MITIGATING EFFECTS OF AQUEOUS EXTRACT OF *HIBISCUS SABDARIFFA* ON LEAD-INDUCED TOXICITY IN THE TESTES AND EPIDIDYMIS OF ADULT WISTAR RATS

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IN PARTIAL FULFILLMENT OF THE REQUIREMENT FORNTHE AWARD OF BACHELOR OF SCIENCE (B.Sc. HONS) IN ANATOMY

JULY, 2021

CERTIFICATION

I certify that this project work was carried out by Idiake Eghonghon Favour with Matriculation number BMS1601636 in the Department of Anatomy, School of Basic Medical sciences, University of Benin, Benin City.

_____ **_____**

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_____
External Examiner

Date:_____

DEDICATION

This work is dedicated to Almighty God for His infinite wisdom, kindness and love during the period of this project work.

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My heartfelt appreciation goes to Almighty God for His mercy, kindness, grace, favour and love for me.

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ABSTRACT

Hibiscus sabdariffa Linn. (Roselle) is an annual shrub which is popular in Asian and African countries including Nigeria. It is used as a medicinal plant for the treatment of various disease conditions such as hypertension and hyperlipidemia [Chukwunonso K. Nwabufo* and O Olusanya, (2017)] This study was undertaken to investigate the mitigating effect of Aqueous extract of *Hibiscus sabdariffa* calyx on lead induced toxicity on testes and epididymis of adult Wistar rats. Twenty male rats weighing 150g - 180g were used for this study, the rats were divided randomly into four groups A, B, C, and D consisting of 5 rats each. Group A served as control group and B, C, D were treatment groups. The rats were fed with Grower's mash animal feed and given water throughout the period of this study (60 days). Group A was just given food and water. Treatment group B was given 0.1ml of lead acetate via orogastric tube for 60 days. Group C was given *Hibiscus sabdariffa* extract 500mg/kg BW for 60 days. Group D was given 0.1ml of lead acetate and *Hibiscus sabdariffa* Aqueous extract 500mg/kg for 60 days. This study suggests that *Hibiscus sabdariffa* extract has some curative and preventive effect against testes and epididymis toxicity caused by lead acetate. These effects were supported by the restoration of histological architecture of the testes.

CHAPTER ONE

INTRODUCTION

The toxic nature of lead has been well known since 2000 BC (Needleman, 2002). Lead is a toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. It is a cumulative toxicant that affects multiple body systems, including the neurological, haematological, gastrointestinal, cardiovascular and renal systems. Children are particularly vulnerable to the neurotoxic effects of lead, and even relatively low levels of exposure can cause serious and, in some cases, irreversible neurological damage WHO (2010) . Such poisoning occurs from different kinds of human related activities such as painting of home, smoking related activities, leaded petrol, contaminated food, and drinking water; smelting; and especially from the industries, which have been carrying out manufacturing processes. It is also found in human milk (Lead Toxicity Where is Lead Found; 2017).

Lead is found at low levels in Earth's crust, mainly as lead sulfide (IARC 2006). .In 2016, the Institute for Health Metrics and Evaluation (IHME) estimated that worldwide 540,000 deaths and loss of 13.9 million healthy life, disability adjusted life years (DALYs), due to lead poisoning were recorded. IHME also predicted that in 63.8% of cases of lead exposure, there was a liability to develop intellectual disability. Low and middle income countries were most commonly affected. It poses a continuous and serious impact on public health. This also adds to burden of these countries, which are already affected with other extensively familiar public health challenges. For a generalization of sustenance and capital rising, high number of DALYs associated with the exposure of lead may be used for the remediation of toxic waste (Lead Poisoning and Health; 2017).

Hibiscus sabdariffa Linn. (Roselle) is an annual shrub which is popular in Asian and African countries including Nigeria. The plant is around 3.5 m tall with alternate leaves, yellow flowers, red calyx, and red fruits after they mature and light brown kidney-shaped seeds. *Hibiscus sabdariffa* (roselle) has been used traditionally as a food, in herbal drinks, in hot and cold beverages, as a flavoring agent in the food industry and as a herbal medicine.

A sweetened drink known as zobo in Nigeria is made from calyx of the *Hibiscus sabdariffa*. This drink is consumed without any thoughts of what effects it will have in the body. The medicinal properties of *Hibiscus sabdariffa* Calyx have been associated with certain phytochemical

constituents. Some of the phytochemical constituents responsible for the pharmacological effect of *Hibiscus sabdariffa* include flavonoids and protocatechuic acid. The following flavonoids have been described in *Hibiscus sabdariffa* extracts: hibiscitrin (hibiscetin-3-glucoside), sabdaritrin, gossypitrin, gossytrin and other gossypetin glucosides, quercetin and luteolin (McKay, 2009; Williamson *et al.*, 2009); as well as chlorogenic acid, protocatechuic acid, pelargonidic acid, eugenol, quercetin, luteolin and the sterols b-sitosterol and ergosterol (McKay, 2009; Williamson *et al.*, 2009)

Kanokwan Sukjail and Ampa Luangpirom (2010) studied aqueous seed extract of *Hibiscus sabdariffa* in male rats, and found that all treated groups showed decreasing of sperm concentration, percentage of normal motile sperms and vital sperms, while the percentage of abnormal sperms increased.5000 mg/kg and doses as high as 4600 mg/kg were administered to rats in drinking water for 12 weeks with no increase in mortality, the extract induced testicular toxicity (reduced sperm counts and spermatogenesis with evidence of marked degenerative histological changes) at all concentrations tested (1150–4600 mg/kg) (Orisakwe, Husaini, & Afonne, 2004). Additionally, deleterious effects on the testis and spermatozoa and an adverse influence in the male reproductive fertility of albino mice were also reported after a c*Hibiscus sabdariffa* WE was administered daily for 4 weeks in a ndose of 200 mg/kg (Y. I. Mahmoud, 2012). In contrast to these studies, long term administration of *Hibiscus sabdariffa* WE for 10 weeks and hibiscus anthocyanins (50–200 mg/kg b.w.) for 5 days did not affect the male reproductive system in rats (Ali *et al.*, 2012).

1.2 JUSTIFICATION OF STUDY

The lead environmental causes represent one of the major factors affecting male fertility (Al-Chalabi *et al,.* 2014). Infusions of the calyces are considered as diuretic, cholerectic, febrifugal and hypotensive, decreasing the viscosity of the blood and stimulating intestinal peristalsis (Salleh *et al.,* 2002). According to an experiment by Asagba (2017) when compared to the organ/body weight ratios obtained from rats exposed to Cd alone the prostate and testis were protected by the extract as shown by enhanced prostate/body weight and testis/body weight ratios of Cd- and extract-treated rats. These data suggest that *Hibiscus sabdarrifa L* might be protective in Cd toxicity. Therefore this experiment is carried out to check how *Hibiscus sabdariffa* extract can protect the tested from toxicity when exposed to lead.

1.3 AIM OF THE STUDY

This study is set out to evaluate the Mitigating effect of Aqueous Extract of *Hibiscus sabdariffa* on lead induced toxicity in the testes and epididymis of adult wistar rats.

1.4 SPECIFIC OBJECTIVES OF THE STUDY

The above aim was achieved through the following specific objectives

1. Body weight changes across the groups
2. Weight of the testes and epididymis across the groups
3. Sperm morphology and motility
4. Histology of testes and epididymis.

CHAPTER TWO

LITERATURE REVIEW

2.1 PLANT OF STUDY

Hibiscus sabdariffa

FIG 2.1 *Hibiscus sabdariffa*

2.1.1 TAXONOMY OF *HIBISCUS SABDARIFFA*

Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta
Superdivision	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Rosanae
Order	Malvales
Family	Malvaceae
Genus	Hibiscus L.
Species	sabdariffa

2.1.2 Botanical description

Roselle (*Hibiscus sabdariffa* L.) with more than 300 species are widely grown and distributed in tropical and subtropical regions around the world (Da-Costa-Rocha *et al.* 2014). Nowadays, roselle is widely cultivated in India, Saudi Arabia, China, Malaysia, Indonesia, Philippines, Vietnam, Sudan, Egypt, Nigeria and Mexico (Riaz and Chopra 2018). The plant has been found to thrive on a wide range of soil conditions. It can perform satisfactorily on relatively infertile soils but for economic purposes, a soil well supplied with organic materials and essential nutrients is essential. It can tolerate relatively high temperature throughout the growing and fruiting periods. The plant requires an optimum rainfall of approximately 45-50 cm distributed over a 90-120 day growing period (Adanlawo and Ajibade, 2006).

Hibiscus sabdariffa ruber is an annual, erect, bushy, herbaceous subshrub that can grow up to 8 ft (2.4 m) tall, with smooth or nearly smooth, cylindrical, typically red stems. The leaves are alternate, 3 to 5 in (7.5–12.5 cm) long, green with reddish veins and long or short petioles. The leaves of young seedlings and upper leaves of older plants are simple; lower leaves are deeply 3 to 5 or even 7 lobed; the margins are toothed. Flowers, borne singly in the leaf axils, are up to 5 in (12.5 cm) wide, yellow or buff with a rose or maroon eye, and turn pink as they wither at the end of the day. At this time, the typically red calyx, consisting of 5 large sepals with a collar (epicalyx) of 8 to 12 slim, pointed bracts (or bracteoles) around the base, begins to enlarge, becomes fleshy, crisp but juicy, 1 1/4 to 2 1/4in (3.2–5.7 cm) long and fully encloses the velvety capsule, 1/2 to 3/4 in (1.25–2 cm) long, which is green when immature, 5-valved, with each valve containing 3 to 4 kidney-shaped, light-brown seeds, 1/8 to 3/16 in (3–5 mm) long and minutely downy. The capsule turns brown and splits open when mature and dry. The calyx, stems and leaves are acid and closely resemble the cranberry (Vaccinium spp.) in flavour (Morton, 1987; Ross, 2003)

2.1.3 Phytochemistry

Roselle is rich in anthocyanins and protocatechuic acid. The dried calyces contain the flavonoids gossypetine, hibiscetine and sabdaretine. The major pigment, formerly reported as hibiscine has been identified as daphniphylline. Small amounts of myrtillin (delphinidin 3-monoglucoside), chrysanthenin (cyanidin 3-monoglucoside) and delphinidin are also present. Roselle seeds are a good source of lipidsoluble antioxidants, particularly -tocopherol (Mohamed *et al.*, 2012). The

anthocyanin content of *Hibiscus sabdariffa* in five strains of the plant reportedly reached 1.7% to 2.5% of the dry weight during calyx growth (Khafaga and Koch, 1980b). *Hibiscus sabdariffa* calyces contain high amounts of organic acids, namely: citric acid, malic acid, tartaric acid and hibiscus protocatechuic acid (Kerharo, 1971; Khafaga and Koch, 1980a; Tseng *et al.*, 1996). The acid content of the calyces increases during growth but decreases when it reaches maturity or ripens. The aqueous extract of *Hibiscus sabdariffa* calyces has a very rich red pigmentation due to the presence of anthocyanins and the colour properties has been the subject of intense scientific investigations (Ali *et al.*, 2005; Salazer *et al.*, 2012; Aishah *et al.*, 2013). *Hibiscus sabdariffa* calyces were found to contain a higher amount of iron content (164.78 mg/kg) (Maregesi *et al.*, 2013). The plant is also found to be rich in minerals especially potassium and magnesium. Vitamins (ascorbic acid, niacin and pyridoxine) were also present in appreciable amounts (Puro *et al.*, 2014).

2.1.4 Medicinal and therapeutic benefits of *Hibiscus sabdariffa*.

The plant *Hibiscus sabdariffa* is also known as Roselle or Rosella. It is reported to be antihypertensive, antiseptic, sedative, diuretic, digestive, purgative, emollient, demulcent and astringent (Odigie *et al.*, 2003). The calyces are used to treat heart ailments, hypertension and leukemia. They are also reported to have diuretic, aphrodisiac, antiseptic, astringent, cholagogue, sedative, laxative, and antimicrobial activity. They are also used as remedy for pyrexia and abscesses. The flowers and fruits are used for treatment of cough and bronchitis (Maregesi *et al.*, 2013). Anthocyanins present in Roselle are dephinidin 3-sambubioside, cyanidin 3-sambubioside, delphinidin 3-glucoside and cyanidin 3-glucoside. They contribute benefit for health as a good source of antioxidants as well as a natural food colourant. The blending of Roselle juice with tropical fruit juices is anticipated to give products with high nutritional value and functional activity (Kilima *et al.*, 2014).

Early studies showed that the alcoholic extract of *Hibiscus sabdariffa* flowers had an antispasmodic effect by relaxing the uterus and intestine stips in vitro (Sharaf, 1962). This was also observed in rabbit aortic smooth muscle (Obiefuna, Owolabi, Adegunloye, Obiefuna, & Sofola, 1994). Interestingly, from various isolated muscle preparations, the extract of *Hibiscus sabdariffa* inhibited the tone of rabbit aortic strip, rhythmically contracting rat uterus, guinea-pig

tracheal chain and rat diaphragms, but it stimulated the tone of solated quiescent rat uterus and frog rectus abdominis (Ali, Salih, Mohamed, & Homeida, 1991).

A study conducted with 10 healthy volunteers also supports the claim for its anti-inflammatory activity. The ingestion of c*Hibiscus sabdariffa* WE (dried) decreased plasma monocyte chemoattractant protein 1 (MCP-1) concentration, a biomarker in the evaluation of inflammatory diseases (Beltran-Debon *et al.*, 2010).

2.1.5 Antimicrobial activity of *Hibiscus sabdariffa* extract

A methanol-water extract of *Hibiscus sabdariffa* was effective against E. coli O157:H7 isolates from food, veterinary and clinical samples (Fullerton, Khatiwada, Johnson, Davis, & Williams, 2011), with the highest concentration (10%) being the most effective. The crude extracts of *Hibiscus sabdariffa* seeds (200 mg/l) also showed antimicrobial effect against three types of Gram-negative bacteria. The extract exhibited higher activity against Salmonella followed by Shigella and Enterobacter (Nwaiwu, Mshelia, & Raufu, 2012).

2.1.6 Other uses

In Sudan and Nigeria, the calyces are boiled with sugar to produce a drink known as ‘‘Karkade’’ or ‘‘Zoborodo’’ (Gibbon & Pain, 1985). In Mexico this drink is called Jamaica or ‘‘agua de Jamaica’’’ or ‘‘té de Jamaica’’. In the West Indies the calyces can also be used as colouring and flavouring ingredient in rum (Ismail, Ikram, & Nazri, 2008) The seeds are eaten roasted or ground in meals, while the leaves and shoots are eaten raw or cooked, or as a sour-flavoured

The calyx is rich in citric acid and pectin and is useful for making jams, jellies (Pacome *et al.*, 2014). vegetable or condiment (Wilson & Menzel, 1964) Calyces extract is also a potential source of natural colourant to replace red synthetic colouring agents for carbonated soft drinks, jams, juices, jellies, sauces, chutneys, wines, preserves and other acidic foods (Delgado and Parcedes, 2003). In Africa, the seeds are roasted or ground into powder and used in meals, such as oily soups and sauces. In China and West Africa, the seeds are also used for their oil (Atta & Imaizumi, 2002). Another use for the seed is as a substitute for coffee (Morton, 1987).

