

**EFFECTS OF *PHOENIX DACTYLIFERA* ON SOME REPRODUCTIVE ORGANS AND  
HORMONAL PROFILES OF MALE WISTAR RATS**

**BY**

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Science in Human Anatomy**

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## DECLARATION

I hereby declare that the research work reported in this dissertation entitled “**Effects of *Phoenix dactylifera* on some Reproductive Organs and Hormonal Profiles of Male Wistar Rats**” was conducted by me in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria, under the supervision of Prof. J. O. Hambolu and Dr. A. A. Buraimoh. The information derived from the literature has been duly acknowledged in the text and a list of references provided and no part of the work has been presented for another degree in any institution.

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**Date**

## CERTIFICATION

This dissertation, entitled: “**Effect of *Phoenix dactylifera* on some Reproductive Organs and Hormonal Profile of Male Wistar Rats**” by Nathan Isaac DIBAL meets the regulation governing the award of Master of Science (M.Sc.) Degree in Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, under the supervision of **Prof. J. O. Hambolu** and **Dr. A. A. Buraimoh**. It is therefore approved for its contribution to knowledge and literary presentation.

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## **DEDICATION**

I dedicate this research work to Almighty God for the strength and courage he bestowed on me to carry out this study.

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## LIST OF ABBREVIATION

AMH	Anti-Mullerian Hormone
ANOVA	Analysis of Variance
ASRM	American Society for Reproductive Medicine
CDC	Center for Disease Control
ELISA	Enzyme-Linked Immunosorbent Assay
FSH	Follicle Stimulating Hormone
H and E	Heamatoxylin and Eosin
LH	Luteinizing Hormone
MIS	Mullerian-Inhibiting Substance
NBF	Neutral Buffered Formalin
SEM	Standard Error of Mean
TDF	Testis-Determining Factor
WHO	World Health Organization

## ABSTRACT

*Phoenix dactylifera* (Date palm) belongs to the family *Arecaceae* and its leaves, barks, pits, fruits and pollens have anticancer, antioxidant, hepatoprotective, antidiabetic, anti-inflammatory, antibacterial, antifungal and antiviral activities. It is rich in antioxidants, vitamins, steroids, flavonoid, saponins and simple sugars. The aim of the study was to evaluate the effects of aqueous extract of dry date palm fruit on some reproductive organs and hormonal profiles of male Wistar rats. Twenty (20) male Wistar rats were divided into four groups of five rats each. Group I serve as the control and received distilled water while three (3) experimental groups (II, III and IV) were treated with aqueous extract of *Phoenix dactylifera* at 250 mg/kg, 500 mg/kg and 1000mg/kg body weight respectively orogastrically once daily for 35 days. At the end of the experiment, the Wistar rats were sacrificed using cervical dislocation and blood samples were collected through cardiac puncture for hormonal assay [testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH)]. Semen were collected from right epididymis and counted; smears were made and stained with cresyl violet and fuelgen stain for sperm morphology. The testes, left epididymis, seminal vesicles and prostate glands were dissected, weighed and processed for light microscopic study; Morphometric analysis was performed to measure seminiferous tubular diameter, size of interstices and epididymal epithelial thickness. The result showed significant decrease in serum testosterone levels, sperm count, sperm motility and sperm morphology with decrease in size of interstices and epididymal epithelial thickness, distortion of spermatogenic cells, epididymal epithelium and prostate gland with degeneration of Leydig cells in rats treated with *Phoenix dactylifera* extract as compared to that of the control at  $P \leq 0.05$  with no effect on FSH and LH. In conclusion, the aqueous extract of dry date palm fruit have the potentials of causing infertility in male Wistar rats by affecting Leydig cells, thereby decreasing serum testosterone levels, sperm count, motility and morphology.

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background of the Study

*Phoenix dactylifera* (Date palm), family *Arecaceae* is cultivated for its edible sweet fruit. Although its place of origin is unknown because of long cultivation, it probably originated from lands around Iraq (Mesopotamia) and its cultivation spread to the Arabian Peninsula, North Africa, and the Middle Eastern Countries, possibly as early as 4000 BC (Morton, 1987; Janick, 2005; Zohary *et al.*, 2012). The tree has a slender trunk covered with the overlapping persistent woody leaf base and can grow to about 24 metres in height either singly or forming a clump with several stems from a single root system (Chandra *et al.*, 1992). The leaves are pinnate, resembling a large feather that can be as long as 6 metres with about 150 leaflets. The date palm is dioecious having separate male and female plants thus, male and female flowers are borne on separate plants and are unlike in appearance, they are naturally wind pollinated but in traditional oasis horticulture and in the modern commercial orchards they are entirely pollinated artificially (Chandra *et al.*, 1992).

The date (Dabino in Hausa, Debino in Yoruba) is a one-seeded fruit (berry) usually oblong but varying much in shape, size, colour (green, bright red to bright yellow), quality, and consistency of flesh, depending on variety (Chao and Krueger, 2007). Only female trees produce fruit, and more than 1,000 dates may appear on a single bunch weighing 8 kg or more. The fruits are highly nutritious and are a staple food for the people of North Africa and Middle East, where hundreds of varieties are grown for

domestic and commercial purposes (Forbes, 1971).The dried fruit has more than 50% sugar by weight although glucose, fructose and sucrose contents depend on fruit type. It also contains about 2% of protein, fat, and mineral matter, 20–70 calories, vitamin C, riboflavin and thiamine depending on size and variety (Khan *et al.*, 2008; Agboola and Adejumo, 2013). The date fruit extract also contains antioxidants such as coumaric and ferulic acids (Ismail and Radzi, 2013). The fruits ripen in four stages, which are known throughout the world by their Arabic names Kimri (Unripe), Khal (full-sized crunchy), rutab (soft ripe) and tamar (ripe sun-dried).Increased sweetness with ripening of dates results from the increase in total sugars and in soft cultivars the conversion of sucrose to fructose and glucose (Kader and Hussein, 2009).

The Ancient Egyptians ate the fruit at harvest and use them to make date wine. Dates are an important traditional crop in Iraq, Arabia, North Africa and Morocco where they are used in the treatment of various ailments/illness (Lim, 2012). They are nutritious, high-energy food, and important part of the diets of people in the Arab countries and are consumed fresh, dried, or in various processed forms (Kader and Hussein, 2009). Dates can also be chopped and used in a range of sweet and savory dishes, from tajines (tagines) in Morocco to puddings, ka'ak (types of Arab cookies) and other dessert items (Das and Sarin, 1936; Vayali, 2002). Date nut bread, a type of cake, is very popular in the United States, especially around holidays, they are also processed into cubes, paste (ajwa), date syrup or honey (dibs or rub) powder (date sugar), vinegar or alcohol in Libya, the Vinegar made from dates is a traditional product of the Middle East (Das and Sarin, 1936; Forbes, 1971). Recent innovations

include chocolate-covered dates (Date bars) and products such as sparkling date juice, used in some Islamic countries as a non-alcoholic version of champagne, for special occasions and religious times such as Ramadan. Date palm kernels have been shown to exhibit anti-aging properties and significant reduction in skin wrinkles in women (Bauza, 2002).



Figure 1.1: Date Palm plant (Date Palm Agro, 2015).

## 1.2 Statement of Research Problems

The prevalence of infertility is higher in developing/underdeveloped countries where limited resources are available for diagnosis and treatment and a significant proportion of couples that experience fertility problems are affected by its social and psychological effects (Hamada *et al.*, 2011; CDC, 2014), while 50% of infertility cases are as a result of male factor (sperm abnormalities) (Jarow *et al.*, 2002).

### **1.3 Significance of the Study**

This study was undertaken to evaluate the possible influence of *Phoenix dactylifera* on some reproductive organs (testes, epididymis, seminal vesicles and prostate gland) administered in aqueous form which is commonly used by the people of Middle East and North Africa (Forbes, 1971; Al Qarawi, 2005). This could be used as a gonadotropic agent or as a male contraceptive.

### **1.4 Study Hypothesis**

Aqueous extract of *Phoenix dactylifera* fruit has negative effects on the histology and morphometric parameters of the testis, epididymis, seminal vesicle and prostate gland, as well as the reproductive hormones and epididymal sperm count, motility and morphology of male Wistar rats.

### **1.5 Aim of the Study**

To evaluate the possible effects of aqueous extract of *Phoenix dactylifera* on the testis, epididymis, seminal vesicles, prostate gland, epididymal sperm count, motility and morphology and hormonal profiles of male Wistar rats.

### **1.6 Objectives of the Study**

The objectives of the study include the following:

- i. To evaluate the effect of aqueous extract of *Phoenix dactylifera* on the levels of testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in the serum of male Wistar rats.

- ii. To evaluate the effects of aqueous extract of *Phoenix dactylifera* on the epididymal sperm count, motility and morphology of Wistar rats.
- iii. To evaluate the histological changes that may occur in the testes, epididymis, seminal vesicles and prostate gland of male Wistar rats following the administration of aqueous extract of *Phoenix dactylifera*.
- iv. To determine the morphometric changes that might occur in the testes and epididymis following the administration of aqueous extracts of *Phoenix dactylifera* to male Wistar rats.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Infertility

Infertility is defined as the inability of couples to achieve pregnancy after one year of continuous unprotected sexual intercourse or six months, if the woman is 35 years or older (ASRM, 2012; Chandra and Copen, 2013). The prevalence varies widely, being less in developed countries where resources for investigation and treatment are readily available and accessible but more in developing countries where limited resources for investigation and treatment are available, infertility is considered also a public problem. It does not affect the couples' life only, but it also affects the healthcare services and social environment (Kamel, 2010).

Infertility can be attributed to any abnormality in the female or male reproductive system and can be of different forms including:

- i. Resolved infertility: Pregnancies occur after one (1) year of trying without medical intervention.
- ii. Primary infertility: Never pregnant.
- iii. Secondary infertility: Failure to conceive after having previously delivered an infant without the use of infertility treatment (CDC, 2014).

The major causes of infertility are male factors (sperm abnormalities), others include ovarian dysfunction, tubal disease, endometriosis, and uterine or cervical factors. A careful history and physical examination of each partner can suggest a single or multifactorial etiology and can direct further investigation but in approximately one fourth of

couples, the cause is uncertain and is referred to as “unexplained infertility” (Jose-Miller *et al.*, 2007). Infertility affects about 15% of couples in reproductive age worldwide and male factor is solely responsible in about 50% of the cases and contributory in 30–40 per cent of cases (Jarow *et al.*, 2002; Raheem and Raph, 2011). Therefore, semen analysis is important in the initial evaluation. The pathogenesis of male infertility can be reflected by defective spermatogenesis due to failure in germ cell proliferation and differentiation (Mitchell *et al.*, 2001).

