Error of Mean
First	1.1550	4	.11206
Second	1.1325	4	.07825
Third	1.2525	4	.09013
Fourth	1.2100	4	.07778
5	1.1775	4	.05498
Total	1.1855	20	.03502

Multiple Comparisons

Dependent Variable: Sample

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
First	Second	.02250	.11979	.854	-.2328	.2778
	Third	-.09750	.11979	.428	-.3528	.1578
	Fourth	-.05500	.11979	.653	-.3103	.2003
	5	-.02250	.11979	.854	-.2778	.2328
Second	First	-.02250	.11979	.854	-.2778	.2328
	Third	-.12000	.11979	.332	-.3753	.1353
	Fourth	-.07750	.11979	.527	-.3328	.1778
	5	-.04500	.11979	.712	-.3003	.2103
Third	First	.09750	.11979	.428	-.1578	.3528
	Second	.12000	.11979	.332	-.1353	.3753
	Fourth	.04250	.11979	.728	-.2128	.2978
	5	.07500	.11979	.541	-.1803	.3303
Fourth	First	.05500	.11979	.653	-.2003	.3103
	Second	.07750	.11979	.527	-.1778	.3328
	Third	-.04250	.11979	.728	-.2978	.2128
	5	.03250	.11979	.790	-.2228	.2878
5	First	.02250	.11979	.854	-.2328	.2778
	Second	.04500	.11979	.712	-.2103	.3003
	Third	-.07500	.11979	.541	-.3303	.1803
	Fourth	-.03250	.11979	.790	-.2878	.2228

Report

Sample

MDA

Group	Mean	N	Std. Error of Mean
First	.4450	4	.08190
Second	.3250	4	.03969
Third	.4400	4	.07036
Fourth	.3500	4	.03697
5	.4675	4	.15261
Total	.4055	20	.03697

Multiple Comparisons

Dependent Variable: Sample

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
First	Second	.12000	.12311	.345	-.1424	.3824
	Third	.00500	.12311	.968	-.2574	.2674
	Fourth	.09500	.12311	.452	-.1674	.3574
	5	-.02250	.12311	.857	-.2849	.2399
Second	First	-.12000	.12311	.345	-.3824	.1424
	Third	-.11500	.12311	.365	-.3774	.1474
	Fourth	-.02500	.12311	.842	-.2874	.2374
	5	-.14250	.12311	.265	-.4049	.1199
Third	First	-.00500	.12311	.968	-.2674	.2574
	Second	.11500	.12311	.365	-.1474	.3774
	Fourth	.09000	.12311	.476	-.1724	.3524
	5	-.02750	.12311	.826	-.2899	.2349
Fourth	First	-.09500	.12311	.452	-.3574	.1674
	Second	.02500	.12311	.842	-.2374	.2874
	Third	-.09000	.12311	.476	-.3524	.1724
	5	-.11750	.12311	.355	-.3799	.1449
5	First	.02250	.12311	.857	-.2399	.2849
	Second	.14250	.12311	.265	-.1199	.4049
	Third	.02750	.12311	.826	-.2349	.2899
	Fourth	.11750	.12311	.355	-.1449	.3799

Report

Sample

LH

Group	Mean	N	Std. Error of Mean
First	3.1000	3	.05774
Second	3.2000	3	.11547
3	3.6000	3	.11547
4	3.2500	3	.08660
5	2.7500	3	.02887
Total	3.1800	15	.08015

Multiple Comparisons

Dependent Variable: Sample

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
First	Second	-.10000	.12383	.438	-.3759	.1759
	3	-.50000*	.12383	.002	-.7759	-.2241
	4	-.15000	.12383	.254	-.4259	.1259
	5	.35000*	.12383	.018	.0741	.6259
Second	First	.10000	.12383	.438	-.1759	.3759
	3	-.40000*	.12383	.009	-.6759	-.1241
	4	-.05000	.12383	.695	-.3259	.2259
	5	.45000*	.12383	.005	.1741	.7259
3	First	.50000*	.12383	.002	.2241	.7759
	Second	.40000*	.12383	.009	.1241	.6759
	4	.35000*	.12383	.018	.0741	.6259
	5	.85000*	.12383	.000	.5741	1.1259
4	First	.15000	.12383	.254	-.1259	.4259
	Second	.05000	.12383	.695	-.2259	.3259
	3	-.35000*	.12383	.018	-.6259	-.0741
	5	.50000*	.12383	.002	.2241	.7759
5	First	-.35000*	.12383	.018	-.6259	-.0741
	Second	-.45000*	.12383	.005	-.7259	-.1741
	3	-.85000*	.12383	.000	-1.1259	-.5741
	4	-.50000*	.12383	.002	-.7759	-.2241

*. The mean difference is significant at the 0.05 level.