2.2 CHEMICAL OF STUDY

Lead is one of the most toxic metals to human, animal and plants, that important environmental contaminant which affects many body organs including male genital system, practically in workers of lead based factories showed disorders in structure and function of male reproductive system and quality of sperms are principal symptoms of exposure to metal (Assi M.A.,etal, 2016.) This substance can be absorbed through respiration, digestion and dermal, and accumulated in body, when, the metal absorption by ingestion depend of factors such as the physical form, particle size, gastro intestinal transit time and nutritional status of as person and patterns of foods intake affect absorption (. Sharma S. and Pandey D. 2011). Lead can show their toxic effects through the inhibition of antioxidant enzyme activity, and generation of reactive oxygen species (ROS) with subsequent stimulation of lipid peroxidation (Al-Saady M.S.M. 2011)

Lead is used mainly in the production of lead-acid batteries, plumbing materials and alloys. Other uses are in cable sheathing, paints, glazes and ammunition. Human occupational exposure can also take place during the application and removal of protective lead-containing paints, during the grinding, welding and cutting of materials painted with lead-containing paints, such as in shipbuilding, construction, demolition industries, and fabrication of heavy lead glass and crystal, and in crystal carving. Mining, smelting, and informal processing and recycling of electric and electronic waste can also be significant sources of exposure. Lead has been used widely in the form of tetraethyl and tetramethyl lead as antiknock and lubricating agents in petrol, although the majority of lead is emitted from vehicles in the form of inorganic particles. This use has been phased out in most countries, which has resulted in a significant reduction of human exposure and mean blood lead levels. In the few parts of the world where leaded petrol is still in use, however, it continues to be a major source of exposure. Old industrial hotspots that have not been cleaned up can also represent a hazard even years after contamination has stopped, particularly to children who might ingest contaminated soil or dust as a result of their hand-to-mouth behaviour. Fewtrell L, Kaufmann R, Prüss-Üstün A (2003).

For the non-smoking general population, the largest contribution to the daily intake of lead is derived from the ingestion of food, dirt and dust. The amount of lead in food plants depends on soil concentrations and is highest around mines and smelters. Cereals can contain high levels of lead, and spices may be contaminated with lead. The use of lead-soldered food and beverage cans (which is now diminishing) may considerably increase the lead content of the food or

beverage, especially in the case of acidic foods or drinks. As alcoholic drinks tend to be acidic, the use of any lead-containing products in their manufacture, distribution or storage will raise lead levels. Migration of lead into food from lead-glazed ceramic or pottery dinnerware is also a source of exposure. Smoking tobacco increases lead intake, (IPCS, 1995)

2.2.1 Lead toxicity

2.2.1.1Cardiovascular toxicity

Lead can cause hypertension and affect blood vessels. Lead blocked blood vessels can lead to immediate heart attack and death. Increasing BLLs significantly correlate with an increase in cardiovascular morbidity and mortality.[Akil L, *et al.,* 2011] Chronic and acute both types of lead poisoning can cause cardiac dysfunction and vascular damage.[Flora G, *et al.,* 2012] Recently, researchers have found an intriguing correlation between low blood leads concentration and cardiovascular toxicity, and recently, some studies suggest that low levels of lead may be associated with high blood pressure. (Kim HC, *et al.,* 2015).

2.2.1.2Nephrotoxicity

When lead affects the kidneys, medical experts call it lead related nephrotoxicity." Due to lead exposure, nephrotoxicity occurs because lead is eliminated through the kidney. In the renal tubules, lead is absorbed by proximal tubular cells, and it binds to specific lead binding proteins. These lead binding proteins produce intercellular inclusions of proximal tubular (Kwon et al 2015)

2.2.1.3 Bone toxicity

A significant reduction in the bone calcium content upon lead intoxication has been observed. This decrease in calcium content may be because of the increased bone resorption. Lead is one of the risk factors for the development of osteoporosis by altering bone mineral metabolism. Osteopenia, osteoporosis, and osteomalacia with increased bone fragility in humans and experimental animals were observed because of lead exposure. Long term exposure to lead damages different body tissues (Bhardwaj P and Rai DV,2016)

2.3 ORGANS OF STUDY; THE TESTIS

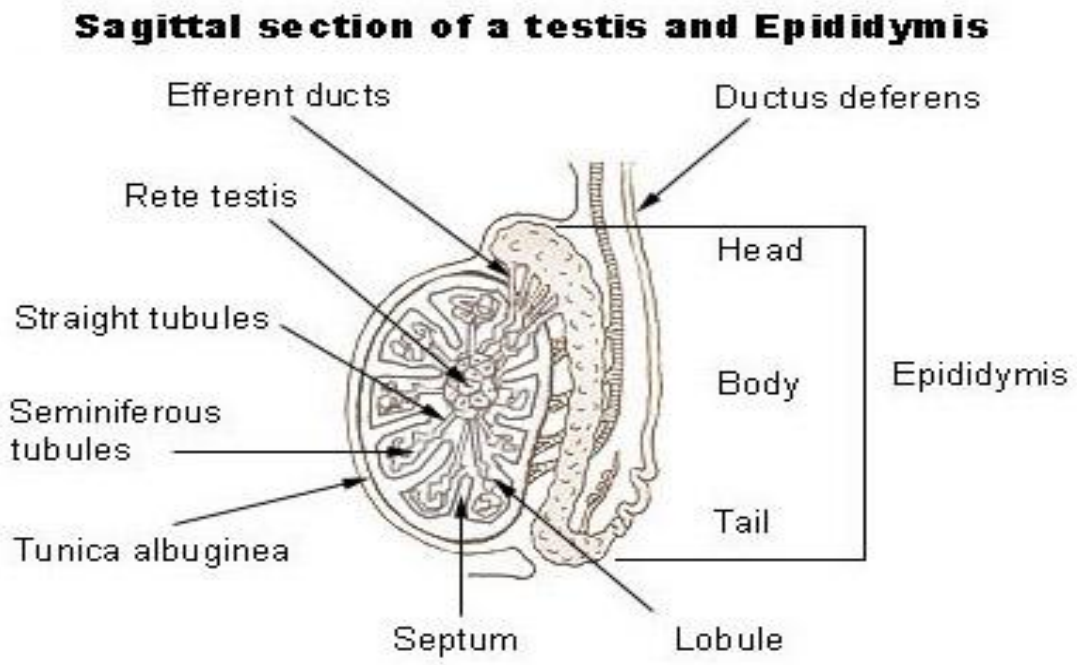


FIG 2.2: sagittal section of testis and epididymis

2.3.1 EMBRYOLOGY OF THE TESTIS

The gonads are derived from three sources: mesothelium (epithelium lining the posterior abdominal wall), underlying mesenchyme (embryonic connective tissue) and primordial germ cells (earliest undifferentiated germ cells), (Moore K.L et al 2016).

Gonads appear initially as a pair of longi-tudinal ridges, the genital or gonadal ridges. They are formed by proliferation of the epithelium and a condensation of underlying mesenchyme. Germ cells do not appear in the genital ridges until the sixth week of development. Primordial germ cells originate in the epiblast, migrate through

h the primitive streak, and by the third week reside among endoderm cells in the wall of the yolk sac close to the allantois. During the fourth week, they migrate by ameboid movement along the dorsal mesentery of the hindgut, arriving at the primitive gonads at the beginning of the fifth week and invading the genital ridges in the sixth week (Saddler, 2015).

2.3.2 HISTOLOGY OF THE TESTIS

Each testis is covered by dense fibrous membrane called the tunica albuginea. In the posterior part of the testis the connective tissue of the tunica albuginea expands into a thick mass that project into the substance of the testis to form the mediastinum testis. Septa pass from the mediastinum testis to the tunica albuginea which divided the substance of the testis into numerous lobules. Each lobule is roughly conical and contains one or more highly convoluted seminiferous tubules. These tubules are lined by cells that are concerned with the production of spermatozoa (Singh I., 2011).

2.3.2.1 INTERSTITIAL CELLS OR LEYDIG CELLS

Within a lobule the spaces between the seminiferous tubules are filled with connective tissue that contains mast cells macrophages, nerves, lymphatics and blood vessels including fenestrated capillaries. During puberty, interstitial cells or Leydig cells develop as large round or polygonal cells with central nuclei and eosinophilic cytoplasm rich in small lipid droplets. These cells produce testosterone which promotes development of the secondary male sex characteristics (Mescher L., 2016).

2.3.2.2 SEMINIFEROUS TUBULES

Each seminiferous tubule is lined with a complex, specialized stratified epithelium called germinal or spermatogenic epithelium. The basement membrane of this epithelium is covered by fibrous connective tissue, with an innermost layer containing flattened, smooth muscle-like myoid cells. The germinal epithelium consists of large non dividing Sertoli cells and dividing cells of spermatogenic lineage (Mescher L., 2016).

Sperm are produced in the seminiferous tubules at a rate of about 2 × 108 per day in the young adult. Each testis has from 250 to 1000 such tubules in its lobules, and each tubule measures 150-250μm in diameter and 30-70cm in length. The combined length of the tubules of one testis totals about 250m (Mescher L., 2016).

2.3.2.3 SERTOLI CELLS

The Sertoli cells are tall columnar epithelial cells that nourish the spermatogenic cells and divides the seminiferous tubules into two (basal and adluminal) compartments. All cells of the

spermatogenic lineage are closely associated with the extended surfaces of Sertoli cells and depend on them for metabolic and physical support. Each Sertoli cells support about 30-50 developing germ cells. Sertoli cells are seen to contain abundant SER, some rough ER, well developed golgi complexes, mitochondria and lysosomes. Their nuclei are typically ovoid or triangular, euchromatic, and have a prominent nucleolus. These features allow Sertoli cells to be differentiated from surrounding germ cells (Mescher L., 2016). The functions of the Sertoli cells include

Support, protection and nutrition of the developing spermatogenic cells (Mescher L., 2016).

Sertoli cells release water that contains new sperm out of the testis into the seminiferous tubules (Mescher L., 2016).

During spermiogenesis, excess cytoplasm shed as residual bodies is phagocytised and digested by Sertoli cell lysosomes (Mescher L., 2016).

2.3.3 SPERMATOGENESIS

Spermatogenesis is the process by which the male gametes (spermatozoa) are formed from the primitive spermatogenic cells (spermatogonia) in the testis. The spermatogenic cells have cytoplasmic attachment with sertoli cells which supply all the necessary materials for spermatogenesis through the cytoplasmic attachment (Sembulingam K., 2012). Spermatogenesis can be divided into four stages;

- Stage of proliferation
- Stage of growth
- Stage of maturation
- Stage of transformation

Stage of Proliferation: Each spermatogonium contains diploid number (23 pairs) of chromosomes. One member of each pair is from maternal origin and the other one from paternal origin (Sembulingam K., 2012). The 23 pairs include 22 pairs of autosomal chromosomes and one pair of sex chromosomes (X or Y chromosome). During the proliferative stage, spermatogonia divide by mitosis, without any change in chromosomal number. There are usually several generations of spermatogonia and the last generation enters the stage of growth as primary spermatocyte. The spermatogonia also migrate along with Sertoli cells towards the lumen of seminiferous tubule (Sembulingam K., 2012).

Stage of Growth: In this stage, the primary spermatocyte grows into a large cell. Apart from growth, there is no other change in spermatocyte during this stage (Sembulingam K., 2012).

Stage of Maturation: After reaching the full size, each primary spermatocyte quickly undergoes meiotic or maturation division, which occurs in two phases: In the first phase, each primary spermatocyte divides into two secondary spermatocytes and receives only haploid number of chromosomes (22 autosomes and an X or a Y chromosome). In the second phase each secondary spermatocyte undergoes second meiotic division, resulting in two haploid cells called spermatids (Sembulingam K., 2012).

Stage of Transformation: There is no further division. Spermatids are transformed into matured spermatozoa (sperms), by means of spermeogenesis and released by spermination (Sembulingam K., 2012).

2.3.3.1 FACTORS AFFECTING SPERMATOGENESIS

Sertoli cells influence spermatogenesis by supporting and nourishing the germ cells, providing hormonal substances necessary for spermatogenesis, secreting androgen-binding protein (ABP), which is essential for testosterone activity, particularly on spermatogenesis. Sertoli cells also release sperms into the lumen of seminiferous tubules (Sembulingam K., 2012).

Hormones also play an important role in spermatogenesis either by acting directly or indirectly. These hormones include follicle-stimulating hormone (FSH), testosterone, estrogen, luteinizing hormone (LH), growth hormone (GH), inhibin, and activin (Sembulingam K., 2012).

Follicule-stimulating hormone initiates spermatogenesis by binding with Sertoli cells and spermatogonia to induce the proliferation of spermatogonia. It also stimulates the formation of estrogen and androgen-binding protein from Sertoli cells (Sembulingam K., 2012).

Testosterone is responsible for the sequence of remaining stages in spermatogenesis. It is also responsible for the maintenance of spermatogenesis. Testosterone activity is largely influenced by androgen-binding protein (Sembulingam K., 2012).

Estrogen is formed from testosterone in Sertoli cells. It is necessary for spermeogenesis (Sembulingam K., 2012).

Luteinizing hormone is essential for the secretion of testosterone from Leydig cells (Sembulingam K., 2012).

Growth hormone is essential for the general metabolic processes in testis. It is also necessary for the proliferation of spermatogonia (Sembulingam K., 2012).

Inhibin is a peptide hormone and serves as a transforming growth factor. It is secreted by Sertoli cells (Sembulingam K., 2012). Its secretion is stimulated by FSH. Inhibin plays an important role in the regulation of spermatogenesis by inhibiting FSH secretion through feedback mechanism. FSH secreted from anterior pituitary induces spermatogenesis by stimulating Sertoli cells (Sembulingam K., 2012). It also stimulates the secretion of inhibin from Sertoli cells. So, when the rate of spermatogenesis increases, there is a simultaneous increase in inhibin secretion also. Inhibin in turn, acts on anterior pituitary and inhibits the secretion of FSH, leading to decrease in the pace of spermatogenesis (Sembulingam K., 2012). It is believed that inhibin also inhibits FSH secretion indirectly by inhibiting GnRH secretion from hypothalamus (Sembulingam K., 2012).

Activin is also a peptide hormone secreted in gonads along with inhibin. The exact location of its secretion in testis is not known. It is suggested that activin is secreted by Sertoli cells and Leydig cells (Sembulingam K., 2012). Activin has opposite actions of inhibin. It increases the secretion of FSH and accelerates spermatogenesis (Sembulingam K., 2012).

2.3.3.2 REGULATION OF TESTOSTERONE SECRETION

Luteinizing hormone (LH) or interstitial cell stimulating hormone (ICSH) stimulates the Leydig cells and the quantity of testosterone secreted is directly proportional to the amount of LH available (Sembulingam K., 2012). Secretion of LH from anterior pituitary gland is stimulated by luteinizing hormone releasing hormone (LHRH) from hypothalamus. Testosterone regulates its own secretion by negative feedback mechanism (Sembulingam K., 2012). It acts on hypothalamus and inhibits the secretion of LHRH. When LHRH secretion is inhibited, LH is not released from anterior pituitary, resulting in stoppage of testosterone secretion from testes (Sembulingam K., 2012). On the other hand, when testosterone production is low, lack of inhibition of hypothalamus leads to secretion of testosterone through LHRH and LH (Sembulingam K., 2012).

2.3.3.3 SPERMEOGENESIS

Spermeogenesis is the process by which spermatids become matured spermatozoa (Sembulingam K., 2012). Changes taking place during spermeogenesis:

1. Condensation of nuclear material
2. Formation of acrosome, mitochondrial spiral filament and tail structures
3. Removal of extraneous (extra volume of nonessential) cytoplasm (Sembulingam K., 2012).

2.3.4 GROSS ANATOMY OF THE TESTIS

The testes are the male gonads homologous to the ovaries in the female. It is a paired ovoid/ellipsoidal reproductive organ that is responsible for the production of spermatozoa and secretion of testosterone (Singh V., 2014). It weigh about 10-15g and measure approximately 4cm in length, 2.5cm in breath, and 3cm in anteroposterior diameter. In rats the general structure of the male reproductive organs is very similar to that of human.

2.3.4.1 EXTERNAL FEATURES

The external features of the testes include: two poles (upper and lower), two borders (anterior and posterior) and two surfaces (medial and lateral) (Singh V., 2014).