## **2.2 Male Reproductive System**

The Male reproductive system consist of both internal organs and external genitalia that functions in the production, storage and conduction of spermatozoa as well as the production of seminal secretions; Themale internal reproductiveorgansinclude the testes, epididymides, *ductus deferentes*, seminal glands, bulbourethral glands, prostate gland and ejaculatory ducts while the external genitalia consist of the penisand scrotum (Moore and Dalley, 2006).

### **2.2.1 The Mammalian Testes**

The testes are the male paired ovoid reproductive glands that produce the male germ cells (sperms or spermatozoa) and male hormones, primarily testosterone, (Jana and Sen, 2012). In humans, the testes are suspended in the scrotum by the spermatic cord with the left testis (testicle) usually suspended more inferiorly than the right testis while in the Wistar rats, the testes may be located within the abdomen or the scrotum because they have open inguinal canal (Treuting and Dintzis, 2012) The testes have tough fibrous outer

surface, the tunica albuginea that thickens into a ridge on its internal, posterior aspect as the mediastinum of the testis, from this internal ridge, fibrous septa extend inward between lobules of minute but long and highly coiled seminiferous tubules in which the sperms are produced. The seminiferous tubules are joined by straight tubules to the *rete testis* (Wajner *et al.*, 2009).

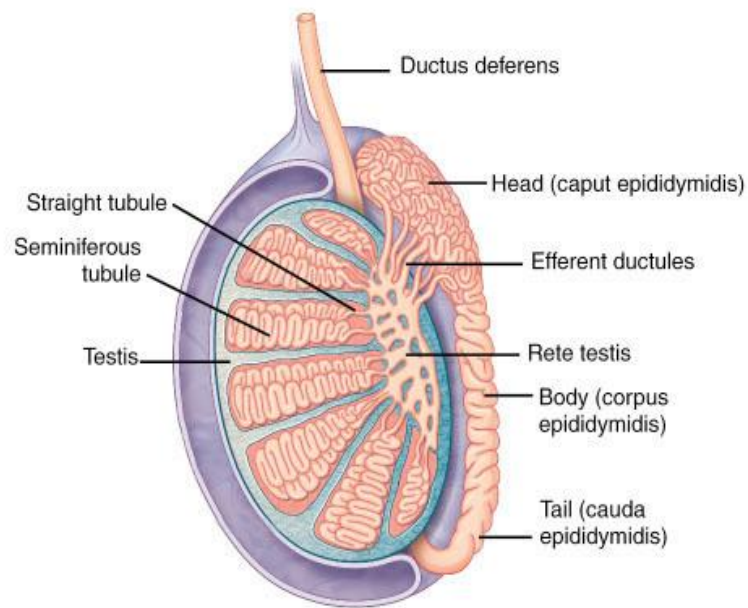


Figure 2.1: The structure of a Mammalian Testis and Epididymis (Koeppen and Stanton, 2009)

The testicular arteries arise from the anterolateral aspect of the abdominal aorta just inferior to the renal arteries; they pass retroperitoneally in an oblique direction, crossing over the ureters and the inferior parts of the external iliac arteries to reach the deep inguinal rings. They pass through the inguinal canals and enter the spermatic cords to supply the testes. The testicular artery or one of its branches anastomoses with the artery

of the *ductus deferens* (Singh *et al.*, 2011; Jyothsna *et al.*, 2012). The veins emerging from the testis and epididymis form the pampiniform venous plexus, a network of 8-12 veins lying anterior to the *ductus deferens* and surrounding the testicular artery in the spermatic cord. The pampiniform plexus is part of the thermoregulatory system of the testis (along with the cremasteric and dartos muscles) helping keep this gland at a constant temperature, the veins of each pampiniform plexus converge superiorly, forming a right testicular vein, which enters the inferior vena cava (IVC), and a left testicular vein, which enters the left renal vein (Singh *et al.*, 2011).

The lymphatic vessels of the testis have occasional smooth muscle cells and valves. At the posterior margin of the testis, the network of lymph vessels merges into collecting ducts, which together with vessels derived from the *rete testis* are drained by the lymphatic system in the spermatic cord while the autonomic nerves of the testis arise as the testicular plexus of nerves on the testicular artery, which contains vagal parasympathetic and visceral afferent fibers and sympathetic fibers from the T7 segment of the spinal cord (Holstein *et al.*, 1979).

### **2.2.1.1 Development and Descent of the Mammalian Testes**

The male primordial germ cells migrate from the yolk sac into the genital ridges and begin to differentiate into gonocytes by the 5<sup>th</sup> and 6<sup>th</sup> weeks after fertilization (Virtanen *et al.*, 2007). The mammalian Sry (sex regulating) gene for testis-determining factor (TDF) on the short arm of the Y chromosome induces development of the undifferentiated gonad into a testis (Kashimada and Koopman, 2010). Studies in rodents (mice) reveal that the major role of Sry is to achieve sufficient expression of the related

gene Sox9, in order to induce Sertoli cell differentiation, which in turn drives testis formation (Kashimada and Koopman, 2010). Testis determining factor (TDF) induces the gonadal cords to condense and extend into the medulla of the undifferentiated gonad, where they branch and anastomose to form the *rete* testis, the connection of the gonadal cords/seminiferous cords with the surface epithelium is lost when a thick fibrous capsule, the tunica albuginea, develops, the development of the dense tunica albuginea is the characteristic feature of testicular development (Hughes, 2001; Sadler, 2012). The genital ridge and testes formation begins at the 49<sup>th</sup> and 56<sup>th</sup> day of gestation respectively in humans while in rodents, genital ridge formation begins between 9<sup>th</sup>-10<sup>th</sup> day of gestation whereas testes formation begins on the 12<sup>th</sup> day of gestation (Klonisch *et al.*, 2004). Gradually the enlarging testis separates from the degenerating mesonephros and becomes suspended by its own mesentery, the mesorchium. The seminiferous cords develop into the seminiferous tubules, *tubuli recti*, and *rete* testis. The seminiferous tubules are separated by mesenchyme that gives rise to the interstitial cells (Leydig cells). In humans, by the 8<sup>th</sup> week, these cells begin to secrete androgenic hormones, testosterone and androstenedione, which induce masculine differentiation of the mesonephric ducts and the external genitalia. Testosterone production is stimulated by human chorionic gonadotropin, which reaches peak amounts during the 8-12 week period. In addition to testosterone, the fetal testes produce a glycoprotein called anti-mullerian hormone AMH or mullerian-inhibiting substance (MIS) (Brennan and Capel, 2004). Anti-mullerian hormone is produced by the sustentacular cells (Sertoli cells), which continues to puberty, after which the levels of anti-mullerian hormone decrease. Anti-mullerian hormone suppresses development of the paramesonephric ducts/mullerian duct, which form the

uterus and uterine tubes (Josso and Picard, 1986; Allard *et al.*, 2000). The seminiferous tubules remain solid (i.e., no lumina) until puberty, at which time lumina begin to develop. The walls of the seminiferous tubules are composed of two kinds of cells:

- i. Sertoli cells, supporting cells derived from the surface epithelium of the testis and
- ii. Spermatogonia, primordial sperm cells derived from the primordial germ cells.

Sertoli cells constitute most of the seminiferous epithelium in the fetal testis. During later fetal development, the surface epithelium of the testis flattens to form the mesothelium on the external surface of the adult testis. The *rete* testis becomes continuous with 15 to 20 mesonephric tubules that become efferent ductules. These ductules are connected with the mesonephric duct, which becomes the duct of the epididymis (Arroteia *et al.*, 2012).

Descent of the testes is the process by which the developing testis moves from its initial position high in the abdomen into the scrotum. The process is generally subdivided into two phases (Hutson, 1985; Hutson *et al.*, 1997). During the first or trans-abdominal phase, which takes place before birth, the testis gains a position at the bottom of the abdomen. The second or inguino-scrotal phase involves movement of the testis from the abdominal bottom to the base of the scrotum (Emmen *et al.*, 2000). In humans, it usually begins during the 26<sup>th</sup> week and takes 2-3 months; they pass external to the peritoneum and *processus vaginalis*. After the testes enter the scrotum the inguinal canal contracts around the spermatic cord (Hughes and Acerini, 2008; Hutson *et al.*, 2009). In rodents, descent of testes begins on the 16<sup>th</sup> day of development to 19 days postpartum (Klonisch *et al.*, 2004). More than 97% of full-term newborn males have both testes in the scrotum, during the first 3 months after birth, most undescended testes descend into the

scrotum. The mode of descent of the testis explains why the *ductus deferens* crosses anterior to the ureter; it also explains the course of the testicular vessels. These vessels form when the testis is high on the posterior abdominal wall. When the testis descends, it carries its *ductus deferens* and vessels with it. As the testis and *ductus deferens* descend, they are ensheathed by the fascial extensions of the abdominal wall (Hutson *et al.*, 2009).

### 2.2.2 The Mammalian Epididymis

The epididymis, located between the efferent ducts and the *vas deferens*, is a male accessory organ characterized by a single coiled tubule duct with an estimated length of 5–7 m in humans (O’Hara *et al.*, 2011). The epididymis stores, concentrates and transport spermatozoa and under androgen control, the epididymal epithelium secretes proteins within the intraluminal compartment that creates a very complex environment surrounding the spermatozoa (Sullivan, 2004). Efferent ductules of the testis transport newly developed sperms to the epididymis from the *rete testis*. The epididymis is formed by minute convolutions of the duct of the epididymis, so tightly compacted that they appear solid, the duct becomes progressively smaller as it passes from the head of the epididymis on the superior part of the testis to its tail, at the tail of the epididymis, the *ductus deferens* begins as the continuation of the epididymal duct (Lesserre *et al.*, 2001; Cornwall, 2009). In the lengthy course of this convoluted duct, the sperms are stored and continue to mature (O’Hara *et al.*, 2011). The epididymis consists of the:

- i. **Head of the epididymis (*Caput*):** the superior expanded part that is composed of lobules formed by the coiled ends of the efferent ductules.

- ii. **Body of the epididymis (*Corpus*):** consists of the convoluted duct of the epididymis.
- iii. **Tail of the epididymis (*Cauda*):** continuous with the *ductus deferens*, the duct that transports the sperm from the epididymis to the ejaculatory duct for expulsion via the urethra during ejaculation.

Although these three anatomical regions of the epididymis are easily identified in most adult male mammals (Smithwick and Young, 2001), histological and ultrastructural segmentation of this organ varies among the different phylogenies of mammals. The rat epididymis is most commonly adopted as an experimental model of study because of its role in the storage of spermatozoa (Arroteia *et al.*, 2012).