FSH

Report

Sample

Group	Mean	N	Std. Error of Mean
First	295.0000	3	37.52777
Second	324.5000	3	37.23909
3	392.0000	3	31.17691
4	307.0000	3	11.54701
5	287.0000	3	19.62991
Total	321.1000	15	14.95359

Multiple Comparisons

Dependent Variable: Sample

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
First	Second	-29.50000	41.40411	.492	-121.7541	62.7541
	3	-97.00000*	41.40411	.041	-189.2541	-4.7459
	4	-12.00000	41.40411	.778	-104.2541	80.2541
	5	8.00000	41.40411	.851	-84.2541	100.2541
Second	First	29.50000	41.40411	.492	-62.7541	121.7541
	3	-67.50000	41.40411	.134	-159.7541	24.7541
	4	17.50000	41.40411	.681	-74.7541	109.7541
	5	37.50000	41.40411	.386	-54.7541	129.7541
3	First	97.00000*	41.40411	.041	4.7459	189.2541
	Second	67.50000	41.40411	.134	-24.7541	159.7541
	4	85.00000	41.40411	.067	-7.2541	177.2541
	5	105.00000*	41.40411	.030	12.7459	197.2541
4	First	12.00000	41.40411	.778	-80.2541	104.2541
	Second	-17.50000	41.40411	.681	-109.7541	74.7541
	3	-85.00000	41.40411	.067	-177.2541	7.2541
	5	20.00000	41.40411	.639	-72.2541	112.2541
5	First	-8.00000	41.40411	.851	-100.2541	84.2541
	Second	-37.50000	41.40411	.386	-129.7541	54.7541
	3	-105.00000*	41.40411	.030	-197.2541	-12.7459
	4	-20.00000	41.40411	.639	-112.2541	72.2541

*. The mean difference is significant at the 0.05 level.

TESTOSTERONE

Report

Sample

Group	Mean	N	Std. Error of Mean
First	4.9000	3	.28868
Second	4.7500	3	.20207
3	5.3000	3	.11547
4	5.0500	3	.14434
5	4.3500	3	.02887
Total	4.8700	15	.10832

Multiple Comparisons

Dependent Variable: Sample

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
First	Second	.15000	.25232	.565	-.4122	.7122
	3	-.40000	.25232	.144	-.9622	.1622
	4	-.15000	.25232	.565	-.7122	.4122
	5	.55000	.25232	.054	-.0122	1.1122
Second	First	-.15000	.25232	.565	-.7122	.4122
	3	-.55000	.25232	.054	-1.1122	.0122
	4	-.30000	.25232	.262	-.8622	.2622
	5	.40000	.25232	.144	-.1622	.9622
3	First	.40000	.25232	.144	-.1622	.9622
	Second	.55000	.25232	.054	-.0122	1.1122
	4	.25000	.25232	.345	-.3122	.8122
	5	.95000*	.25232	.004	.3878	1.5122
4	First	.15000	.25232	.565	-.4122	.7122
	Second	.30000	.25232	.262	-.2622	.8622
	3	-.25000	.25232	.345	-.8122	.3122
	5	.70000*	.25232	.020	.1378	1.2622
5	First	-.55000	.25232	.054	-1.1122	.0122
	Second	-.40000	.25232	.144	-.9622	.1622
	3	-.95000*	.25232	.004	-1.5122	-.3878
	4	-.70000*	.25232	.020	-1.2622	-.1378

*. The mean difference is significant at the 0.05 level.

REGRESSION ANALYSIS OF DPPH

Formula: $y = 0.0002x + 0.3607$