Both poles are convex and smooth and the upper pole provides attachment to the spermatic cord. The appendix of the testis is a small oval body often found attached to the upper pole of the testis (Singh V., 2014).

The anterior border is round and completely covered by tunica vaginalis while the posterior border is straight, partially covered by tunica vaginalis and provides attachment for the epididymis (Singh V., 2014). The sinus of the epididymis is an extension of the cavity of the tunica vaginalis and separates the epididymis from the testis on the lateral aspect (Singh V., 2014).

Both surfaces are smooth and slightly convex. (Singh V., 2014).

2.3.4.2 COVERINGS OF THE TESTIS

Each testis is enclosed by three coverings.

Tunica Vaginalis

The tunica vaginalis is the most superficial covering of the testis. It is a serous sac that represents the persistent lower portion of the processus vaginalis. It is invaginated by the testis from behind and as a result presents outer parietal and inner visceral layers with a potential cavity between them (Singh V., 2014). The outer parietal layer lines the scrotum and the inner visceral layer covers the tunica albuginea on the anterior and lateral sides of the testis (Mescher L., 2016).

Tunica Albuginea

It is a deep, dense layer of fibrous tissue enclosing the testis. It is covered by tunica vaginalis except superiorly and posteriorly where it is in direct contact with the epididymis (Singh V., 2014). The tunica albuginea is thickened on the posterior side of the testis to form the mediastinum testis (Mescher, 2016).

Tunica Vasculosa

It is the innermost vascular layer of the testis, lining the lobules of the testis (Singh V., 2014).

2.3.4.3 ARTERIAL SUPPLY

The arterial supply of the testis and epididymis is derived from the testicular artery which originates from the abdominal aorta and descends into the scrotum through the inguinal canal to supply the testis (Drake R.L, Vogl A., Mitchell W.M., 2015). At the posterior border of the testis, it divides into a number of small branches and two large branches (medial and lateral) branches. The medial and lateral branches pierce the tunica albuginea and ramify on the surface of the lobules of the testis to form the tunica vasculosa (Singh V., 2014).

2.3.4.4 VENOUS DRAINAGE

Venous blood from the testis is drained by the pampiniform plexus of veins which is made up of veins emerging from the testis. This plexus ascends up and at the superficial inguinal ring condenses to form four veins, which pass through the inguinal canal within the spermatic cord. At the level of the deep inguinal ring, they join to form two testicular veins which accompany the testicular artery. On the right side the testicular vein drains into the inferior vena cava at an oblique angle while on the left it drains into the left renal vein at a right angle (Singh V., 2014).

2.3.4.5 LYMPHATIC DRAINAGE

The lymph vessels from the testis drain into the pre-aortic and para-aortic group of lymph nodes at the level of the second lumbar vertebra (Singh V., 2014).

2.3.5 THE EPIDIDYMIS

2.3.5.1 GROSS ANATOMY OF THE EPIDIDYMIS

The epididymis courses along the posterolateral side of the testis. It has two distinct components:
The efferent ductules, which forms the head of the epididymis; and
the true epididymis, which is a single, long coiled duct into which the efferent ductules all drain, and continues as the body of the epididymis at the posterolateral margin of the testis and enlarges to form the tail of the epididymis at the inferior pole of the testis. The end of the epididymis is continuous with the ductus deferens (Drake R.L, Vogl A., Mitchell W.M., 2015).
The functions of epididymis includes:
Storage and maturation of spermatozoa.
Absorption of the fluid.
Addition of substances to the seminal fluid to nourish the maturating spermatozoa. (Singh V., 2014).

2.3.5.2 HISTOLOGY OF THE EPIDIDYMIS

The epididymis is about 4-5m in length and consists of a head region where the efferent ductules enter, a body and a tail entering into the ductus deferens (Mescher L. 2016). The head is formed by highly convoluted continuations of the efferent ductules lined by ciliated columnar epithelium. The body and tail of the epididymis are made up of the duct of the epididymis, greatly coiled on itself (Singh I., 2011). The epididymal duct is lined by pseudostratified columnar epithelium consisting of columnar principal cells with characteristic long sterocilia with small round stem cells. The duct epithelium is surrounded by a few layers of smooth muscle cells, arranged as inner and outer longitudinal layers as well as a circular layer in the tail of the epididymis (Mescher, 2016). Fluid within the epididymis contains glycolipid “decapacitation factors” that bind the plasma membranes of sperm and block acrosomal reactions and fertilizing ability until these factors are removed as part of the capacitation process in the female reproductive tract (Mescher, 2016).

2.3.5.3 EMBRYOLOGY OF THE EPIDIDYMIS

Genital ducts in the male are stimulated to develop by testosterone and are derived from parts of the mesonephric kidney system (Saddler T.W 2015). Testosterone stimulates the mesonephric ducts to form male genital ducts, whereas antimullerian hormone (AMH) causes the paramesonephric ducts to regress. Under the influence of testosterone produced by the fetal testes in the eighth week, the proximal part of each mesonephric duct becomes highly convoluted to form the epididymis. As the mesonephros degenerates, some mesonephric tubules persist and are transformed into efferent ductules. These ductules open into the duct of the epididymis (Moore K.L et al 2016).

CHAPTER THREE

MATERIALS AND METHODS\

Animals: 20 Adult Male Wistar Rats

Extract: Aqueous extract of *Hibiscus sabdariffa*

Feed: Growers mash

Instruments: weighing balance, microtome, surgical blades, forceps, 5ml syringe, microscope, specimen bottles, cotton wool, orogastric tube, disposable gloves, tissue embedding station, laptop,

Reagents: 10% formal saline, chloroform, distilled water, eosin, hematoxylin, paraffin wax, xylene.

3.1 ANIMAL CARE AND MANAGEMENT

Twenty male adult Wistar rats weighing were used for this experiment. The animals were obtained and bred from the animal house of the department of Anatomy, University of Benin, Benin City. The animals were allowed to acclimatize for 1 week and were fed with grower mash manufactured by top feed limited and were allowed access to water.

3.2 PREPARATION OF PLANT EXTRACT

Zobo flowers was purchased in Uselu market in Egor local government area of Edo state, Nigeria. lt was identified as *Hibiscus sabdariffa* in Department of Plant Biology and Biotechnology (PBB) in University of Benin, Benin city. It was air dried for two weeks and pulverized using British grinding machine in pharmacognosy department in faculty of pharmacy University of Benin .The fine powder of the *Hibiscus sabdariffa* was weighed and soaked with distilled water for 24 hrs .after 24hrs it was filtered and the residue was discarded then the filterate was poured into a container .water bath was used to concentrate the filterate at 45°c .

3.3 EXPERIMENTAL DESIGN

The experimental animals were divided into four groups; A, B, C, D, of five rats each. The experimental periods lasted for 60 days. The rats were administered with lead acetate, Aqueous *Hibiscus sabdariffa*

GROUPS	DESCRIPTION
A	CONTROL
B	0.1ml of lead acetate
C	Dose of *Hibiscus sabdariffa* extract, 500mg/kg
D	0.1ml of lead acetate plus *Hibiscus sabdariffa* extract, 500mg/kg

Table 3.1 Showing the groups of experimental animals and their treatment

Group A; Animals served as control, given only feed and water.

Group B; Animals were administered lead acetate daily for 60 days.

Group C; Animals were administered *Hibiscus sabdariffa* extract 500mg/kg for 60 days.

Group D; Animals were administered lead acetate and *Hibiscus sabdariffa* Aqueous extract 500mg/kg for 60 days.

3.4 METHOD OF SACRIFICE AND TISSUE COLLECTION

3.4.1Method of sacrifice

During sacrifice, the final weight of the rats was taken using compact electric weighing scale. After taking the final weights, the rat was put into an enclosed container with Cotton wool and about 50ml of chloroform for anaesthesizing, after about 2-3 min of the rat inside the enclosed container it was brought out and placed on a dissection table in supine position. Abdominal thoracic incision was made with dissection scissors and surgical blade to expose the abdominal visceral. Thread was used to litigate the two sides of vas deferens to be able to collect semen for sperm analysis. Epididymis and testes were harvested for histological analysis

3.4.2 HISTOLOGICAL TECHNIQUE

Paraffin Embedding

1. The testes and epididymis were fixed in 10% formal saline. They were dehydrated by subjecting to ascending grades of alcohol 70%,90%,95% and absolute alcohol for one hour
2. The tissues containing alcohol were cleared in xylene for one hour

3. The tissues were infiltrated with molten paraffin wax for 1 hour at 30'C -60'C
4. They were embedded in molten paraffin wax and allowed to solidify in plastic cassette
5. The resulting blocks of tissues were sectioned at five microns with the aid of a rotary microtome.

3.4.3 HEMATOXYLIN AND EOSIN STAINING METHOD

The sections of tissues were placed in 40% alcohol and allowed to float in a water bath vat at temperature of 50°C-55°C.

The sections were picked from the water bath with albumin greased slides and allowed to dry the tissues were dewaxed in xylene for 2 minutes

They were passed through descending grades of alcohol. Absolute alcohol for 2 minutes, 95%,90%, and 70% for one minute each and then rinsed in water

The tissues were stained in hematoxylin for 30 minutes

Tissues were rinsed in running water for 10 minutes

The sections were stained in eosin for 2-3 minutes, then rinsed in water for 30 seconds

The sections were passed through ascending grades of alcohol 70%, 90%, 95% for 30 seconds and in absolute alcohol for 2 seconds

The sections were immersed in xylene for 1 minute

The sections were mounted in Distrene plasticizer and xylene (DPX), covered with coverslip. Sections were viewed under a microscope.

3.5 PHOTOMICROGRAPHY

The sections were obtained and examined under Leica DM750 research microscope with a digital camera (LeicaCC50) attached. Digital PHOTOMICROGRAPHY of the tissue sections was taken at ×40, ×100, ×400 magnifications

3.6 STATISTICAL ANALYSIS

All data collected was presented as graph tables and figures also as mean $\pm$ standard deviation of the mean (SDM) of control and experimental groups. The data was subjected to analysis of variance (ANOVA) using statistical package for social sciences, (SPSS), version 17. A value of $p< 0.05$ was taken as significant

CHAPTER FOUR
RESULTS

The following results below were obtained after a period of administration of lead acetate, *Hibiscus sabdariffa*, feed and water to four different groups of rats

Table 4.1; shows the comparison between initial and final body weights across the groups

Table 4.2; shows the Total sperm cell count: $x10^6$cells/mm^3, Motility (Progressive, Non progressive and Immotile) = % percentage, Morphology (normal and Abnormal) = % Percentage across the group

Table 4.3; shows the organ weight and gonadosomatic index across the groups

Plate 1-16; Photomicrography showing the studies on the histology of the testes and epididymis

Figure 1-4; shows changes in sperm morphology across the groups.

TABLE 4.1; shows the comparison between initial and final body weights across the groups

GROUPS	**INITIAL BODY WEIGHT**	**FINAL BODY WEIGHT**	**P-VALUE**
Control	154.67±12.67	180.00±21.66	0.532
Lead only	158.33±1.33	213.33±9.35*	0.032
H. sabdariffa only	165.00±8.54	214.33±11.57*	0.009
H. sabdariffa + Lead	171.67±6.33	206.33±4.41*	0.006

*Significantly different from the initial body weight

TABLE 4.2; shows the Total sperm cell count: x10^6cells/mm^3, Motility (Progressive, Non progressive and Immotile) = % percentage, Morphology (normal and Abnormal) = % Percentage

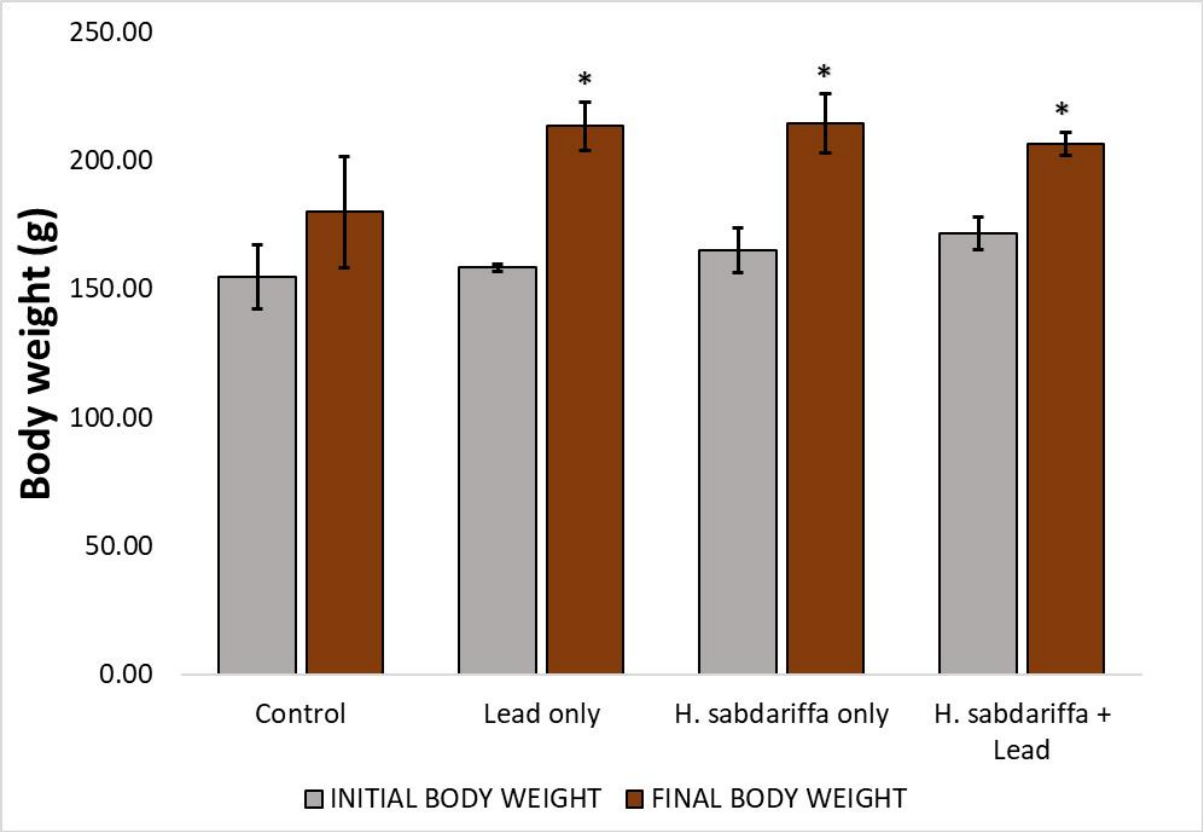
	Control	**Lead only**	***H. sabdariffa* only**	***H. sabdariffa* + Lead**	**F**	**P-value**
Total sperm count (x10^6cells/mm^3)	510.00±5.77	413.33±5.77*	512.50±11.09	390.00±12.91*	23.549	0.000
Progressive motility (%)	72.50±2.50	66.67±3.33	72.50±2.50	70.00±10.00	0.545	0.664
Non-progressive motility (%)	12.50±2.50	10.00±0.00	10.00±0.00	15.00±5.00	1.062	0.412
Immotile sperm cells (%)	15.00±2.89	23.33±3.33	17.50±2.50	57.50±24.62*	2.272	0.137
Normal sperm cells (%)	90.00±0.00	80.00±0.00*	78.75±3.15*	78.75±3.15*	5.442	0.015
Abnormal sperm cells (%)	10.00±0.00	20.00±0.00*	21.25±3.15*	21.25±3.15*	5.442	0.015

* Significantly different from the control group

TABLE 4.3; shows the organ weight and gonadosomatic index across the groups

	CONTROL	**Lead only**	**H. sabdariffa only**	**H. sabdariffa + Lead**	**F**	**P-value**
Testicular weight (g)	1.29±0.06	1.39±0.04	1.42±0.06	1.34±0.09	0.724	0.559
Gonadosomatic index (%)	0.75±0.05	0.65±0.05	0.65±0.06	0.68±0.03	0.898	0.483