### 2.2.3 Mammalian Seminal Vesicles

The seminal vesicles are elongated structures about 5 cm long that lies between the fundus of the bladder and the rectum in most mammals (Treuting and Dintzis, 2012). In rodents, the anterior lobe (coagulating gland) of the prostate is bilaterally attached to the lesser curvature of the seminal vesicles and the ducts opens at the seminal colicle with secretions of the seminal vesicles, prostate and bulbourethral glands forming the copulatory plug (Treuting and Dintzis, 2012). In humans, the seminal vesicles are obliquely placed glands superior to the prostate and do not store sperms but secrete a thick alkaline fluid with fructose and a coagulating agent that mixes with the sperms as they pass into the ejaculatory ducts and urethra (Kim and Kim, 2013). The superior ends of the seminal glands are covered with peritoneum and lie posterior to the ureters, where the peritoneum of the rectovesical pouch separates them from the rectum; the inferior

ends of the seminal vesicles are closely related to the rectum and are separated from it only by the rectovesical septum. The duct of the seminal vesicle joins the *ductus deferens* to form the ejaculatory duct and the relevant functions commonly ascribed to mammalian seminal fluid components include maintenance of sperm viability, enhancement of sperm motility, and hormonal stimulation of the female reproductive tract and suppression of female chemical and immunological challenges to the sperm (Ramm *et al.*, 2005).

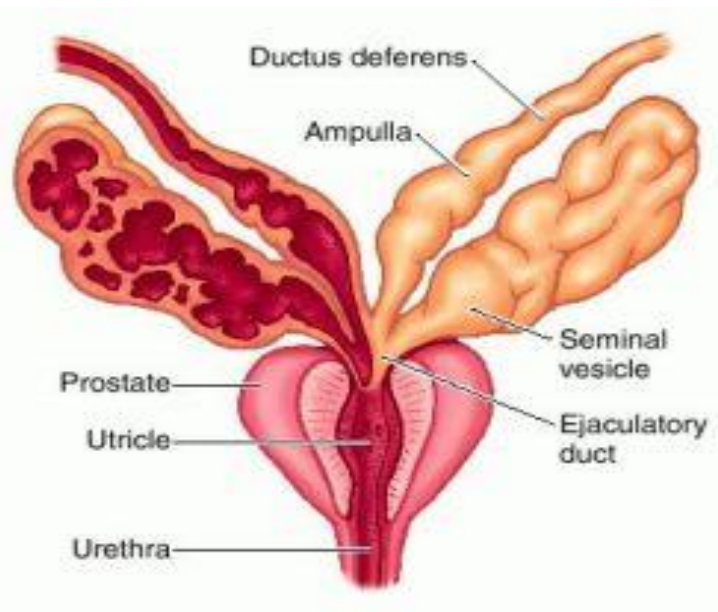


Figure 2.2: The structure of a mammalian seminal vesicle and prostate gland (Anderson *et al.*, 2007)

The arteries to the seminal vesicles are derived from the inferior vesical and middle rectal arteries. The veins accompany the arteries and have similar names. The iliac lymph nodes receive lymph from the seminal glands: the external iliac nodes from the superior part, and the internal iliac nodes from the inferior part (Moore and Dalley, 2006).

#### 2.2.4 The Mammalian Prostate Gland

The prostate is the largest accessory gland of the male reproductive system in humans (Moore and Dalley, 2006), it is a walnut-size organ that surrounds the prostatic urethra, located between the base of the urinary bladder and the rectum, measuring approximately 3 cm long, 4 cm wide, and 2 cm in antero-posterior depth, the glandular part makes up approximately two thirds of the prostate; the other third is fibromuscular, the fibrous capsule of the prostate is dense and neurovascular, incorporating the prostatic plexuses of veins and nerves, all this is surrounded in turn by the visceral layer of the pelvic fascia, forming a fibrous prostatic sheath that is thin anteriorly, continuous anterolaterally with the puboprostatic ligaments, and dense posteriorly where it blends with the rectovesical septum (Donkol and Al Nammi, 2012). In the mouse the prostate is divided grossly into four distinct lobes; anterior, dorsal, ventral and lateral, the dorsal and lateral lobes are sometimes grouped as dorsolateral lobe, the lobes surround the urethra and are invested in membrane (Treuting and Dintzis, 2012). The prostate gland develops from epithelial invaginations of the posterior urogenital sinus under the influence of the underlying mesenchyme during the third month of gestation; it requires the presence of  $5\alpha$ -dihydrotestosterone, which is synthesized from fetal testosterone by the action of  $5\alpha$ -reductase (Moore and Persaud, 2003). This enzyme is localized in the urogenital sinus and external genitalia of humans. Consequently, deficiencies of  $5\alpha$ -reductase will cause a rudimentary or undetectable prostate in addition to severe abnormalities of the external genitalia (Hammerich *et al.*, 2009). During the prepubertal period, the constitution of the human prostate remains more or less identical but begins to undergo morphologic changes into the adult

phenotype with the beginning of puberty and the continuous enlargement of the gland to reach the adult weight of approximately 20g by 25–30 years of age ( Hammerich *et al.*, 2009). The Human prostate gland consist of

- i. A base closely related to the neck of the bladder.
- ii. An apex that is in contact with fascia on the superior aspect of the urethral sphincter and deep perineal muscles.
- iii. A muscular anterior surface, featuring mostly transversely oriented muscle fibers forming a vertical, trough-like hemisphincter (rhabdosphincter), which is part of the urethral sphincter, separated from the pubic symphysis by retroperitoneal fat in the retropubic space.
- iv. A posterior surface that is related to the ampulla of the rectum.
- v. Inferolateral surfaces that are related to the levator ani.

The prostatic ducts (20-30) open chiefly into the grooves, the prostatic sinuses that lie on either side of the seminal colliculus on the posterior wall of the prostatic urethra. Prostatic fluid, a thin, milky fluid, provides approximately 20% of the volume of semen and plays a role in activating the sperms (Donkol and Al Nammi, 2012).

The prostatic arteries are mainly branches of the internal iliac artery especially the inferior vesical arteries but also the internal pudendal and middle rectal arteries. The veins join to form a plexus around the sides and base of the prostate. This prostatic venous plexus, between the fibrous capsule of the prostate and the prostatic sheath, drains into the internal iliac veins. The prostatic venous plexus is continuous superiorly with the vesical venous plexus and communicates posteriorly with the internal vertebral venous

plexus. The lymphatic vessels terminate chiefly in the internal iliac lymph nodes, but some drainage may pass to the sacral nodes (Kisileuzky *et al.*, 2014).

### **2.3 Phytochemistry of Date Palm Fruit**

Phytochemical screening of date palm fruit revealed the presence of flavonoids, tannins, saponins, cardiac glycosides, carotenoids and steroids (Anjumet *et al.*, 2012; Sadiq *et al.*, 2013).

#### **2.3.1 Flavonoids**

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones (Ren *et al.*, 2003; Tapas *et al.*, 2008). They are responsible for the major organoleptic characteristics of plant-derived foods and beverages, particularly color and taste properties and they also contribute to the nutritional qualities of fruits and vegetables (Ren *et al.*, 2003; Anderson and Markham, 2006). Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages and they have been reported to have antiviral, anti-allergic (Tapas *et al.*, 2008), anti-inflammatory (Marzocchella *et al.*, 2011), antiplatelet (Rechner and Kroner, 2005), anticancer (Ren *et al.*, 2003; Dai and Mumper, 2010) and antioxidant activities (Tapas *et al.*, 2008).

#### **2.3.2 Tannins**

Tannins are astringent, bitter plant and water soluble polyphenolic compounds that either bind and precipitate or shrink proteins (Ashok and Upadhyaya, 2012). The astringency

from the tannins is that which causes the dry and puckery feeling in the mouth following the consumption of red wine, strong tea, or an unripen fruit. The sensation apparently results from the interaction between tannin constituents and proteins of the saliva and/or the mucous tissue of the mouth (Amarowicz, 2007). The term tannin refers to the use of tannins in tanning animalhides into leather. They are found as shapeless yellowish or light brown masses like powder, flakes or sponge, they are found almost in all plants and in all climates all over the world (Ashok and Upadhyaya, 2012). Their percentage in the plants, however, varies; while they are present in significant proportions in some plants, many others have too little of them (Ashok and Upadhyaya, 2012). They are usually found in large quantities in the bark of trees where they act as a barrier for micro-organisms and protect the tree. Tannins are defined as antinutrients of plant origin because they can precipitate proteins, inhibit digestive enzymes, and decrease the utilization of vitamins and minerals (Ashok and Upadhyaya, 2012). On the other hand, tannins have also been considered “health-promoting” components in plant-derived foods and beverages as they have been reported to possess anticarcinogenic, antioxidant, antiradical (Amarowicz and Toszynska, 2004) and antimicrobial properties (Akiyama *et al.*, 2001), antimutagenic as well as antiparasitic potentials (Kolodziej and Kinderlen, 2005). They are also used in dyeing, photography, refining beer and wine and form a vital element of tea (Wheeler, 1979; Kolodziej and Kinderlen, 2005).

### **2.3.3 Saponins**

Saponins are a class of natural products which are structurally constructed of aglycone (triterpene or steroid) and sugars (hexose and/or uronic acid) (Tamura *et al.*, 2012). They

derive their name from their ability to form stable, soap-like foams in aqueous solutions (Francis *et al.*, 2002). They are widely distributed in many plant species both wild and cultivated and are relatively widespread in our foodstuffs and herbal preparations (Francis *et al.*, 2002). Saponins traditionally used as a natural detergent. In addition to this physical property, plant-derived triterpenoid and steroidal saponins have historically received a number of industrial and commercial applications ranging from their use as sources of raw materials for the production of steroid hormones in the Pharmaceutical Industry, to their use as food additives and as ingredients in photographic emulsions, fire extinguishers and other industrial applications which take advantage of their generally non-ionic surfactant properties (Leung and Foster, 1996). In cultivated crops the triterpenoid saponins are generally predominant, while steroid saponins are common in plants used as herbs or for their health-promoting properties (Fenwick *et al.*, 1991). Extensive research has been carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals (Francis *et al.*, 2002). They also act as antioxidant, antifungal, antiyeast and antiviral agents (Apers *et al.*, 2000; Tamura *et al.*, 2012).

#### **2.3.4 Carotenoids**

Carotenoids are a family of pigmented compounds that are synthesized by plants and microorganisms but not animals; they contribute to the photosynthetic machinery in plants and protect them against photo-damage (Rao and Rao, 2007). They are present in all living organisms, from bacteria, yeast, algae to higher plants and animals (Jaswir *et*

*al.*, 2011). Fruits and vegetables constitute the major sources of carotenoid in human diet where they are present as micro-components and are responsible for their yellow, orange and red colors (Mangels *et al.*, 1993; Johnson, 2002). Carotenoids are thought to be responsible for the beneficial properties of fruits and vegetables in preventing human diseases including cardiovascular diseases, cancer and other chronic diseases (Eldahshan and Singab, 2013). They are also thought to have a variety of different actions, including possible antioxidant activity, vitamin A activity, immune-enhancement, inhibition of mutagenesis and transformation, inhibition of premalignant lesions. They function in plants and in photosynthetic bacteria as accessory pigments in photosynthesis and protect against photosensitization in animals, plants, and bacteria (Rodriguez-Amaya, 2001).