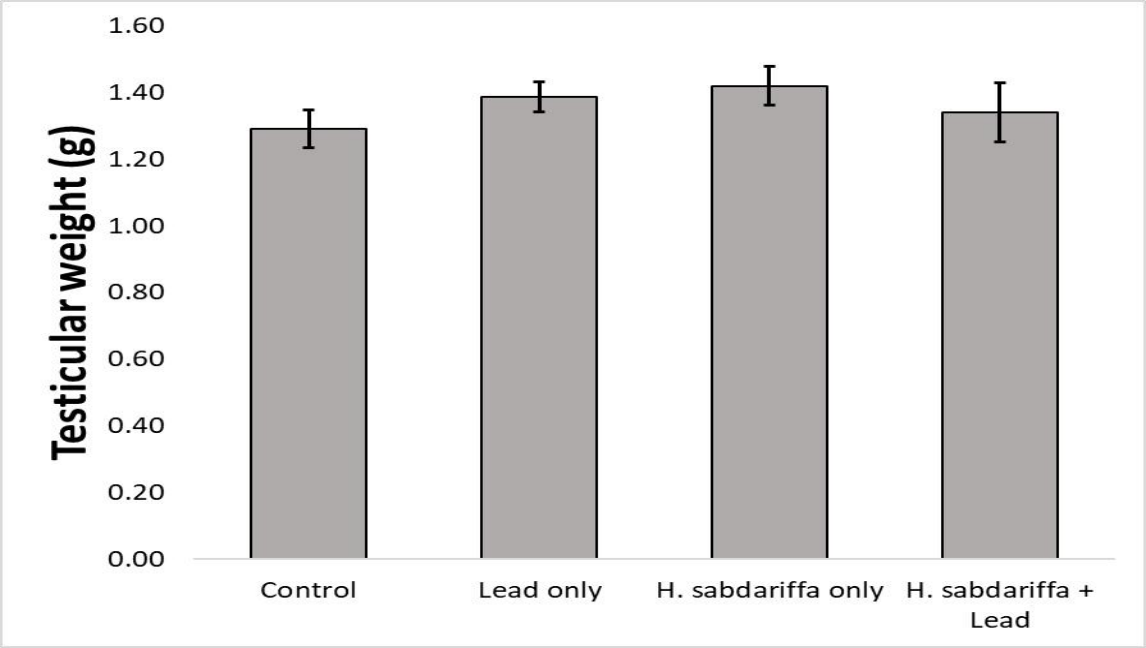
Chart showing the initial body weight in comparison to the final body weight.



*Significantly different from the initial body weight

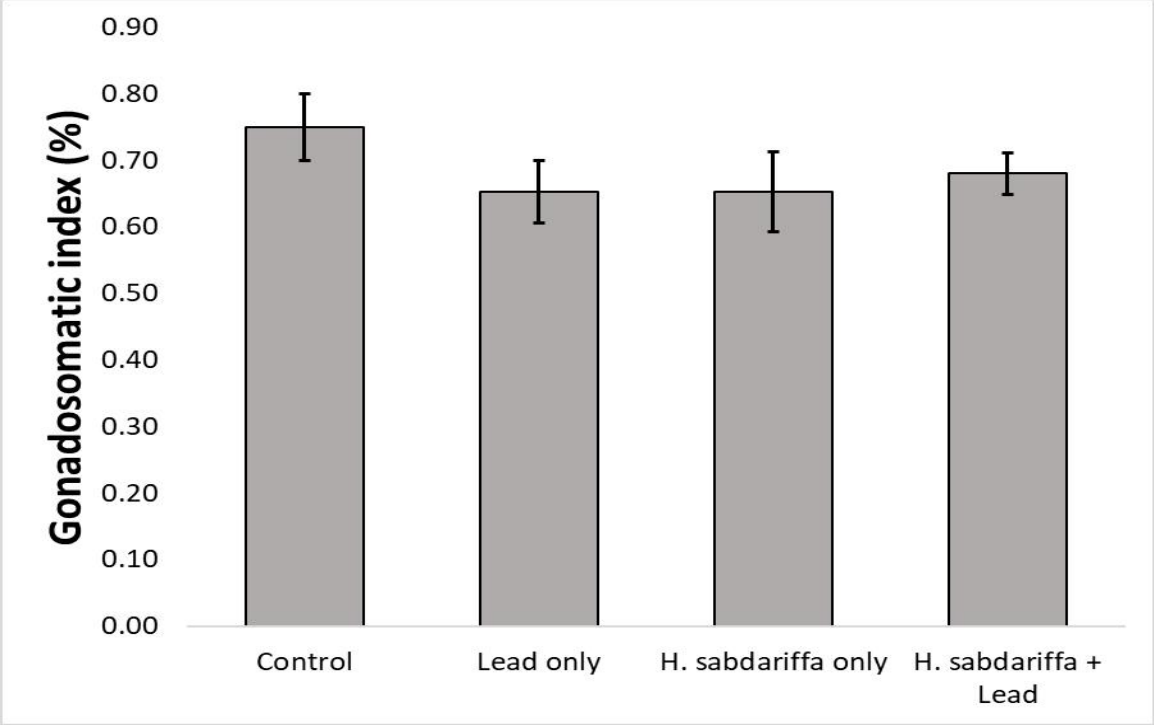
There were statistically significant increases ($P<0.05$) of body weights in groups B, C and D, when the initial body weights were compared to the final body weights.

Chart showing testicular weight



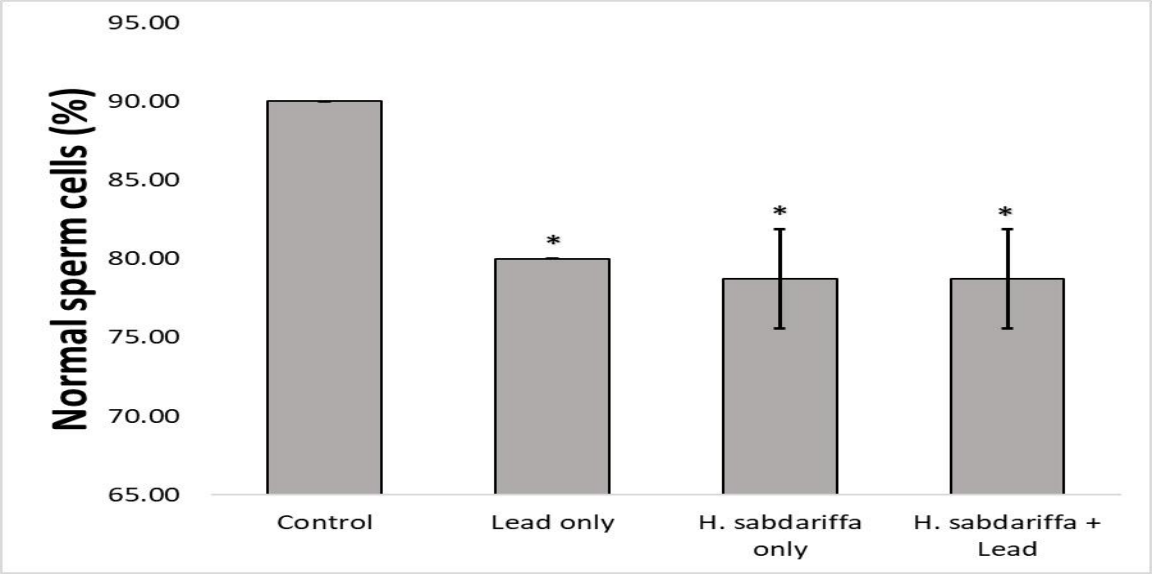
There were no statistically significant differences (P>0.05) in testicular weight across the groups.

Chart showing gonadosomatic index



There were no statistically significant differences (P>0.05) in gonadosomatic index across the groups.

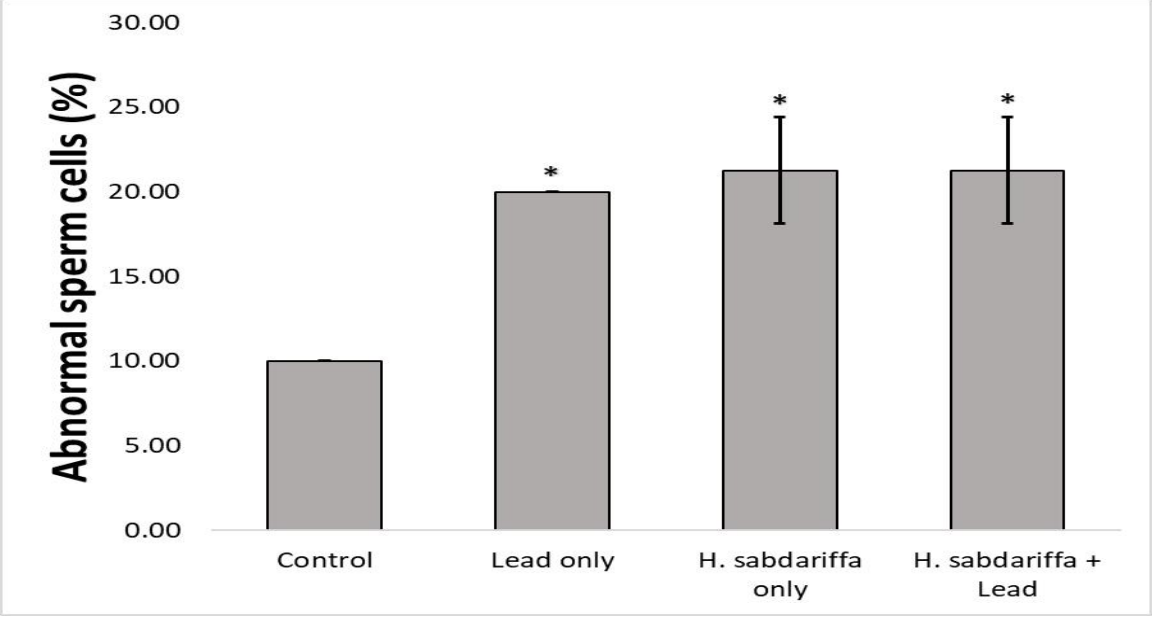
Chart showing percentage of normal sperm cells



*significantly different from the control group

There were statistically significant decreases ($P<0.05$) of normal sperm cells in all the groups, when compared to the control group.

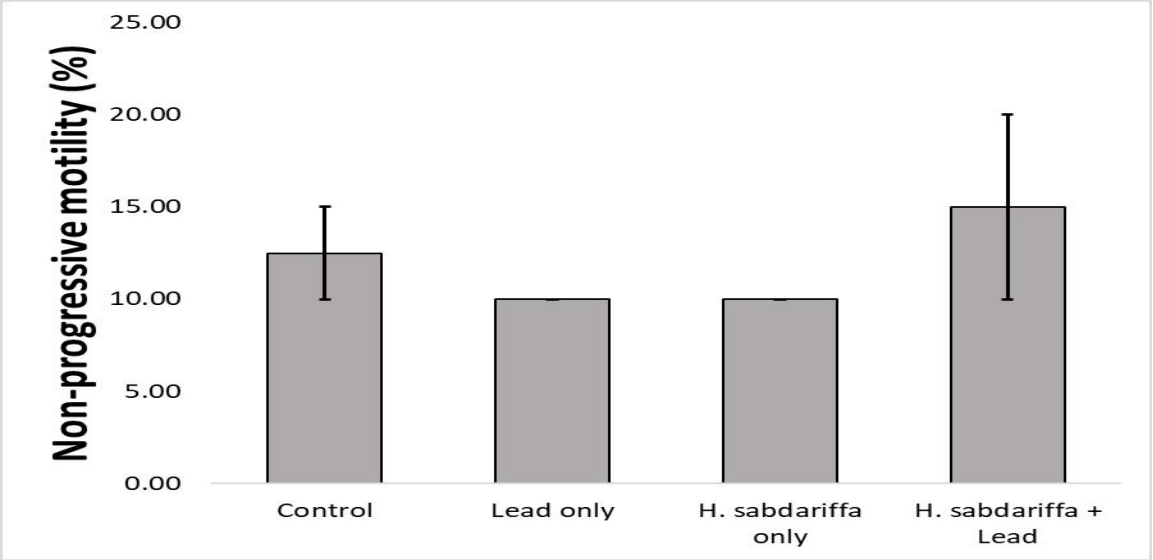
Chart showing percentage of abnormal sperm cells



*significantly different from the control group

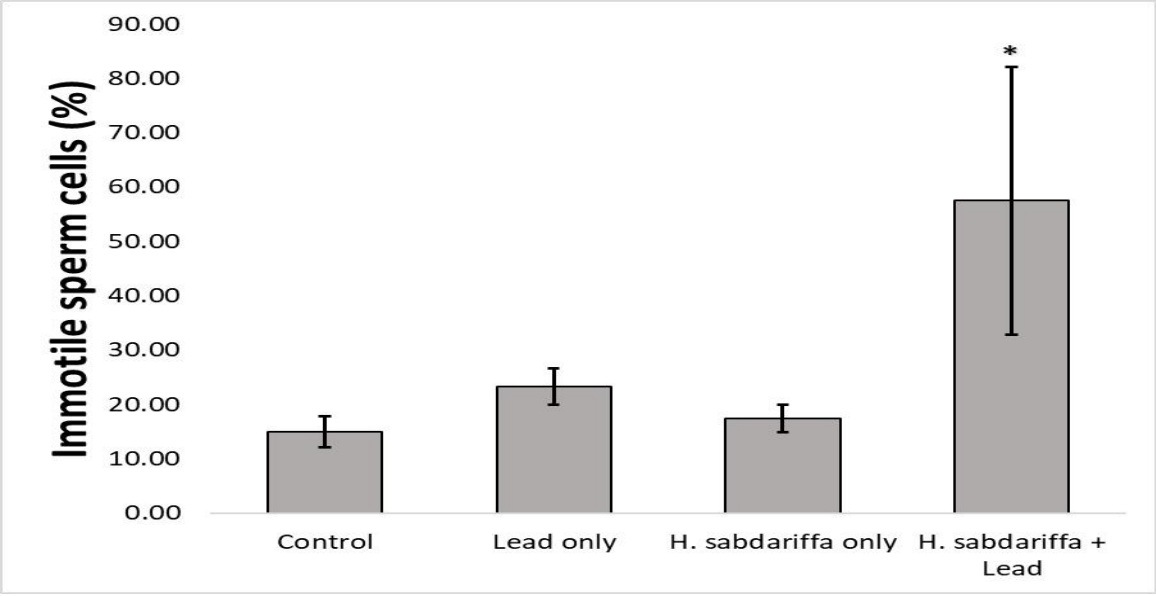
There were statistically significant increases ($P<0.05$) of abnormal sperm cells in all the groups, when compared to the control group.

Chart showing percentage of non-progressive motility



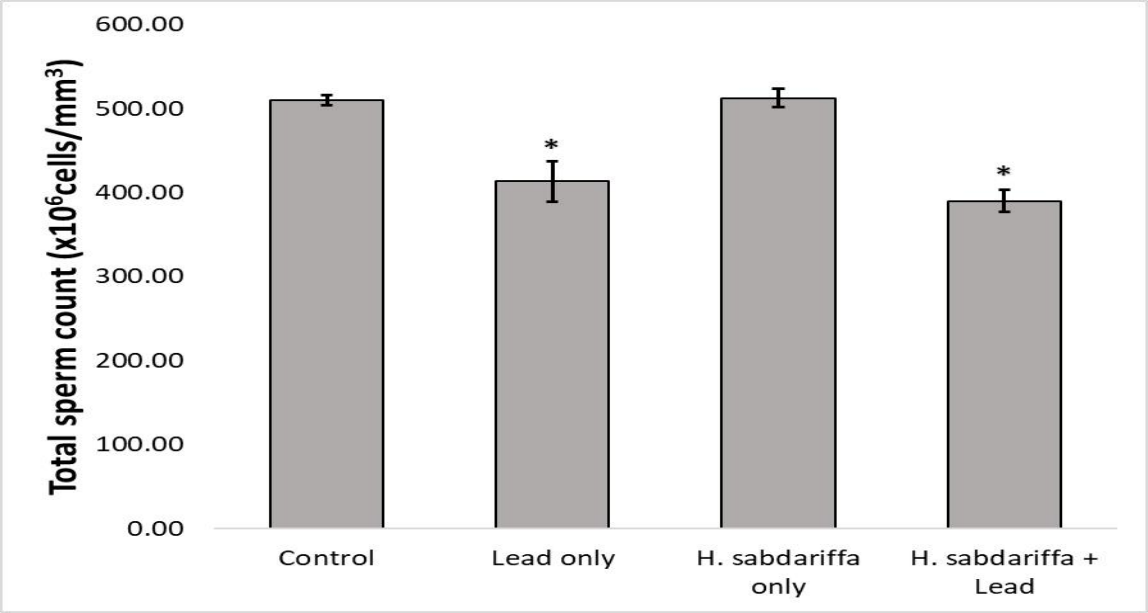
There were no statistically significant differences (P>0.05) in non-progressive motility across the groups

Chart showing percentage of immotile sperm cells



There was a statistically significant increase (P<0.05) of immotile sperm cells in Group D, when compared to the control group.

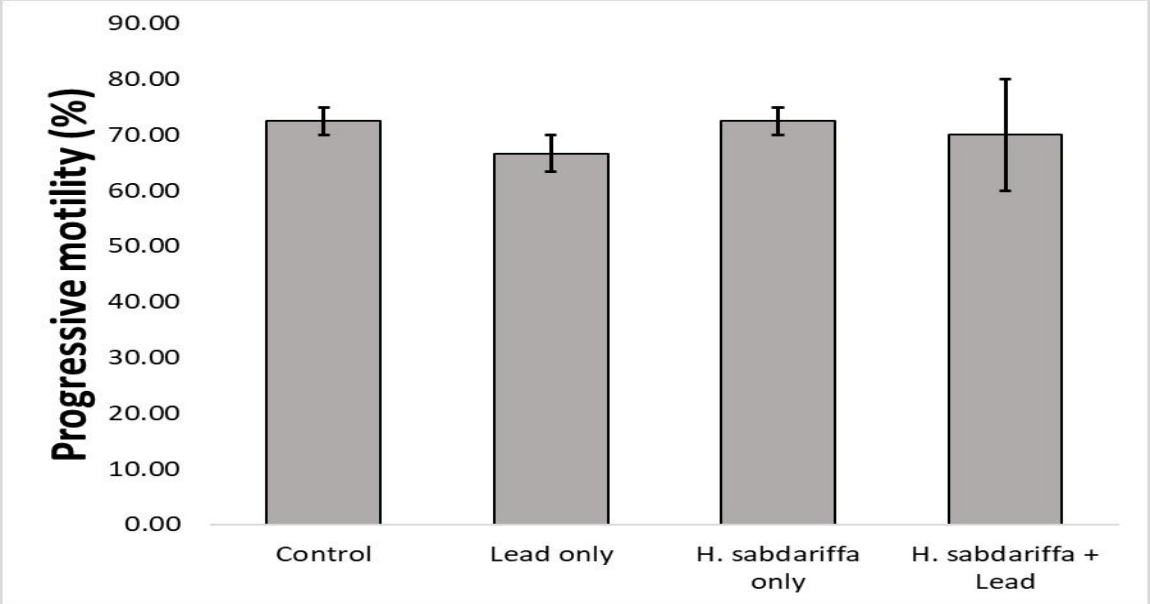
Chart showing total sperm count



*significantly different from the control group

There were statistically significant decreases ($P<0.05$) of total sperm count in groups B and D, when compared to the control group.

Chart showing percentage of progressive motility



There were no statistically significant differences ($P>0.05$) in progressive motility across the groups.

PHOTOMICROGRAPHS

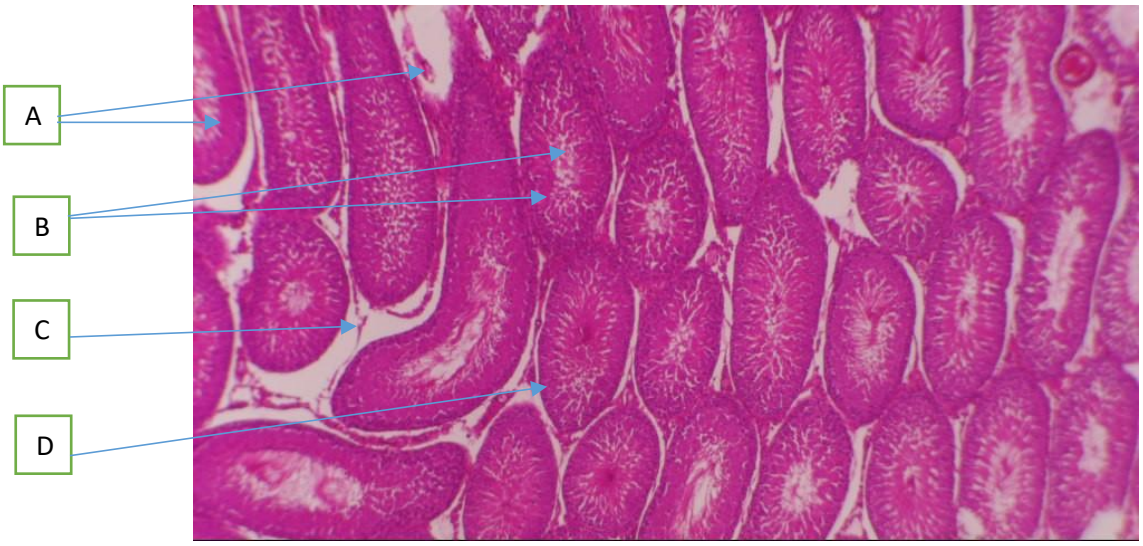


Plate 1. Testis. Control. A: seminiferous tubules lined by B, developing spermatocytes, C, interstitial cells of leydig and D, sertoli cells (H&E x 40)

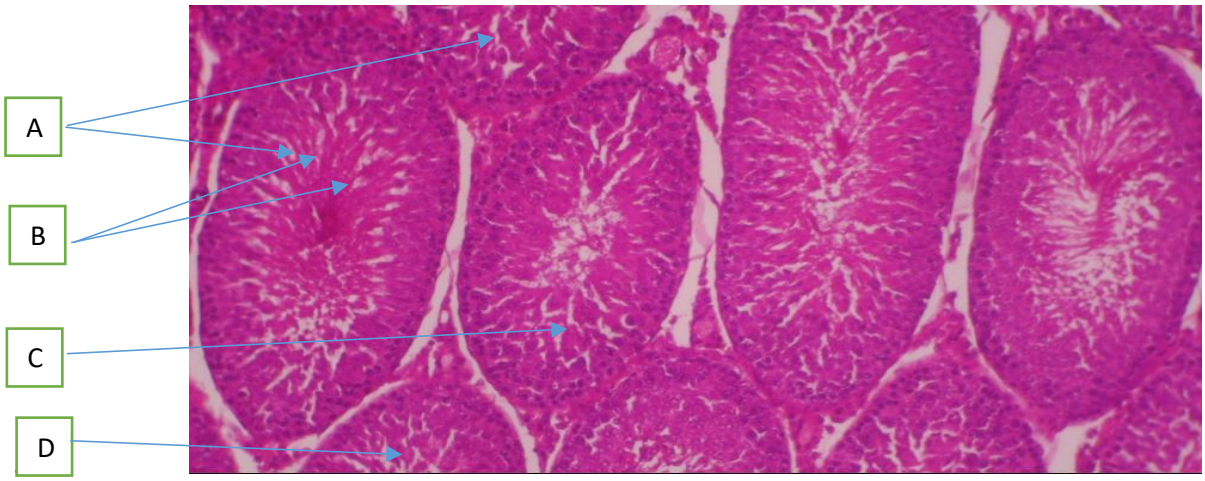


Plate 2. Higher magnification of the above: A, B, C, D (H&E x 100)

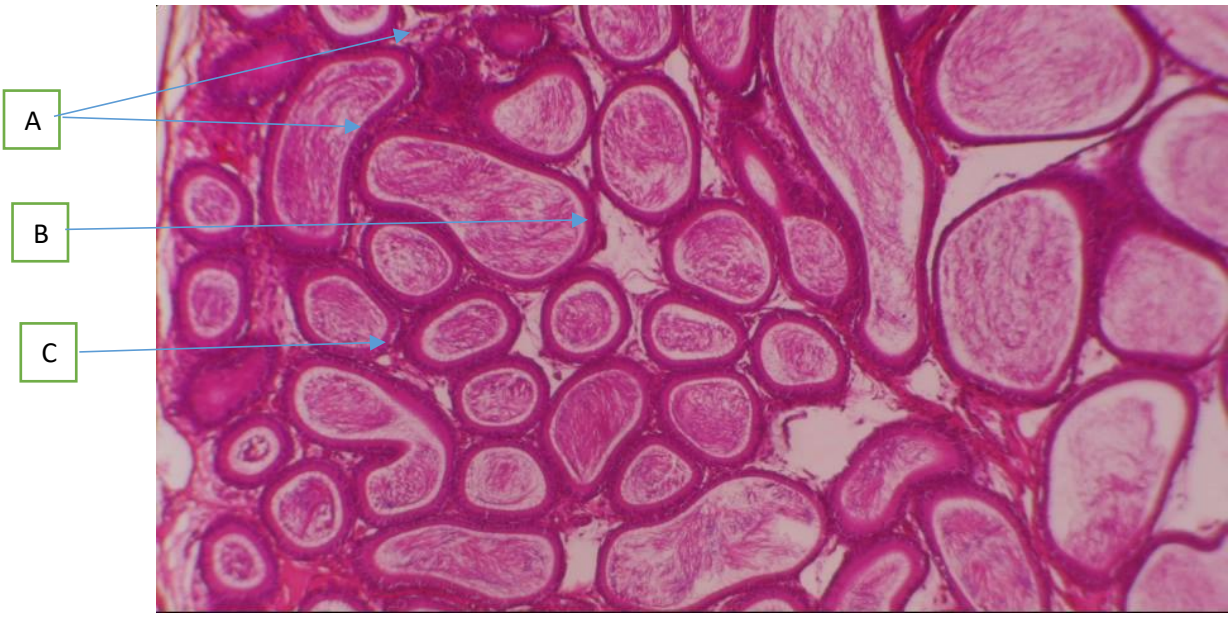


Plate 3. Epididymis. Control. A: ducts lined by low columnar epithelium, surrounding B, a lumen packed with spermatozoa and C, interstitial space (H&E x 40)

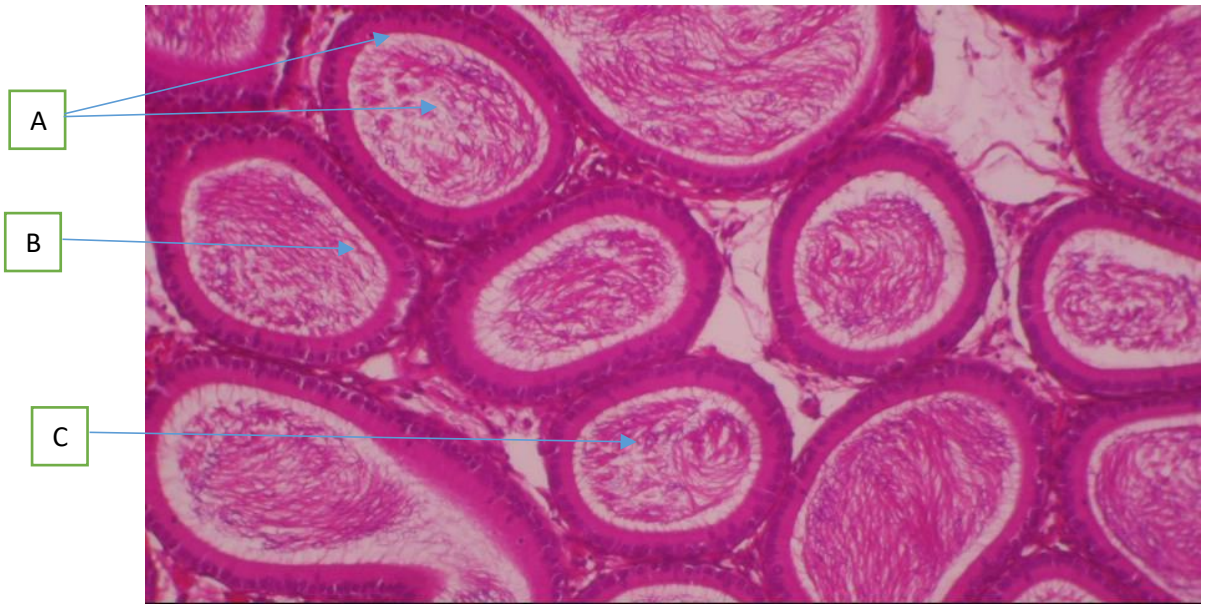


Plate 4. Higher magnification of the above: A, B, C (H&E x 100)

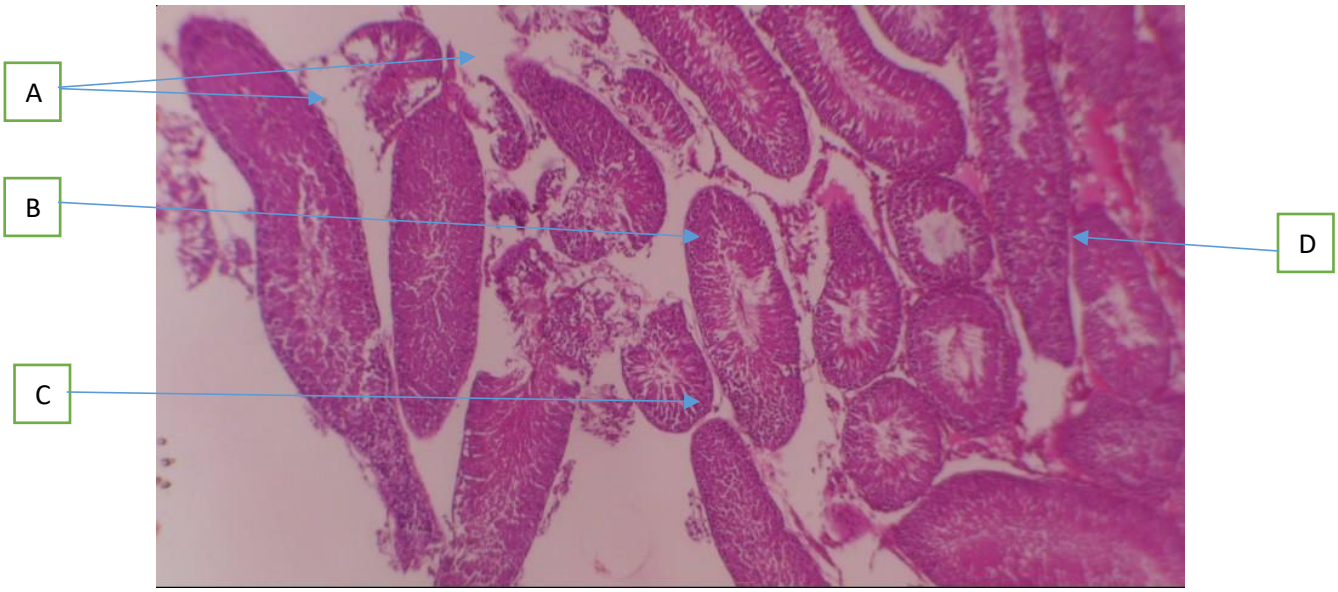


Plate 5. Testis of rat given Pb only showing A, distorted tubules, B, degenerating spermatocytes, C, degenerating leydig cells and D, patchy maturation arrest (H&E x 40)