### **2.3.5 Steroids**

Steroids are substances that are naturally produced in the body by the adrenal glands (Guyton and Hall, 2006). They help control many different functions in our bodies, like the way the body uses fats, proteins and carbohydrates, they regulate our immune system and the balance of salt and water in our bodies and also help to reduce inflammation (Kersey *et al.*, 2012). There are many kinds of steroids, including estrogens, testosterone. They can be synthetically produced and are abused in an attempt to promote muscle growth, enhance athletic or other physical performance, and improve physical appearance (Hartgens and Kuipers, 2004). The adverse effects of steroids on the male reproductive function compromise the athlete's fertility resulting in oligozoospermia, azoospermia, a decrease in testis size, concomitant to a decrease in serum gonadotropin and testosterone levels (Torres-Calleja *et al.*, 2001; Leme de Souza and Hallak, 2011). Anabolic

androgenic steroid-induced lowered male infertility has been found to be reversible, other adverse effects of anabolic androgenic steroids use includes; ventricular tachycardia, transient infertility, atherogenic changes in lipoprotein profile and pathological remodelling of the myocardium (Karila, 2003). In both men and women, anabolic steroid use can cause high cholesterol levels, which may increase the risk of coronary artery disease, strokes, and heart attacks, can also cause acne and fluid retention (Hassan *et al.*, 2009).

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1 MATERIALS**

##### **3.1.1 Experimental Animals**

Twenty (20) male Wistar rats (122-134g) were purchased from the Animal House, Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University (ABU), Zaria, Kaduna State, Nigeria.

##### **3.1.2 Plant Material**

The date palm fruit was purchased from Samaru market in Zaria Kaduna State. Identification and authentication of the fruit was done in the herbarium section of the Department of Biological Sciences, Ahmadu Bello University Zaria, Kaduna State and was given Voucher number of 3253.

##### **3.1.3 Equipment and other Materials**

The equipment used include: Rotary Microtome (Leica RM 2125RT Austria), Centrifuge Machine (MSE 846307 England), Chemical balance (ACCULAB Sartorius group United States of America USA. Sensitivity = 0.001g), Microscope (Leitz Wetzlar HM-Lux Wetzlar, Germany), Am Scope (3.0.0.5 USA), Ocular/Stage micrometer (Graticules Ltd. Tombridge Kent England), Haemocytometer (Hawksley Christalite), Heamatoxylin, eosin, Bouin's fluid, neutral buffered formalin, Dissecting set, needles and syringes (1

ml, 5ml and 10 ml), Beakers, pipettes, centrifuge machine, glass slides, Spatula, pestle, mortar, cages, feeders, hand gloves, cotton wool, cover slips and surgical blades.

## **3.2 METHODS**

### **3.2.1 Experimental Design**

The Wistar Rats were housed in metal cages in the Animal House, Department of Human Anatomy, Ahmadu Bello University Zaria. (12 hours light/12 hours dark cycle) and were fed with standard feeds pellets (Growers mash, Vital Feed, Grand Cereal, Nigeria) and water *ad libitum*. They were acclimatized at the animal house for two weeks prior to the start of experiment. The Rats were randomized into four groups of five rats each. The rats in group I (control) were given distilled water while Group II-IV received the extract at (250mg/kg, 500 mg/kg and 1000mg/kg that corresponds with 5%, 10% and 20% of the LD<sub>50</sub> respectively) by oral intubation once daily for 35 days (Mehraban *et al.*, 2014).

### **3.2.2 Preparation of Extract**

The aqueous extraction of *Phoenix dactylifera* fruit was done in the Department of Pharmacognosy, Ahmadu Bello University Zaria, Kaduna State according to the method described by Al Quarawi, (2005). The fruit was opened and the fleshy part was oven dried at 50°C and grounded to powder. It was soaked in maceration apparatus with distilled water for 24 hours, filtered and allowed to settle down, it was then decanted and oven dried at 50°C.

### **3.2.3 Acute Toxicity (LD<sub>50</sub>) Study**

The acute toxicity (LD<sub>50</sub>) study of the aqueous extract of *Phoenix dactylifera* fruit was conducted as earlier described by Akunna *et al.*,(2012) using the Organization for Economic Cooperation and Development (OECD) guidance document on humane end points that should lessen the overall suffering of animals used in toxicity test. Four Wistar rats were divided into two groups of two rats each and were given aqueous extract of *Phoenix dactylifera* at 2500mg/kg and 5000mg/kg respectively and observed for 72 Hours whether there will be mortality.

**Table 3.1: Animal grouping, concentration and frequency of administration of *Phoenix dactylifera* to Male Wistar Rats**

<b>Groups</b>	<b>Number of Rats</b>	<b>Dosage</b>	<b>Duration</b>
<b>Group I</b>	5 Rats	Distilled Water	35 days
<b>Group II</b>	5 Rats	250mg/kg	35 days
<b>Group III</b>	5 Rats	500mg/kg	35 days
<b>Group IV</b>	5 Rats	1000mg/kg	35 days

### **3.2.4 Histology**

All Animals were sacrificed on the 36<sup>th</sup> day by cervical dislocation and the testes, epididymis, seminal vesicle and prostate gland were dissected and weighed using chemical balance (ACCULAB Sartorius group USA. Sensitivity =0.001g). The testes were fixed in bouin's fluid while the epididymides, seminal vesicles and prostate glands were fix in neutral buffered formalin (NBF), all the tissue were embedded in paraffin

wax, sectioned at 5µm using rotary microtome (Leica RM 2125 RT Austria) and stained with heamatoxylin and eosin (H and E).

#### **3.2.4.1 Tissue processing**

The tissues were processed in the Department of Histopathology, Ahmadu Bello University Zaria Kaduna State, Nigeria.

- i. Tissues were placed in graded Alcohol 30%, 50%, 70% and 90% for 2 minutes each.
- ii. Tissues were embedded in molten paraffin wax using tissue blocks and allowed to cool and solidify.
- iii. Tissues were sectioned using rotary microtome at 5µm.
- iv. The sections were cleared in two changes of xylene for 3 minutes each.
- v. The sections were placed in graded alcohol 90%, 70% and 50% for 2 minutes each.

#### **3.2.4.2 Heamatoxylin and Eosin staining**

- i. The sections were placed in water.
- ii. Sections were stained in Mayer Heamatoxylin for 10 minutes.
- iii. Washed in water.
- iv. Bleached in tap water for 2 minutes.
- v. Washed in tap water.
- vi. The sections were counter stained with eosin for 60 seconds.
- vii. The sections were differentiated through changes of ethanol.
- viii. Placed in absolute alcohol for 2 minutes.

- ix. Cleared in 2 changes of xylene for 2 minutes each.
- x. Mounted with DPX.

### **3.2.5 Morphometric Study**

The seminiferous tubular diameter, size of interstices and epididymal epithelial thickness was measured using a standardized ocular micrometer (Graticules Ltd. Tonbridge Kent England) in the Department of Microbiology, Ahmadu Bello University Zaria Kaduna State, Nigeria. Five slides were studied for each rat and five different measurements were taken on each slide. The average of measurements from the five slides was taken as the value of each rat. Seminiferous tubular diameter was measured at X100 while size of interstices and epididymal epithelial thickness were measured at X400.

- i. A stage micrometer was placed on the microscope with the ocular micrometer in eyepiece.
- ii. The number of grids/lines on the ocular micrometer that corresponds to X $\mu$ m on the stage micrometer was noted.
- iii. The stage micrometer was removed and a slide was placed on the microscope.
- iv. The seminiferous tubules, Interstices and epididymal epithelium were aligned with the grids/lines of the ocular micrometer when viewed under the microscope for each slide.

- v. The number of grids/lines were counted and recorded for seminiferous tubule, interstices and epididymal epithelium. (for the interstices the average of length and breadth was taken as the measurement)
- vi. The values obtained were used to calculate the seminiferous tubular diameter, size of interstices and epididymal epithelial thickness in  $\mu\text{m}$ .

### 3.2.6 Semen Analysis

The semen analysis was conducted according to the method described by Mehraban *et al.*, (2014) right epididymis was teased and placed in a vessel containing 5ml (9.5%) normal saline to make a suspension, a pipette was used to introduce the suspension onto an improved Neubauer haemocytometer (Hawksley Christalite) that was fitted with cover slip and placed under the microscope for counting. A smear was made on a glass slide from which cresyl fast violet staining and Feulgen nuclear reaction was done to study sperm morphology by counting in different fields.

#### 3.2.6.1 Cresyl fast violet Staining: Adapted from Kellet, (1963)

##### Solutions

- |      |                     |          |
|------|---------------------|----------|
| i.   | Cresyl fast violet  | 1 g      |
| ii.  | Distilled water     | 100 mls  |
| iii. | Glacial Acetic Acid | 0.25 mls |

##### Methods

- i. Slides were placed in water.

- ii. Stained in Cresyl fast violet solution for 30 minutes.
- iii. Rinsed in water.
- iv. Differentiated in 96% alcohol.
- v. Cleared in 2 changes of xylene for 2 minutes each.
- vi. Mounted with DPX.

### **3.2.6.2 Feulgen Nuclear Reaction:** Adapted from fuelgen and Rossenbeck, (1924)

#### **Solution A** (1 M Hydrochloric acid)

- |     |                                  |         |
|-----|----------------------------------|---------|
| i.  | Hydrochloric acid (concentrated) | 8.5 ml  |
| ii. | Distilled water                  | 91.5 ml |

#### **Solution B** (Schiff reagent)

#### **Methods**

- i. Slides were placed in water.
- ii. Rinsed in 1 M HCL at room temperature.
- iii. Placed in 1 M HCL at 60°C for 8 minutes.
- iv. Rinsed in 1 M HCL at room temperature for 60 seconds.
- v. Slides were transferred to schiff's reagent for 45 minutes.
- vi. Rinsed in distilled water.
- vii. Dehydrated through graded alcohol 50%, 70%, 95% for 2 minutes each.
- viii. Cleared in xylene.
- ix. Mounted with DPX.

### **3.2.7 Hormonal Profile**

Blood sample was collected in a plain bottle from the heart using the cardiac puncture method, it was centrifuged at 2500 rpm xg for 5 minutes using centrifuge machine (MSE 846307 England) and the serum obtained was used to determine the levels of testosterone, FSH and LH using enzyme-linked immunosorbent assay(ELISA) kit (Testosterone) Crystal Chem Inc. USA and Elabscience Biothecnology Ltd. China (FSH and LH) according to the manufacturer's instruction.