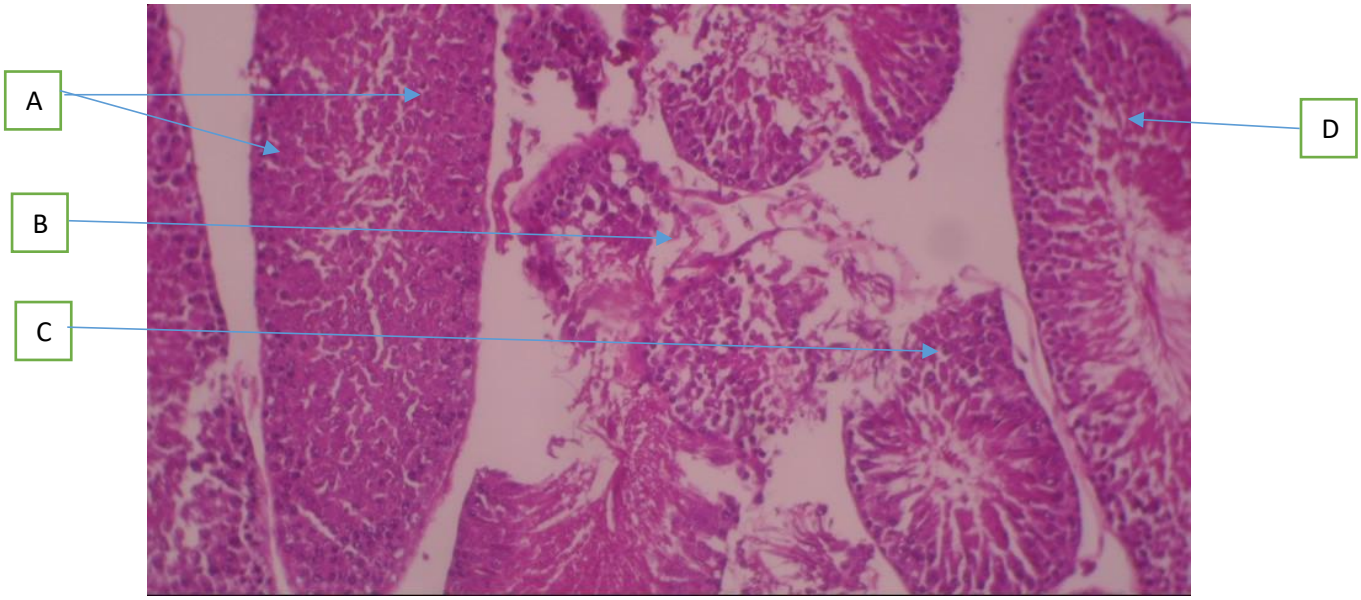


Plate 6. Higher magnification of the above: A, B, C & D (H&E x 100)

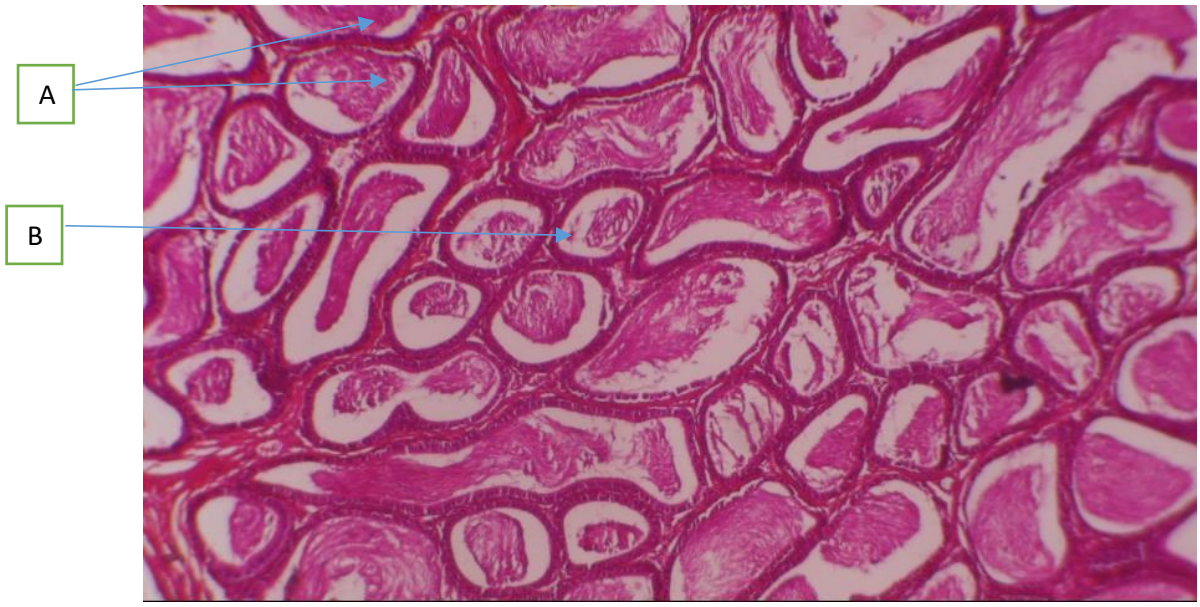


Plate 7. Epididymis of rat given Pb only showing A, ducts with B, reduction in the population of spermatozoa in the lumen (H&E x 40)

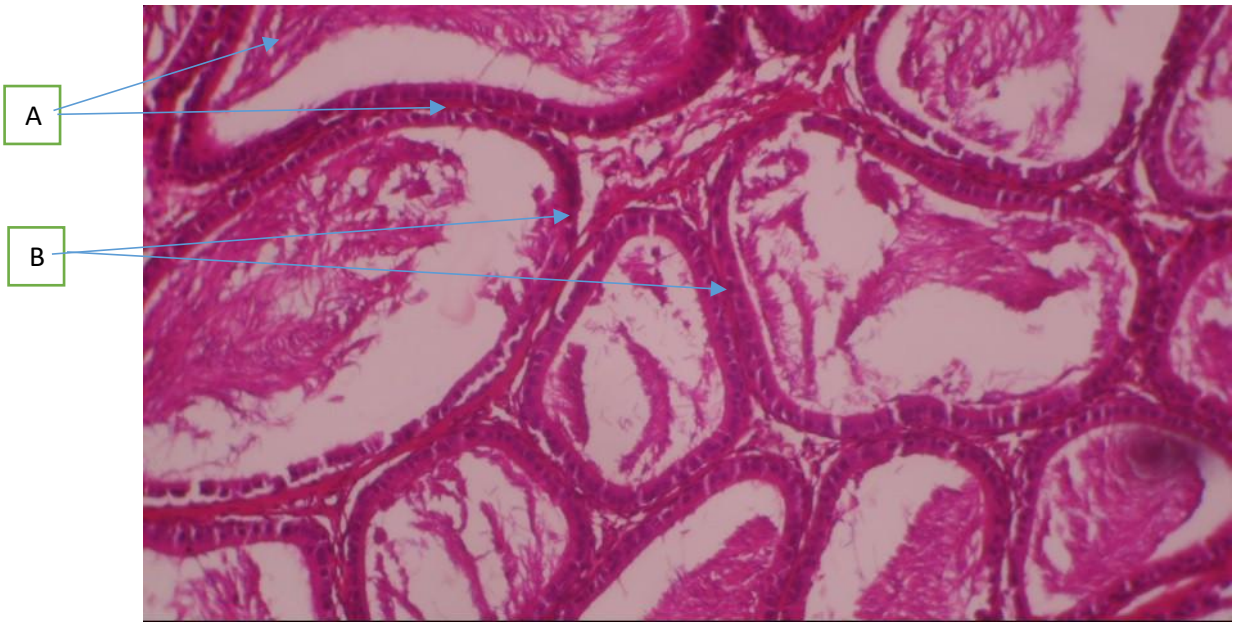


Plate 8. Higher magnification of the above: A, B (H&E x 100)

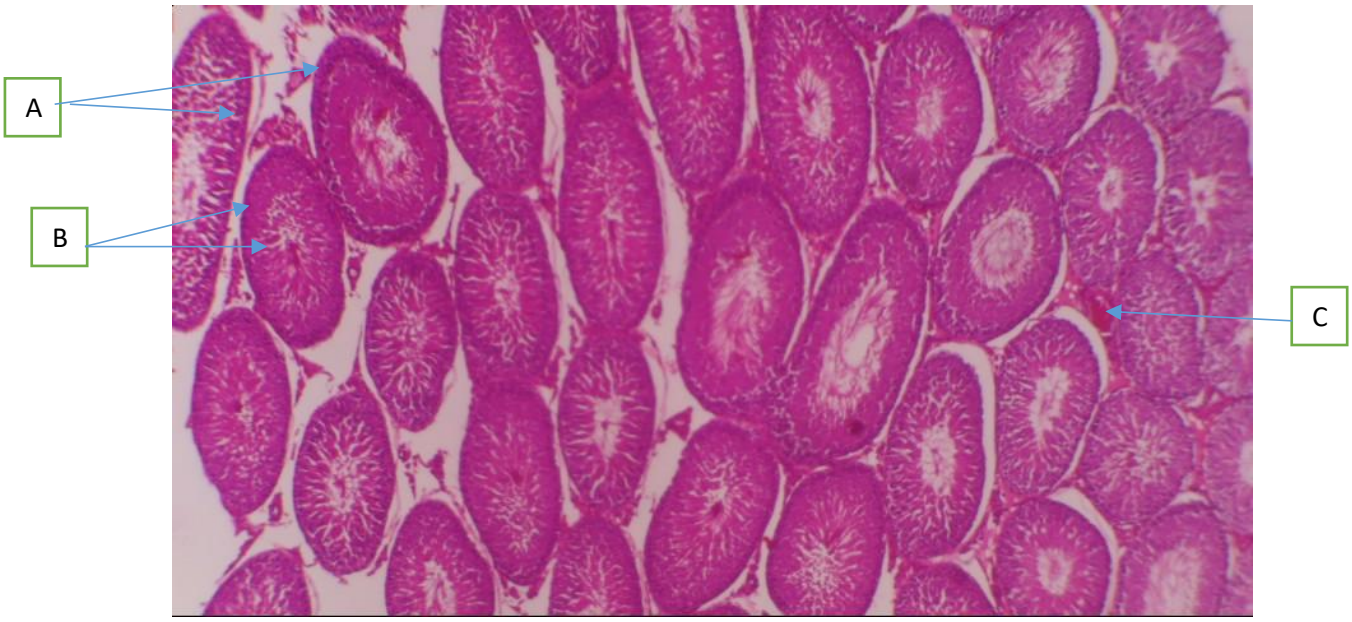


Plate 9. Testis of rat given extract only. A, normal tubules lined by B, normal developing spermatocytes and C, active interstitial congestion (H&E x 40)

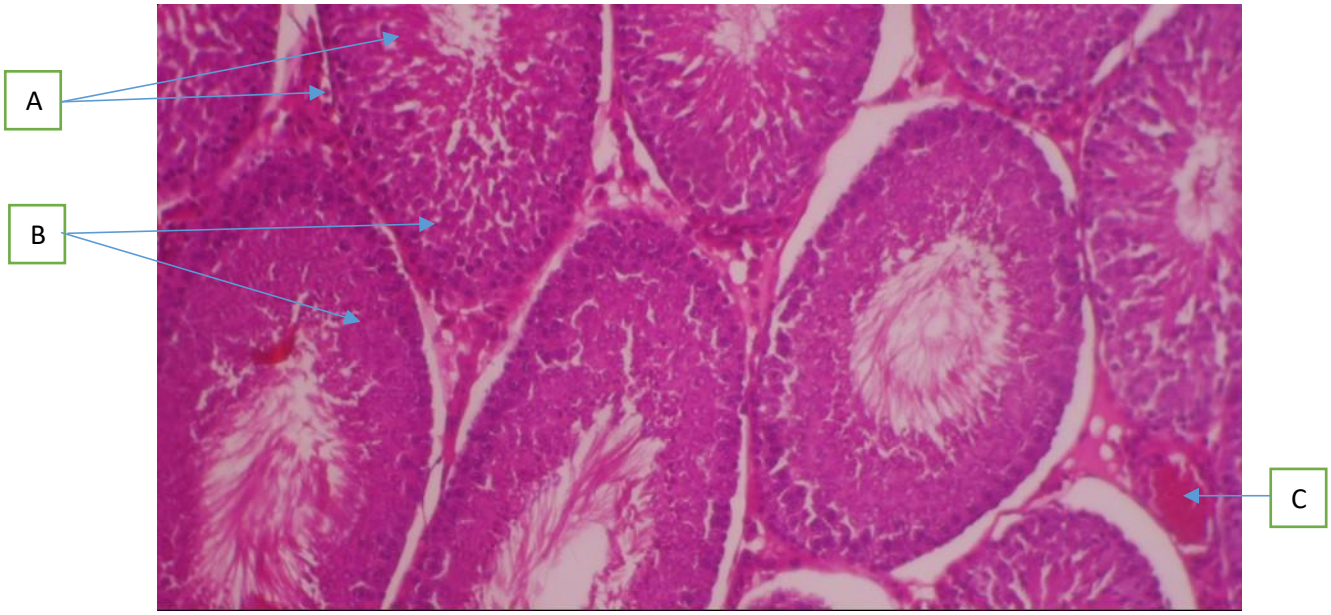


Plate10. Higher magnification of the above: A, B & C (H&E x 100)

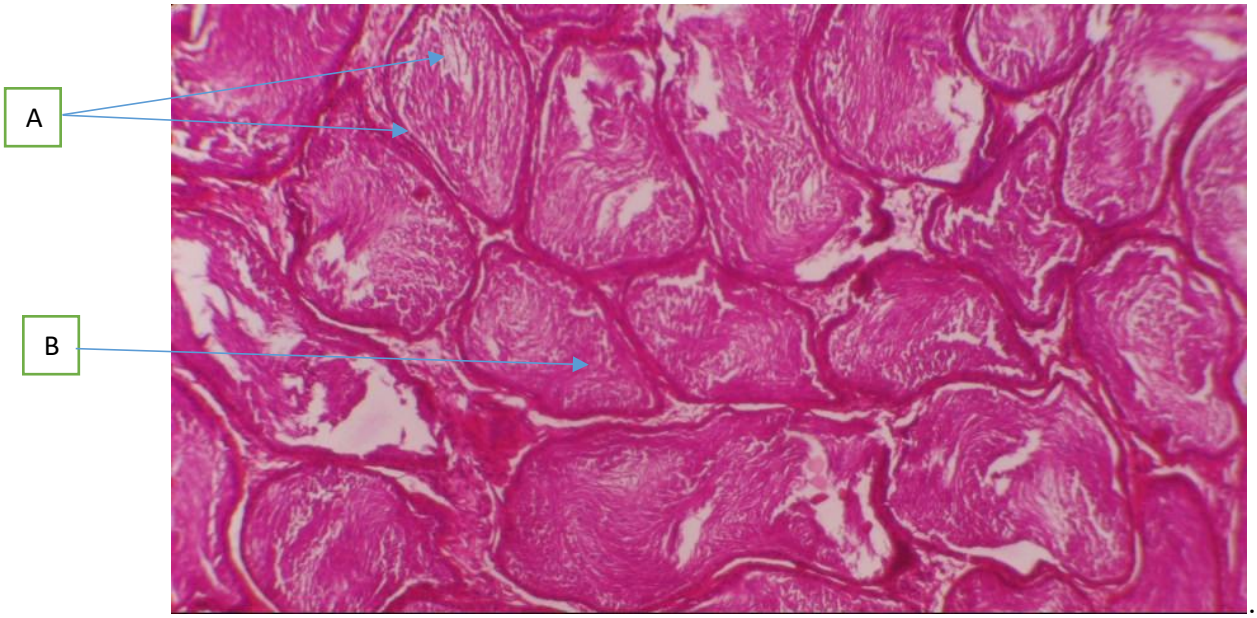


Plate 11Epididymis of rat given Extract only. A, normal ducts packed with B, spermatozoa (H&E x 40)

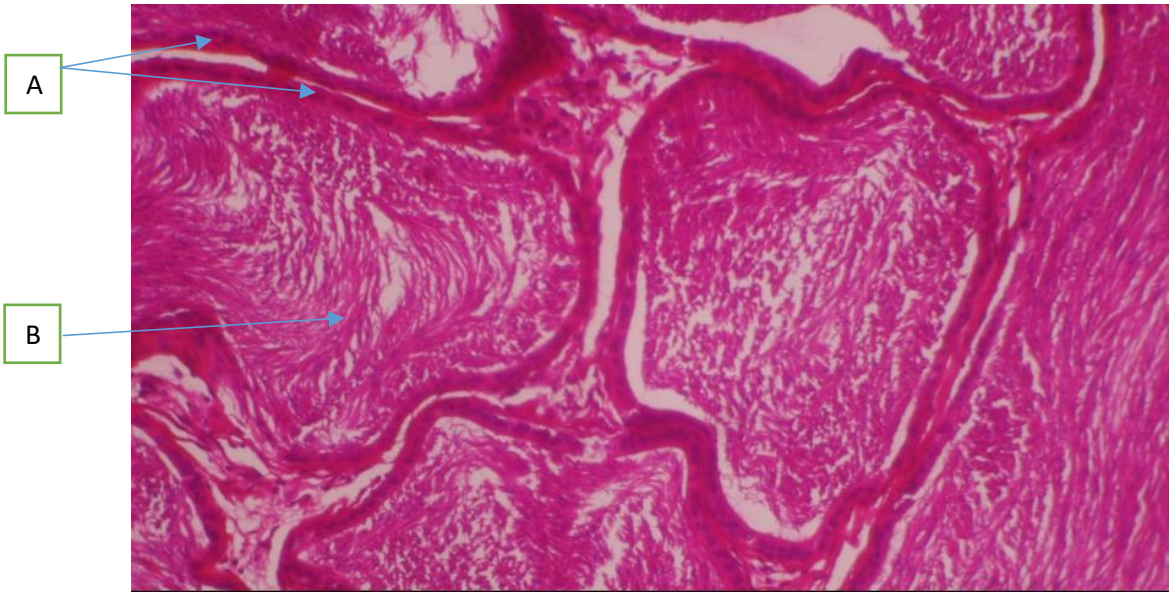


Plate 12. Higher magnification of the above: A, B (H&E x 100)

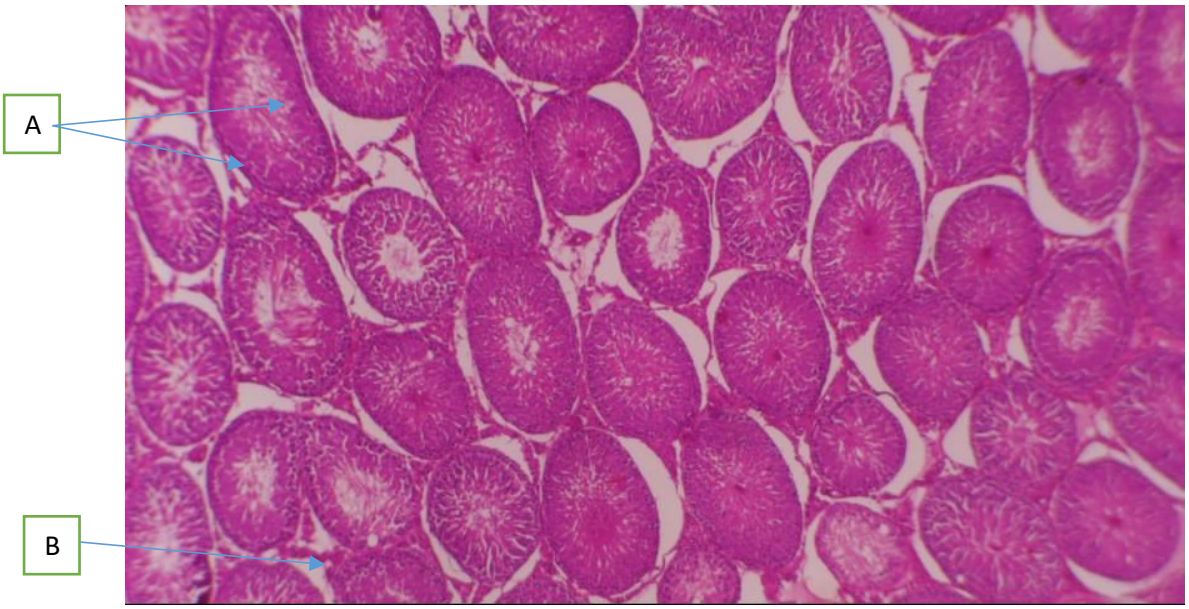


Plate 13. Testis of rat given Extract + Pb showing A, tubules lined by normal maturing spermatocytes and B, leydig cell hyperplasia (H&E x 40)

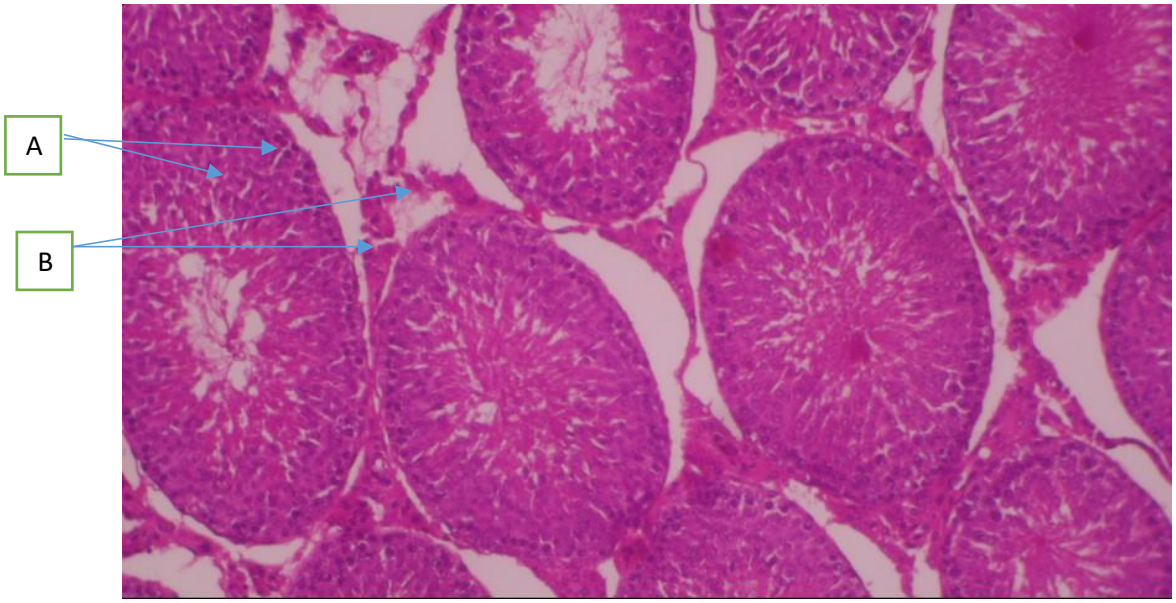


Plate 14. Higher magnification of the above: A, B (H&E x 100)

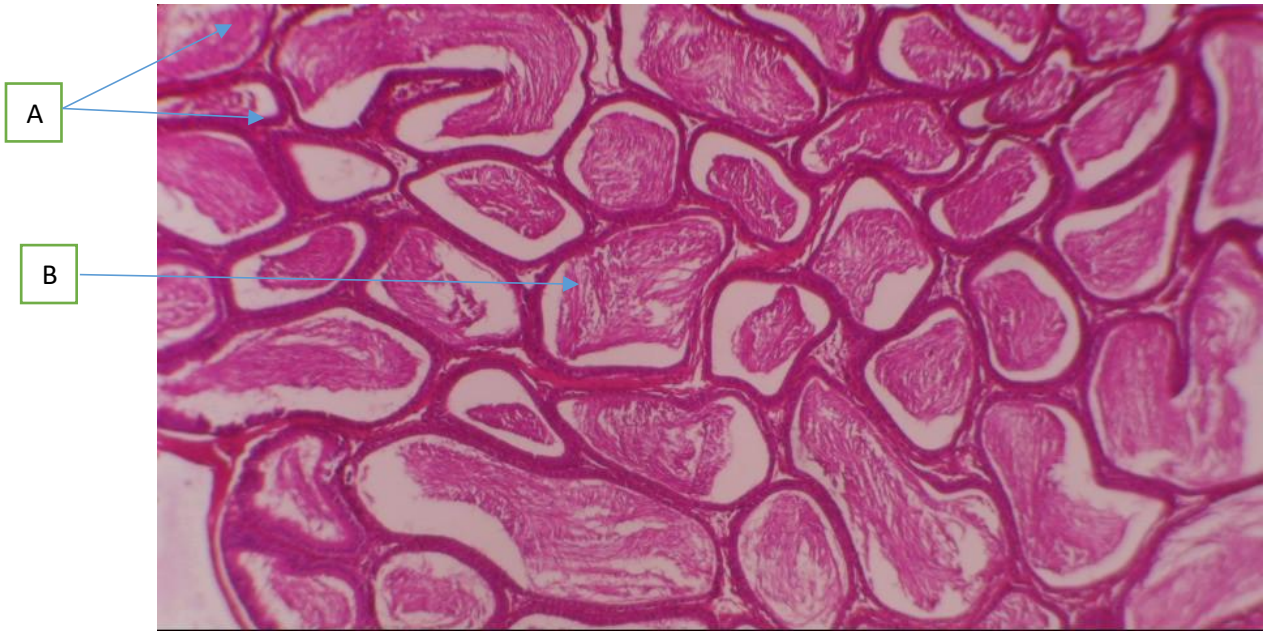


Plate 15. Epididymis of rat given Extract + Pb showing A, ducts with B, lumen fairly packed with spermatozoa (H&E x 40)

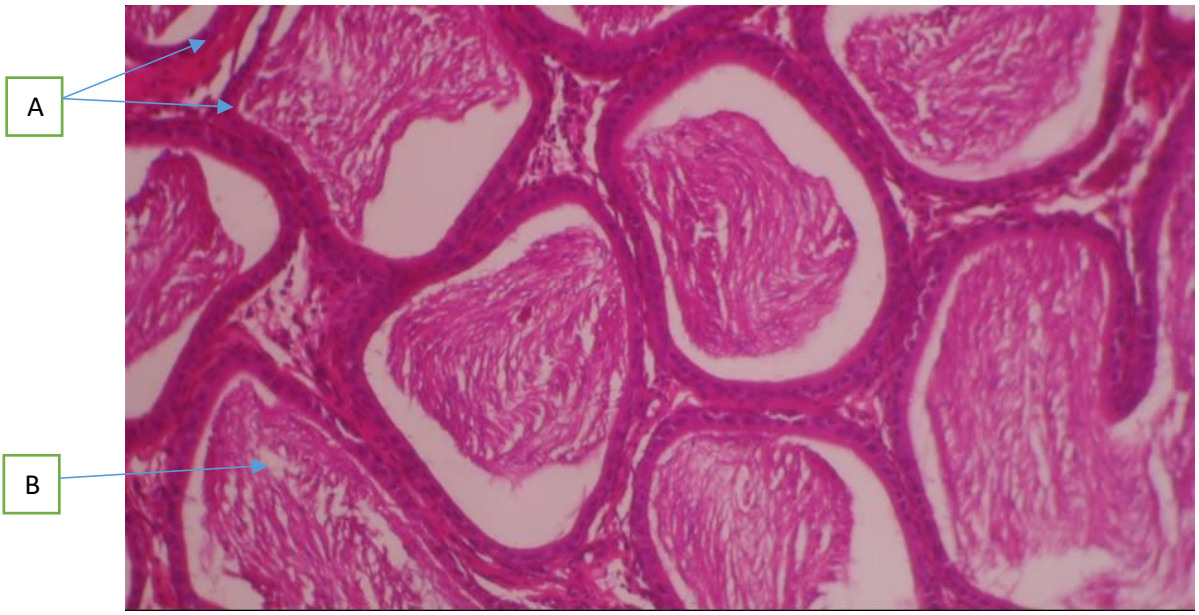
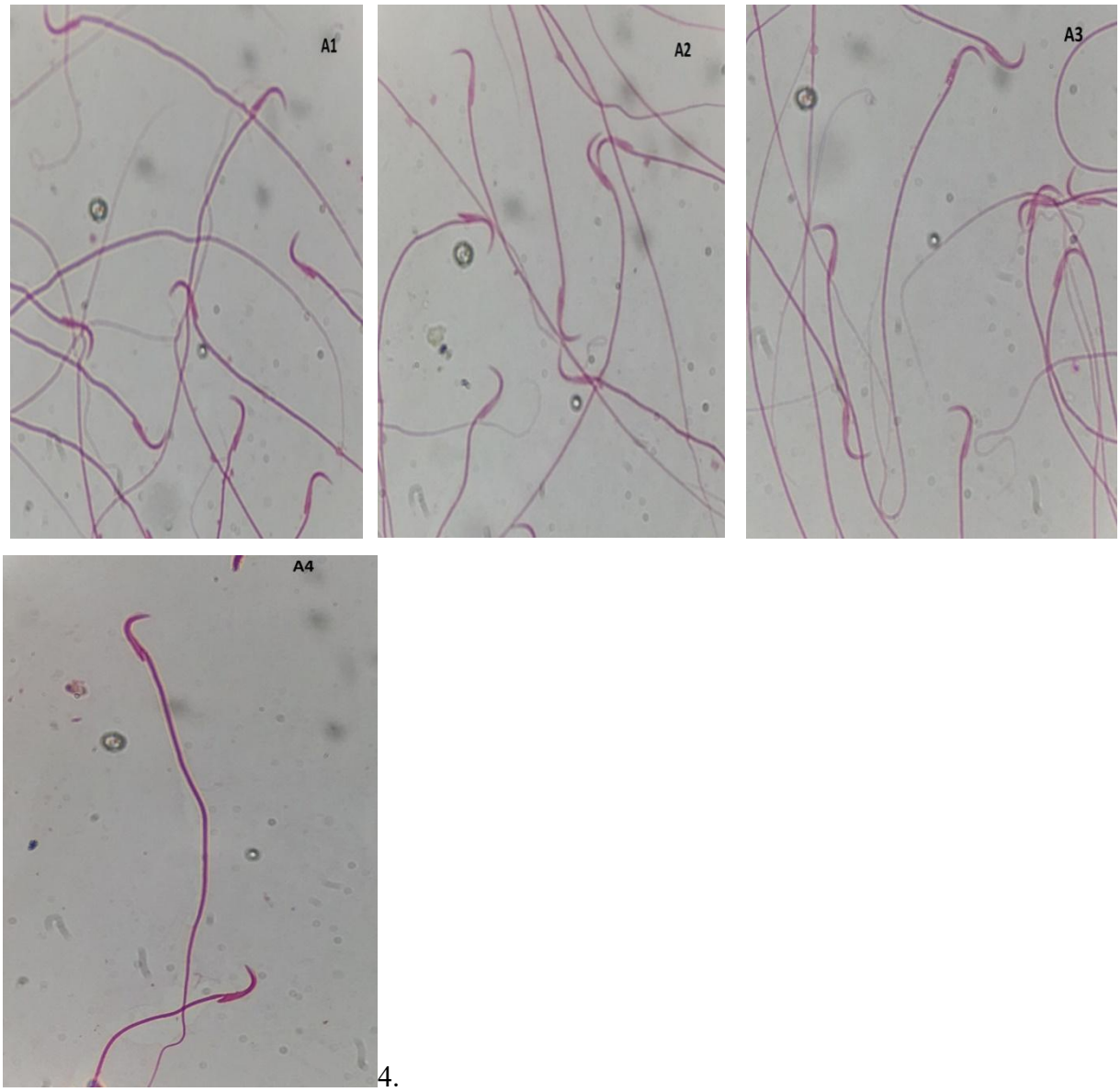


Plate 16. Higher magnification of the above: A, B (H&E x 100)

SPERM MORPHOLOGY

GROUP (A) 1. 2. 3.