### **3.2.8 Statistical Analysis**

The data are expressed as Mean  $\pm$  SEM (standard error of the mean) and were analyzed using Instat Statistic Package version 3 (Graph Pad). One way analysis of Variance (ANOVA) was used to compare the mean difference between and within the groups and a P-value(P<0.05) was considered statistically significant.

## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 Acute Toxicity (LD<sub>50</sub>) Study

There was no death in rats treated with aqueous extract of *Phoenix dactylifera* at 2500mg/kg and 5000mg/kg after 72 hours of treatment. The extract has wide safety margin.

#### 4.2 Effect of Aqueous Extract of *Phoenix dactylifera* on the Hormonal Profiles of Male Wistar Rats.

Table 4.1 showed a significant decrease in the levels of testosterone between the control (2.02±0.07ng/ml) and the rats treated with aqueous *Phoenix dactylifera* extract at 250mg/kg, 500mg/kg and 1000mg/kg (1.14±0.09ng/ml, 1.00±0.11 ng/ml and 1.14±0.09ng/ml, respectively) at P≤0.05 with no significant difference in FSH and LH levels between the control and the treated groups.

**Table 4.1: Levels of Testosterone, FSH and LH of Rats treated with Aqueous Extract of *Phoenix dactylifera***

<b>Groups</b>	<b>Testosterone (ng/ml)</b>	<b>FSH (mlμ/ml)</b>	<b>LH (mlμ/ml)</b>
<b>Control</b>	2.02±0.07 <sup>a</sup>	0.41±0.015	0.59±0.012
<b>250 mg/kg</b>	1.14±0.09 <sup>b</sup>	0.39±0.014	0.58±0.012
<b>500 mg/kg</b>	1.00±0.11 <sup>b</sup>	0.39±0.017	0.58±0.012
<b>1000 mg/kg</b>	1.14±0.09 <sup>b</sup>	0.40±0.013	0.58±0.008

All values expressed as Mean±SEM. Values in the same column with different superscript are significantly different at  $P \leq 0.05$  using one-way ANOVA.

#### **4.3 Effect of Aqueous Extract of *Phoenix dactylifera* on Body Weight, Organ Weight and Epididymal Sperm Count, Motility and Morphology of Male Wistar Rats**

Table 4.2 showed no significant difference between the control and treated groups for the body and organ weights (testes, epididymis, seminal vesicles and prostate glands) of the rats treated with aqueous extract of *Phoenix dactylifera* but there was significant decrease in sperm count between the control group ( $130.14 \pm 8.18 \times 10^6/\text{ml}$ ) and groups treated with the extract at 250mg/kg, 500mg/kg and 1000mg/kg body weight  $93.90 \pm 3.08 \times 10^6/\text{ml}$ ,  $91.15 \pm 3.33 \times 10^6/\text{ml}$  and  $93.55 \pm 10.59 \times 10^6/\text{ml}$ , respectively at  $P \leq 0.05$ . There was a significant decrease in sperm motility in the rats treated with the extract at 250mg/kg, 500mg/kg and 1000mg/kg ( $79.50 \pm 8.51 \times 10^6/\text{ml}$ ,  $70.75 \pm 4.03 \times 10^6/\text{ml}$  and  $68.60 \pm 8.91 \times 10^6/\text{ml}$ , respectively) compared to that of the control ( $116.60 \pm 7.43 \times 10^6/\text{ml}$ ) at  $P \leq 0.05$ . There was also significant decrease in sperm morphology (normal sperm cells) in the rats treated with the extract at 250mg/kg, 500mg/kg and 1000mg/kg ( $74.49 \pm 1.38\%$ ,  $79.59 \pm 0.75\%$  and  $70.58 \pm 1.50\%$ , respectively) compared to that of the control group ( $87.16 \pm 1.94\%$ ) at  $P \leq 0.05$ .

**Table 4.2: Body Weight, Organs Weight and Epididymal Sperm Count, Sperm Motility and Sperm Morphology of Rats treated with Aqueous Extract of *Phoenix dactylifera***

Parameters	Groups			
	Control	250mg/kg	500mg/kg	1000mg/kg
<b>Body weight (g)</b>	77.20±2.69	60.00±4.52	82.00±2.98	71.80±7.23
<b>Left Testis (g)</b>	0.740±0.033	0.748±0.012	0.692±0.016	0.638±0.024
<b>Right Testis (g)</b>	0.748±0.028	0.740±0.027	0.686±0.018	0.618±0.023
<b>Left Epididymis (g)</b>	0.506±0.292	0.196±0.014	0.160±0.014	0.180±0.014
<b>Right Epididymis (g)</b>	0.226±0.023	0.212±0.013	0.156±0.016	0.190±0.018
<b>Left Seminal gland (g)</b>	0.094±0.016	0.136±0.025	0.118±0.017	0.140±0.014
<b>Right Seminal gland(g)</b>	0.088±0.017	0.112±0.019	0.096±0.016	0.144±0.028
<b>Prostate gland (g)</b>	0.278±0.013	0.288±0.055	0.228±0.028	0.316±0.058

<b>Sperm count <math>\times 10^6/\text{ml}</math></b>	130.14 $\pm$ 8.18 <sup>a</sup>	93.90 $\pm$ 3.08 <sup>b</sup>	91.15 $\pm$ 3.33 <sup>b</sup>	93.55 $\pm$ 10.59 <sup>b</sup>
<b>Sperm Motility<math>\times 10^6/\text{ml}</math></b>	116.60 $\pm$ 7.43 <sup>a</sup>	79.50 $\pm$ 8.51 <sup>b</sup>	70.75 $\pm$ 4.03 <sup>b</sup>	68.60 $\pm$ 8.91 <sup>b</sup>
<b>Sperm Morphology</b>	87.16 $\pm$ 1.94 <sup>a</sup>	74.49 $\pm$ 1.38 <sup>b</sup>	79.59 $\pm$ 0.75 <sup>b</sup>	70.58 $\pm$ 1.50 <sup>b</sup>
<b>Normal sperm (%)</b>				

All values expressed as Mean $\pm$ SEM. Values with different super script in the same row are significantly different at  $P \leq 0.05$  using one-way ANOVA.

#### 4.4 Sperm Morphology

The result showed significant decrease in percentage of sperm cells with normal morphology in rats treated with aqueous extract of *Phoenix dactylifera* at 250mg/kg, 500mg/kg and 1000mg/kg compared to that of control rats. The abnormal sperm cells observed include no tail, no head and coiled tails (Plates 4.1, 4.2 and 4.3).



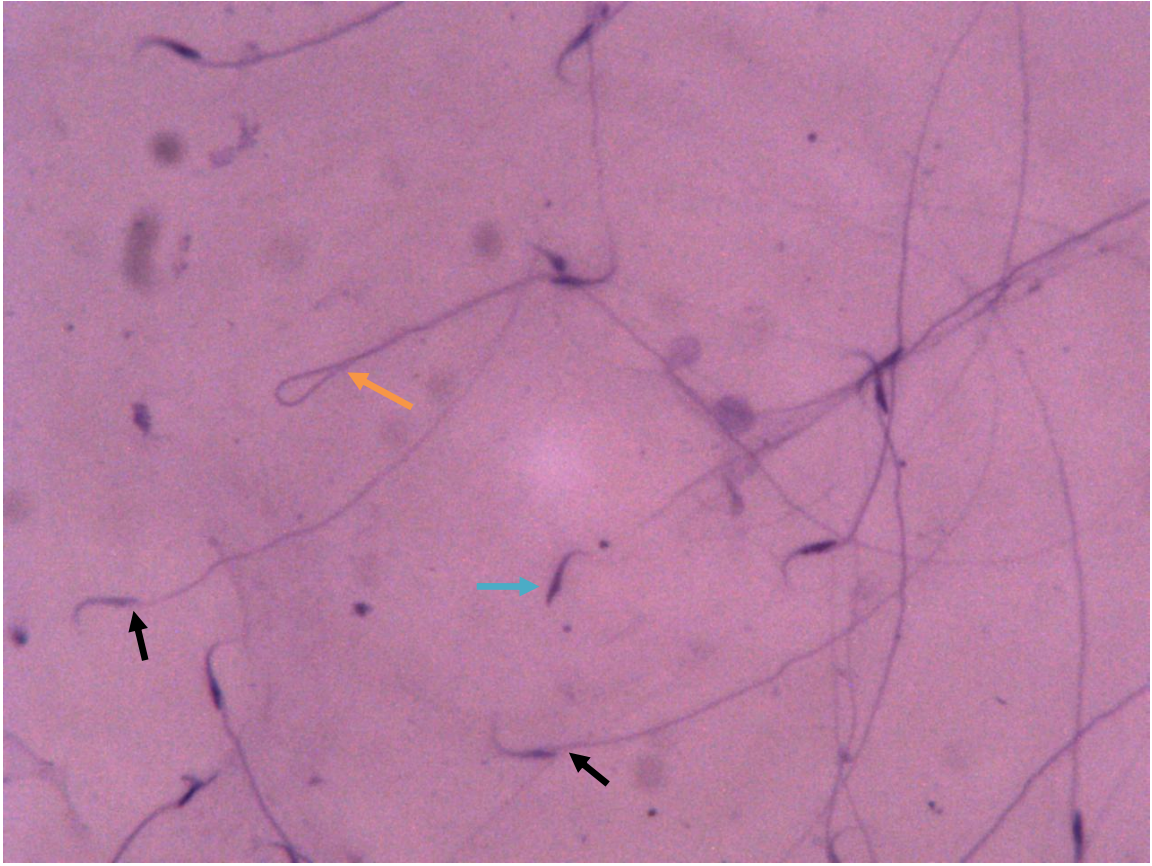


Plate 4.1: Photomicrograph of sperm cells of rats treated with *Phoenix dactylifera extract* at 1000 mg/kg in a field; showing normal sperm cells (black arrows), no tail (light blue arrow) and coiled tail (orange arrow) cresyl fast violet stain x400.