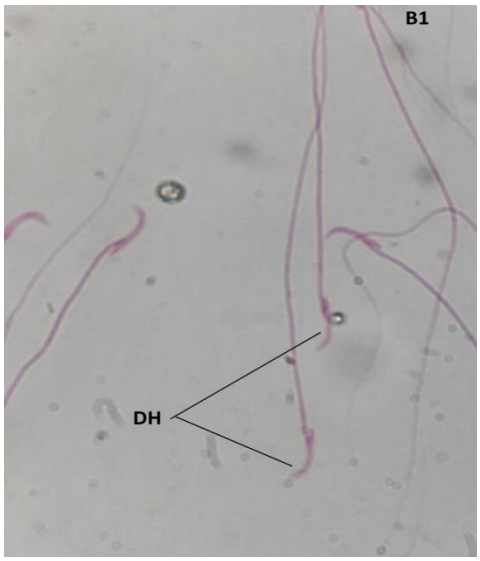


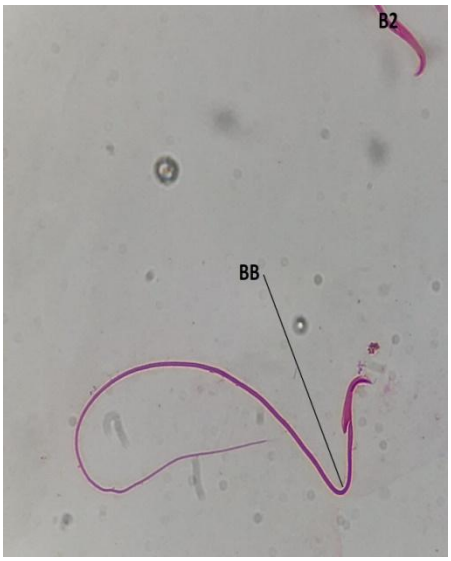
4.

Figure 1.0: Wister rats sperm cells stained with the Leishman and Eosin stain (ibeh *et al.*, 2018) and viewed with the X100 magnification lense for the total sperm cell count and morphology.

Interpretation: All rats in this group showed normal sperm cell morphology, with normal shape size and density, Sperm cells showed good motility and count. The heads of the sperm cell is properlly curved with distinct body and tail, which would aid in its motility as a sign of virility

GROUP (B) 1. 2.





3.

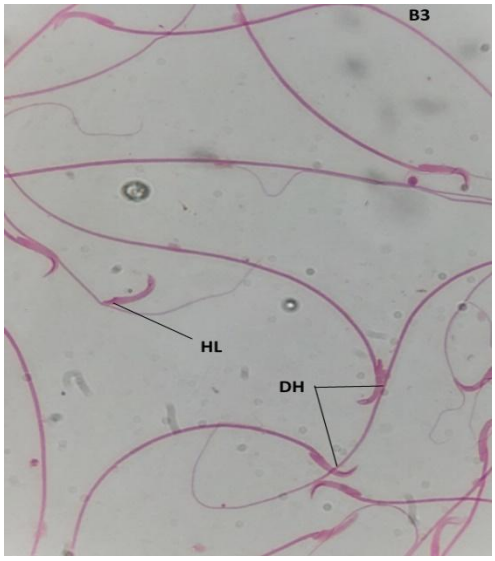


Figure 2.0: Wister rats sperm cells stained with the Leishman and Eosin stain (ibeh *et al.*, 2018) and viewed with the X100 magnification lense for the total sperm cell count and morphology

Interpretation: Increased Tetratozoospermia with normal motility, and sperm cell count, the observable Tetratozoospermia as seen in (1), Deformed head, head not properly bent (DH), (2) Bent middle section which may impact on motility (BB) and (3) increased tetratozoospermia, Tailless sperm cells (TL) as a result of stress, poorly formed headed with poor curve (DH). The motility seemed normal the count reduced when compared with the control group (A) this may be due to the extract administered

Group (C.) 1. 2. 3.

4.

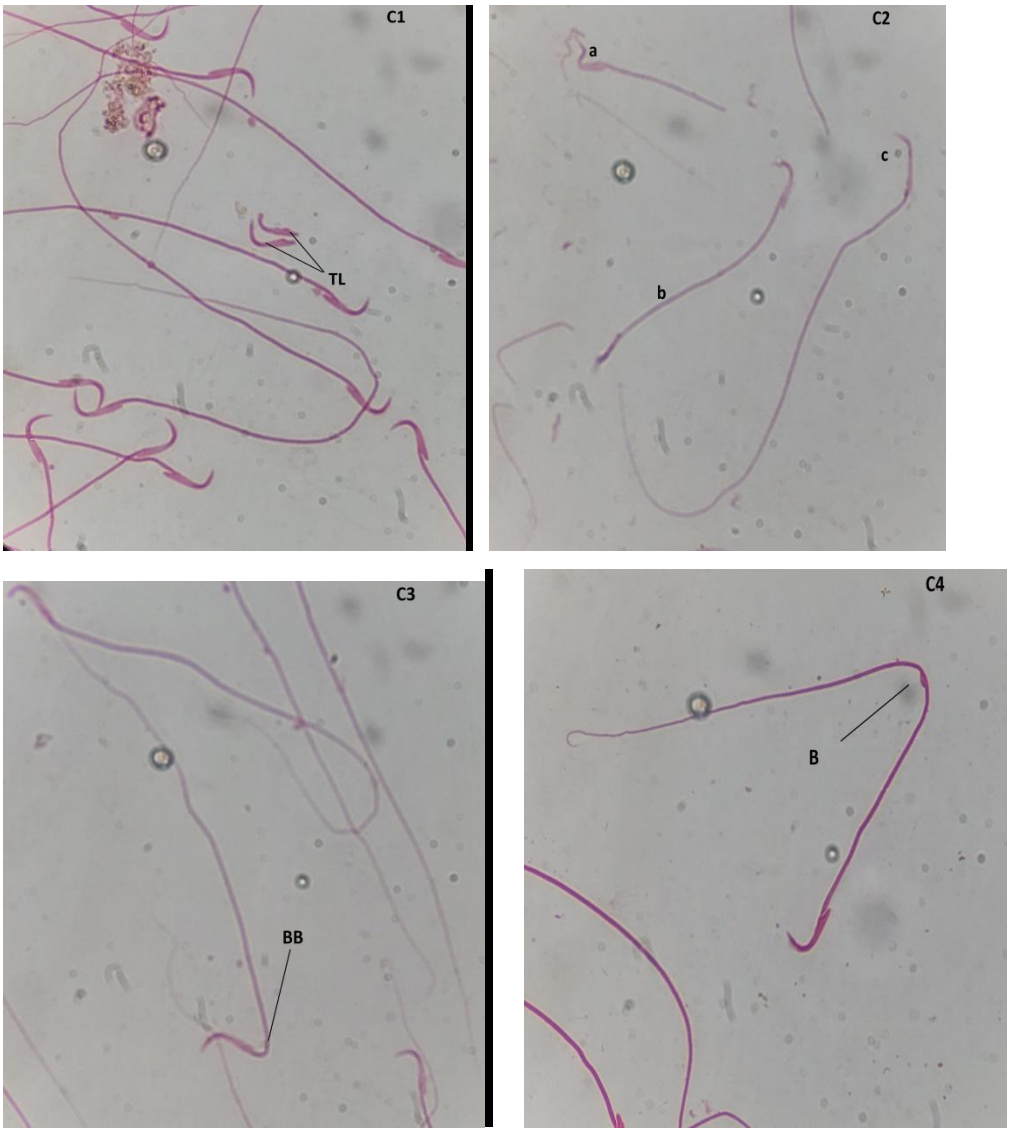


Figure 3.0: Wister rats sperm cells stained with the Leishman and Eosin stain (ibeh *et al.*, 2018) and viewed with the X100 magnification lense for the total sperm cell count and morphology

Interpretation: There Is an increased sperm cell count with high motility, but observable Tetratozoospermia (1) there are observable abnormalities tailless sperm cells (TL) with debris (2) Deformed sperm cells, (a) Short tailled sperm cells (b) and poorly formed head (c) (3) Bent body which may impact negatively on motility (bb), (4) Bulgy middle section, this would prevent the sperm cells from penetrating and fertilizing the eggs. The sperm cell count is high so also the motility as a sign of its virility and quality

Group (D) 1. 2.

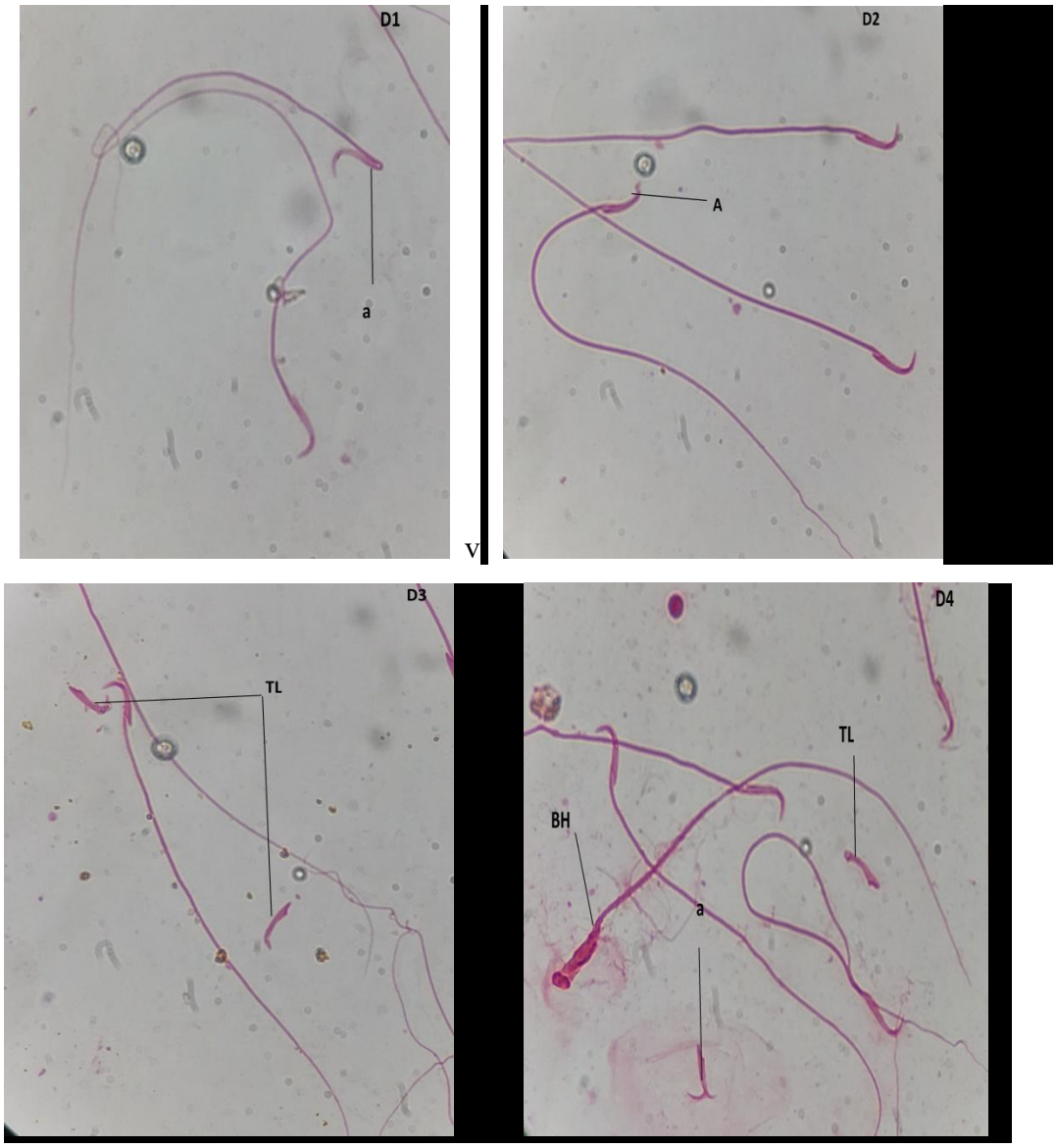


Figure 4.0: Wister rats sperm cells stained with the Leishman and Eosin stain (ibeh *et al.*, 2018) and viewed with the X100 magnification lense for the total sperm cell count and morphology

Interpretation: Reduced sperm cell count when compared with the control Group (A) and Treatment Group (C.), there is presence of asthenozoozpermia and Tetratozoospermia (1) Bent neck (tertratozoozpermia and Asthenozoospermia) (a) with poor motility (2) Poorly formed head (A) (3) Tailless sperm cells (TL) (4) Tailless sperm cells (TL), Bulgy head (BH), Double headed tailless sperm, cell (a). There is marked Asthenozoozpermia and tertratozoospermia.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Discussion

There was statistically significant increase ($p<0.05$) of body weights in groups B, C, and D, when the initial body weights were compared to the final body weights. There were no statistically significant differences in testicular weight across the groups, this suggests that neither the extract nor the lead acetate disrupted their feeding habits

The histological results as seen in plate 5 shows that administration of lead can cause degeneration in the rat testes, this was shown by distorted tubules, degenerating spermatocyte, degenerating leydig cells and patchy maturation arrest and also reduction in the population of spermatozoa in the lumen of epididymis in agreement with Hamadouche A et al 2009 experiment which showed reduced spermatogenic cells from basement membrane, thickening and irregular basement membrane of most seminiferous tubules with less spermatogenic layers.

Group C administered *Hibiscus sabdariffa* extract shows normal tubules lined by normal developing spermatocyte and active interstitial congestion and also normal ducts packed with spermatozoa in the epididymis this suggests that *Hibiscus sabdariffa* extract has a positive effect on the testes in agreement with Ali *et al.*, 2012, long term administration of *Hibiscus sabdariffa* WE for 10 weeks and hibiscus anthocyanins (50–200 mg/kg b.w.) for 5 days did not affect the male reproductive system in rats

The histology slides of group D which was used to investigate the curative effect of *Hibiscus sabdariffa* extract on lead toxicity of the testes as seen in plates 13, 14, 15 and 16 showed recovery of seminiferous tubules back to normal shape lined with maturing spermatocyte and epididymis showing ducts with lumen fairly packed with spermatozoa, this suggests that Hibiscus sabdariffa has protective effects on the histological architecture of the testes and epididymis

The semen analysis of rats treated with Hibiscus sabdariffa showed a reduction in percentage in normal sperm cells, sperm motility and the morphology of sperm cells showed some deformity, this suggests that the extract given had some deleterious effect on sperm parameters in agreement with Kanokwan Sukjail and Ampa Luangpirom (2010) who studied aqueous seed extract of *Hibiscus sabdariffa* in male rats, and found that all treated groups showed decreasing of sperm concentration, percentage of normal motile sperms and vital sperms

Conclusion

This study shows that *Hibiscus sabdariffa* extract has some curative and preventive effects against testes toxicity caused by lead.

Recommendation

After consideration of the results of this study, it is recommended that more studies should be carried out on the response of *Hibiscus sabdariffa* to testis toxicity and testicular damage

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