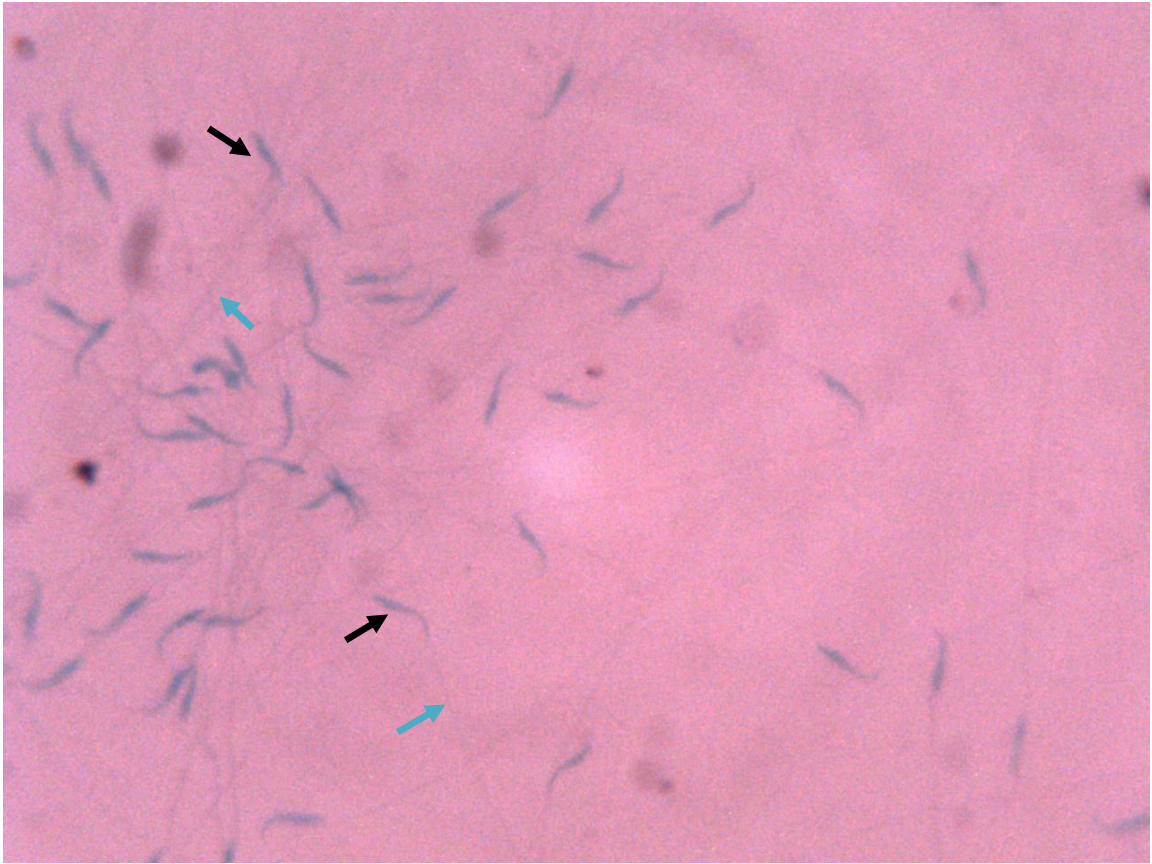


Plate 4.2: Photomicrograph of sperm cells of control rats in a field; showing normal cells with the head/mid piece (black arrows) stained green and a pale tail (light blue arrows) fuelgen stain x400.

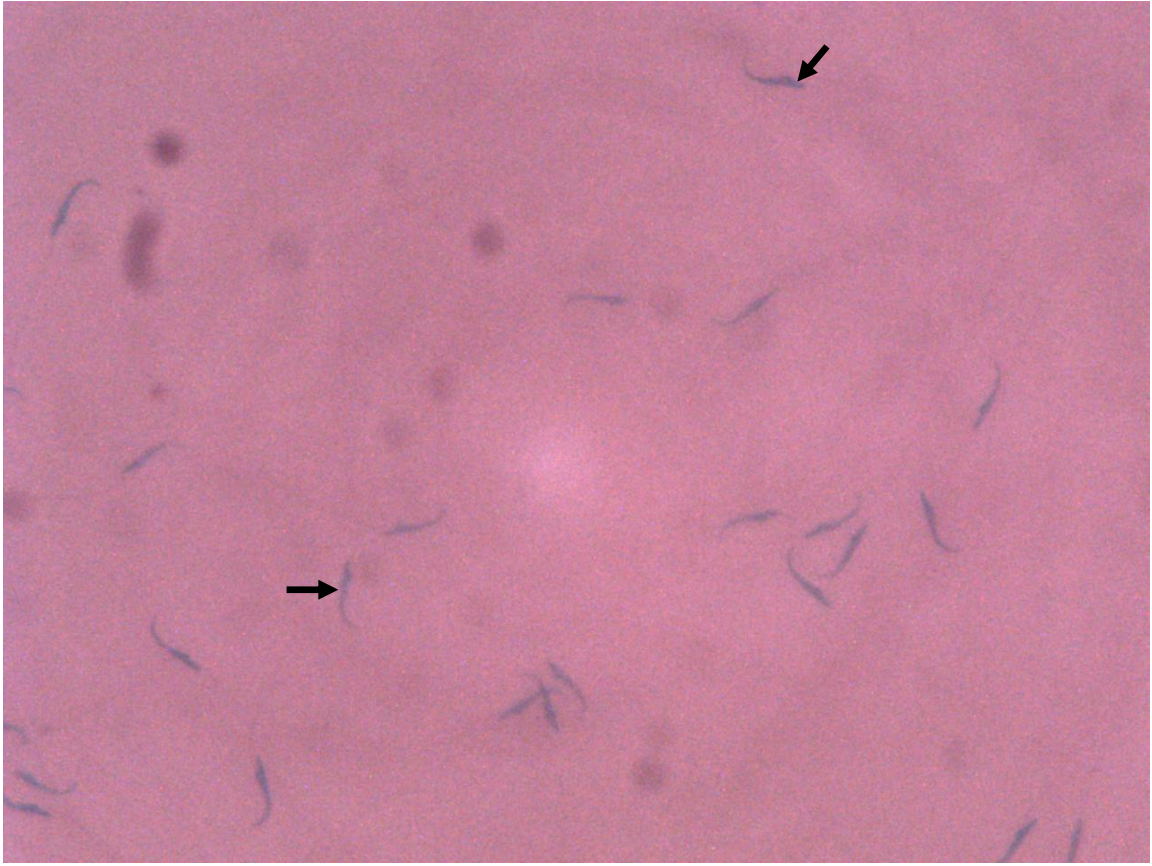


Plate 4.3: Photomicrograph of sperm cells of rats treated with *Phoenix dactylifera* at 1000 mg/kg in a field; showing scanty sperm cells without tail (black arrows) fuelgen stain x400.

#### 4.5 Histopathology

The photomicrograph of the testes of control rats showed the typical structure of the seminiferous tubules illustrating the stages of spermatogenesis from spermatogonia to mature sperm cells (Plate 4.4). The testes of rats treated with *Phoenix dactylifera* at 250 mg/kg and 500mg/kg showed distortion of spermatogenic cells (Plates 4.5 and 4.6) compared to that of the control while the rats treated with the extract at 1000 mg/kg showed degeneration of Leydig cells and seminiferous tubular capsule (Plate 4.7) compared to that of the control (Plate 4.4).

The epididymis of control rats showed the normal architecture with the ducts and epithelial lining of simple columnar epithelium and normal sperm cells within the lumen of ducts (Plate 4.8). The epididymis of rats treated with *Phoenix dactylifera* extract at 250 mg/kg, 500 mg/kg & 1000 mg/kg showed degeneration of sperm cells and vacuolated epithelial cells (Plates 4.9, 4.10 and 4.11).

The seminal vesicles of the control rats showed no difference from that of the rats treated with *Phoenix dactylifera* extract at 250 mg/kg, 500 mg/kg and 1000mg/kg with all illustrating normal glands and smooth muscle layers (Plates 4.12, 4.13, 4.14 and 4.15).

The prostate glands of control rats showed normal glands, connective tissues and smooth muscle layers (Plate 4.16). The prostate of rats treated with *Phoenix dactylifera* at 250mg/kg showed degeneration of connective tissues and distorted glands (Plate 4.17) while that of rats treated with the extract at 500 mg/kg and 1000mg/kg showed distorted glands without the normal convolutions (Plates 4.18 and 4.19).

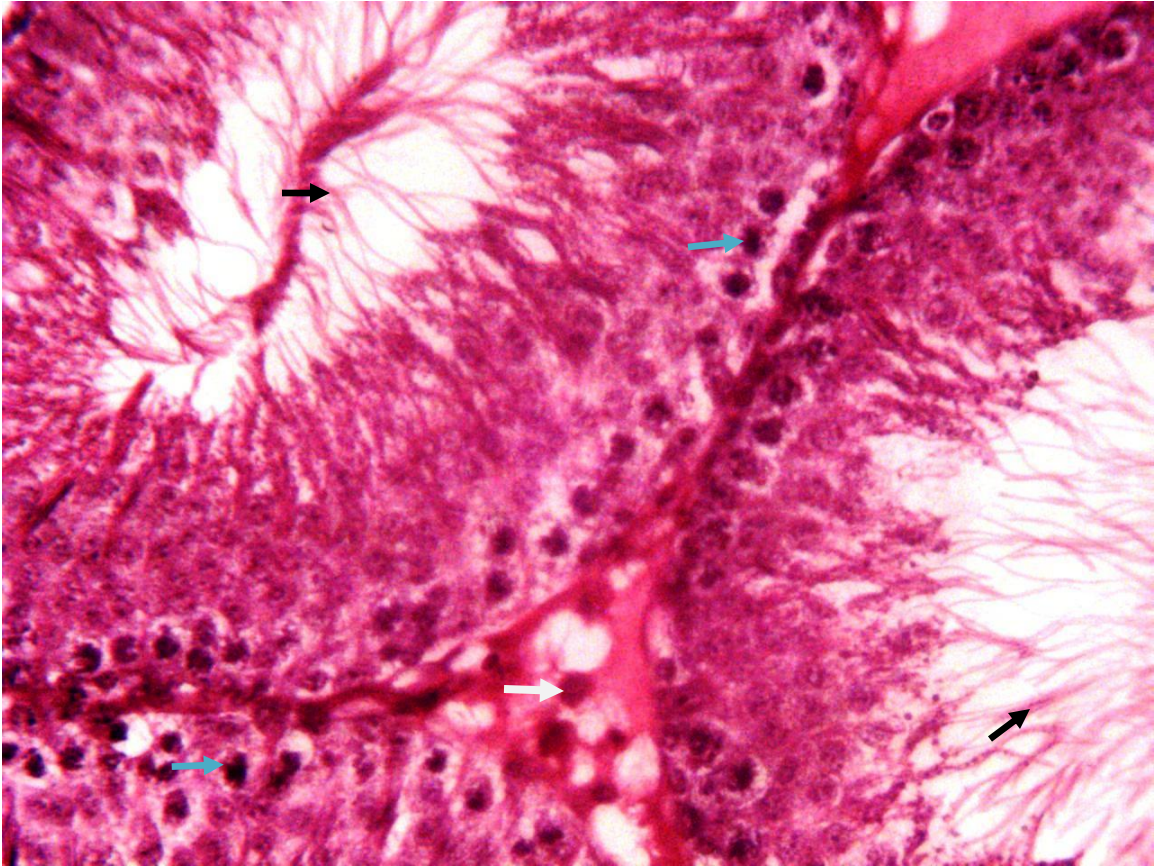


Plate 4.4: Photomicrograph of transverse section of the testis of control rats; illustrating the typical structure of the seminiferous tubule showing the stages of spermatogenesis, spermatogonia (light blue arrows), sperm cells (black arrows), and the interstitial cells of Leydig (white arrow) H and E x250.

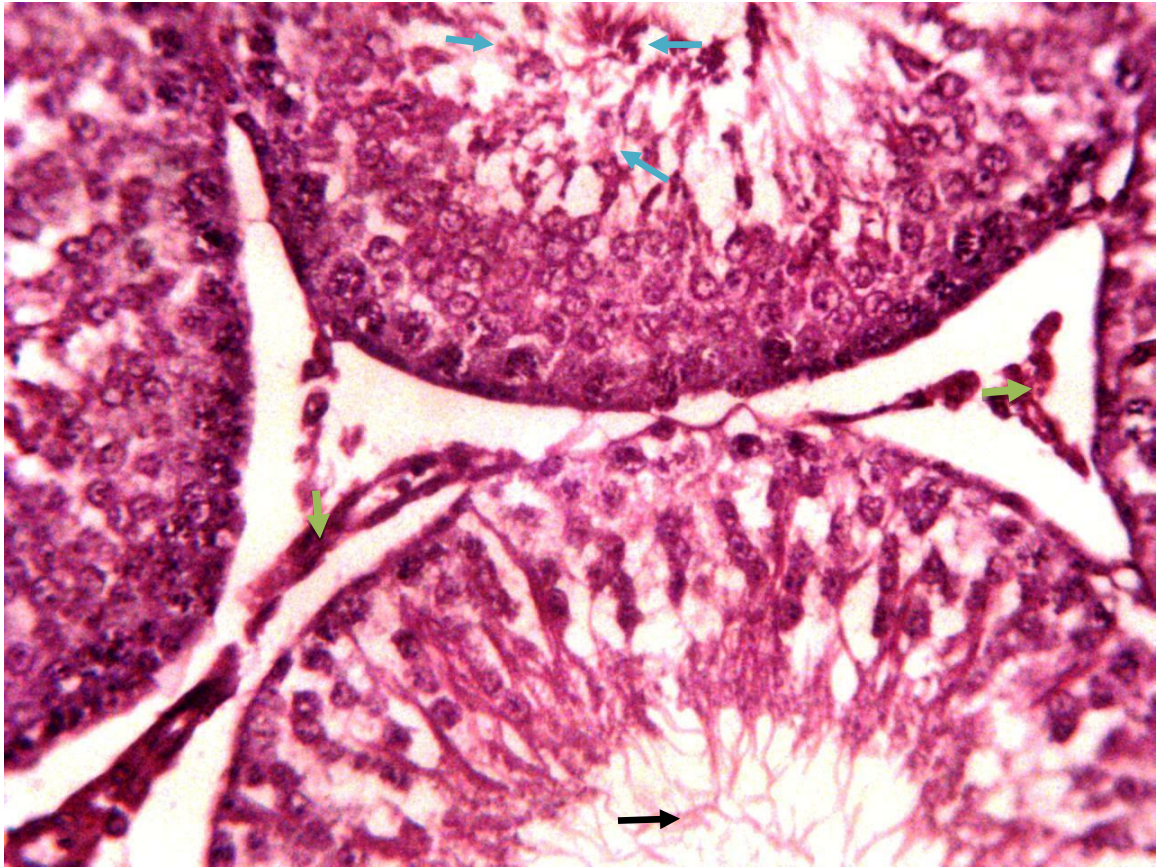


Plate 4.5: Photomicrograph of transverse section of testis of rats treated with aqueous *Phoenixdactylifera* extract at 250mg/kg; showing scanty sperm cells in the lumen of seminiferous tubule (black arrow), distortion of spermatogenic cells (light blue arrows) and compacted interstices (green arrows) H and E x250.

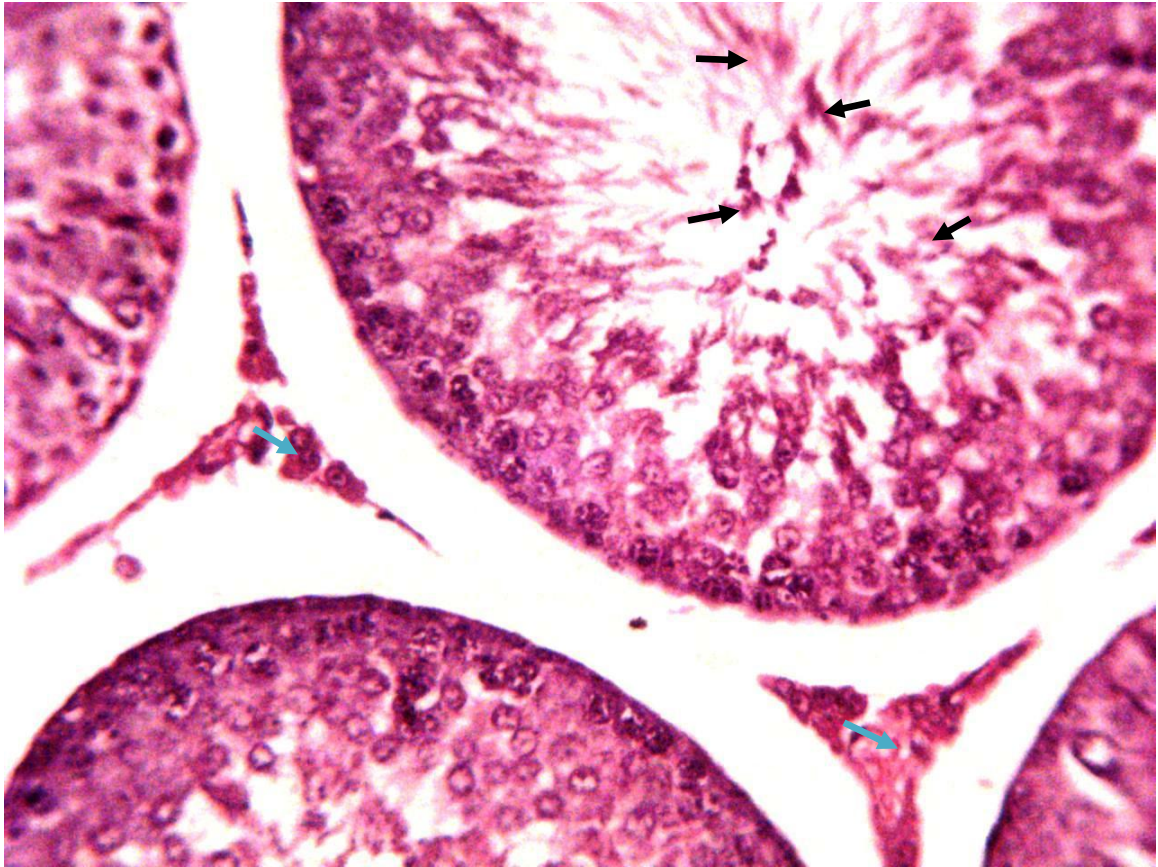


Plate 4.6: Photomicrograph of transverse section of the testis of rats treated with aqueous extract of *Phoenixdactylifera* at 500mg/kg; showing distorted sperm cells (black arrows) within the lumen of seminiferous tubule and compacted interstices (Light blue arrows) H and E x250.

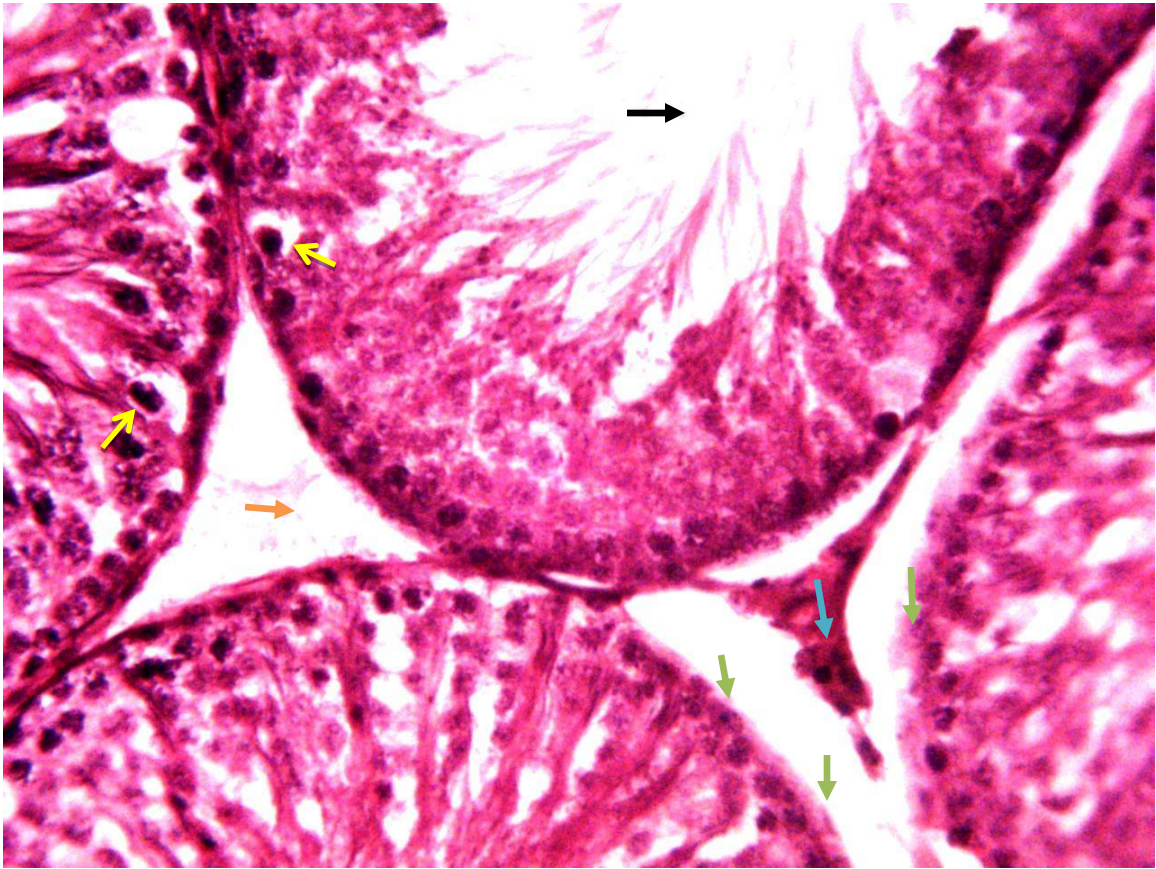


Plate 4.7: Photomicrograph of transverse section of testis of rats treated with aqueous *Phoenix dactylifera* extract at 1000mg/kg; showing seminiferous tubule with scanty sperm cells (black arrow), degeneration of capsule (green arrows), compacted interstices (light blue arrow), vacuolated cells (yellow arrows) and degeneration of leydig cells (orange arrow)H and E x250.

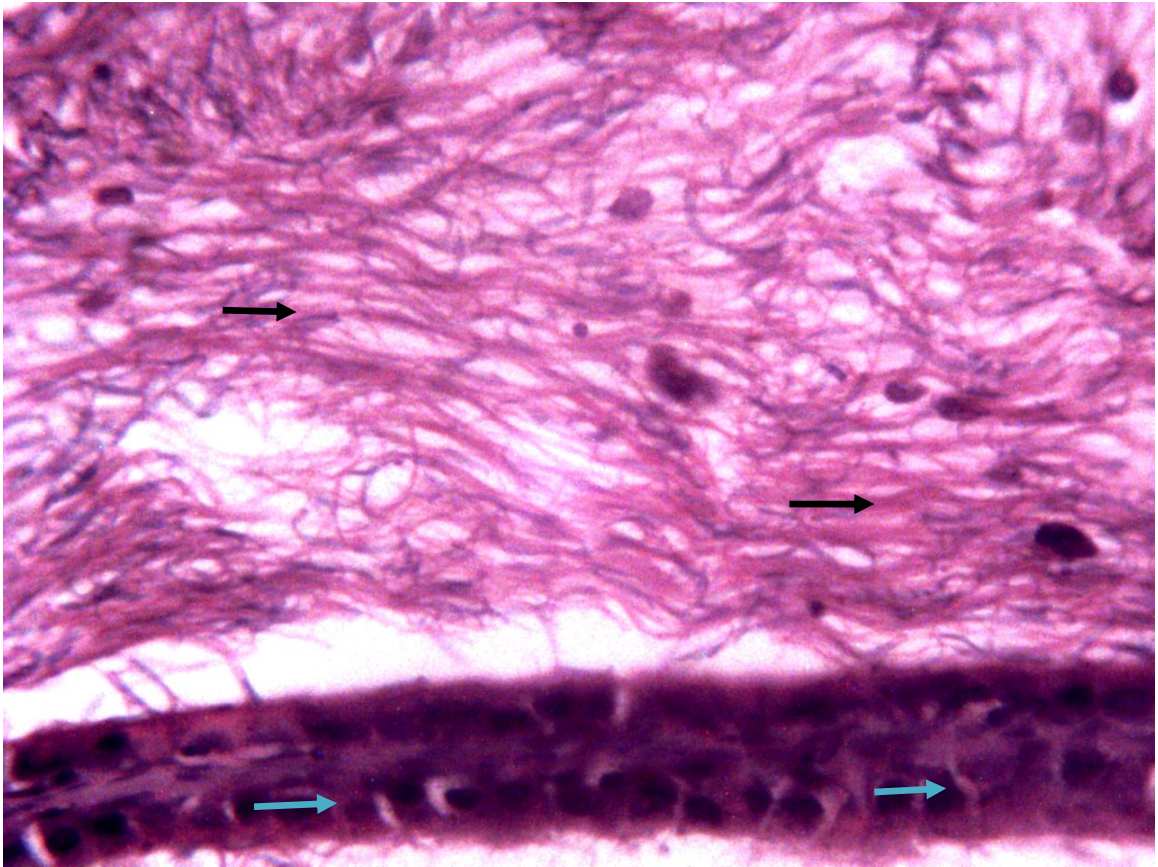


Plate 4.8: Photomicrograph of transverse section of epididymis of control rats; showing the normal architecture with normal sperm cells (black arrows) and epithelial lining of simple columnar epithelium (light blue arrows) H and E x400.

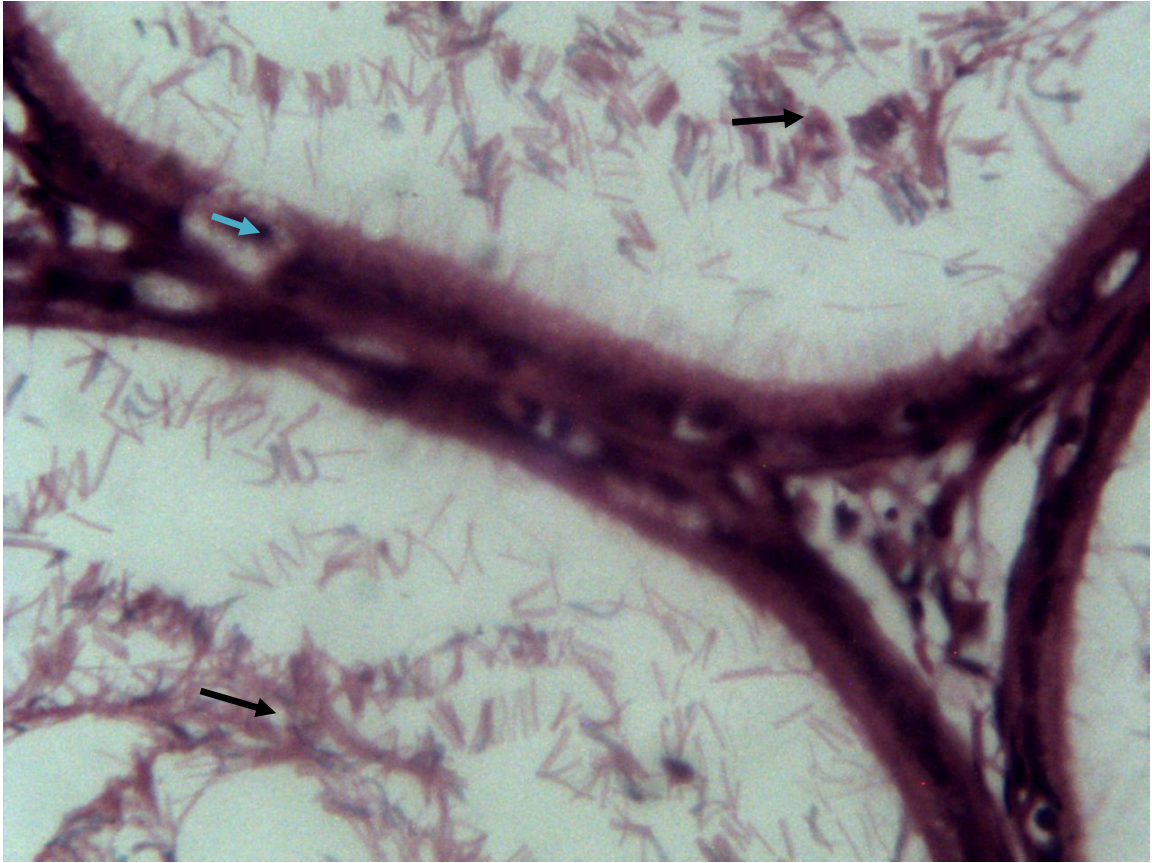


Plate 4.9: Photomicrograph of transverse section of epididymis of rats treated with aqueous extract of *Phoenix dactilifera* at 250mg/kg showing scanty and distorted sperm cells (black arrows) and vacuolated epithelial cell (Light blue arrow) H and E x400.

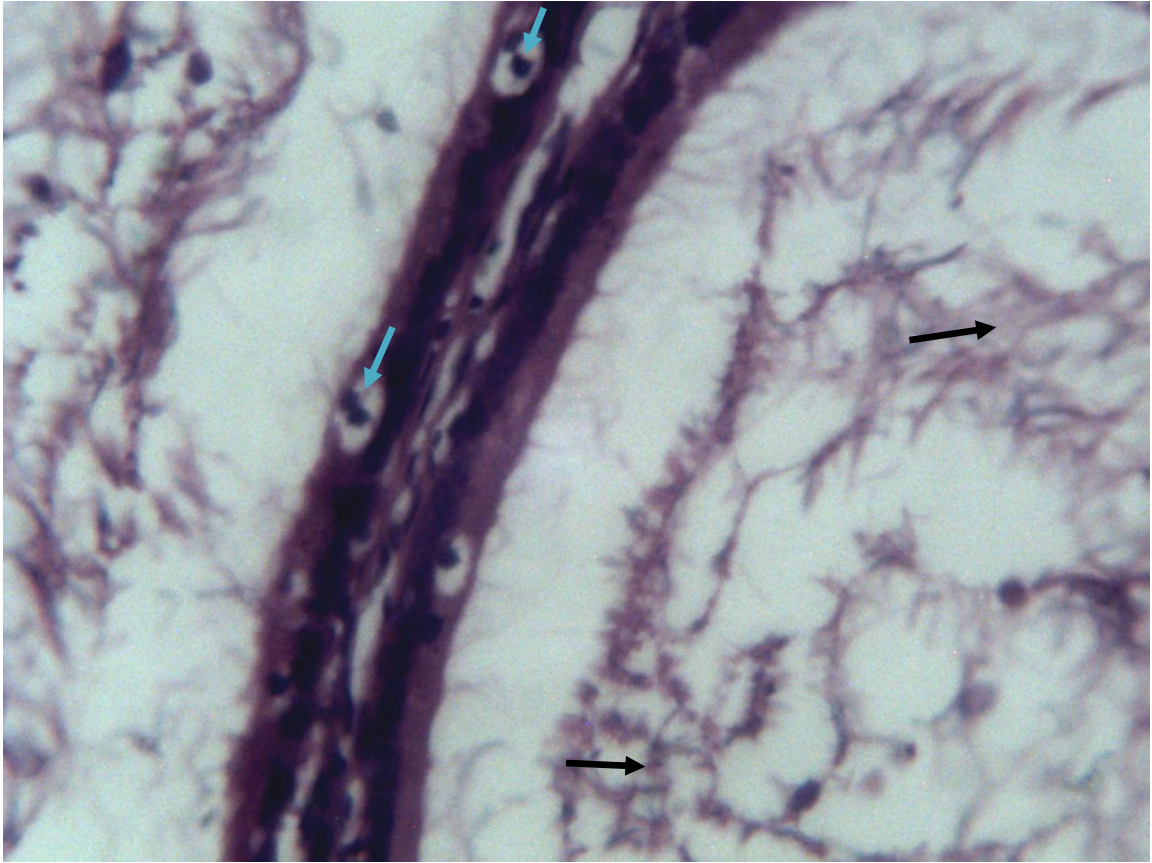


Plate 4.10: Photomicrograph of transverse section of epididymis of rats treated with *Phoenix dactylifera* at 500mg/kg; showing the lumen with scanty and distorted sperm cells (black arrows) and vacuolated epithelial cells (light blue arrows) H and E x400.

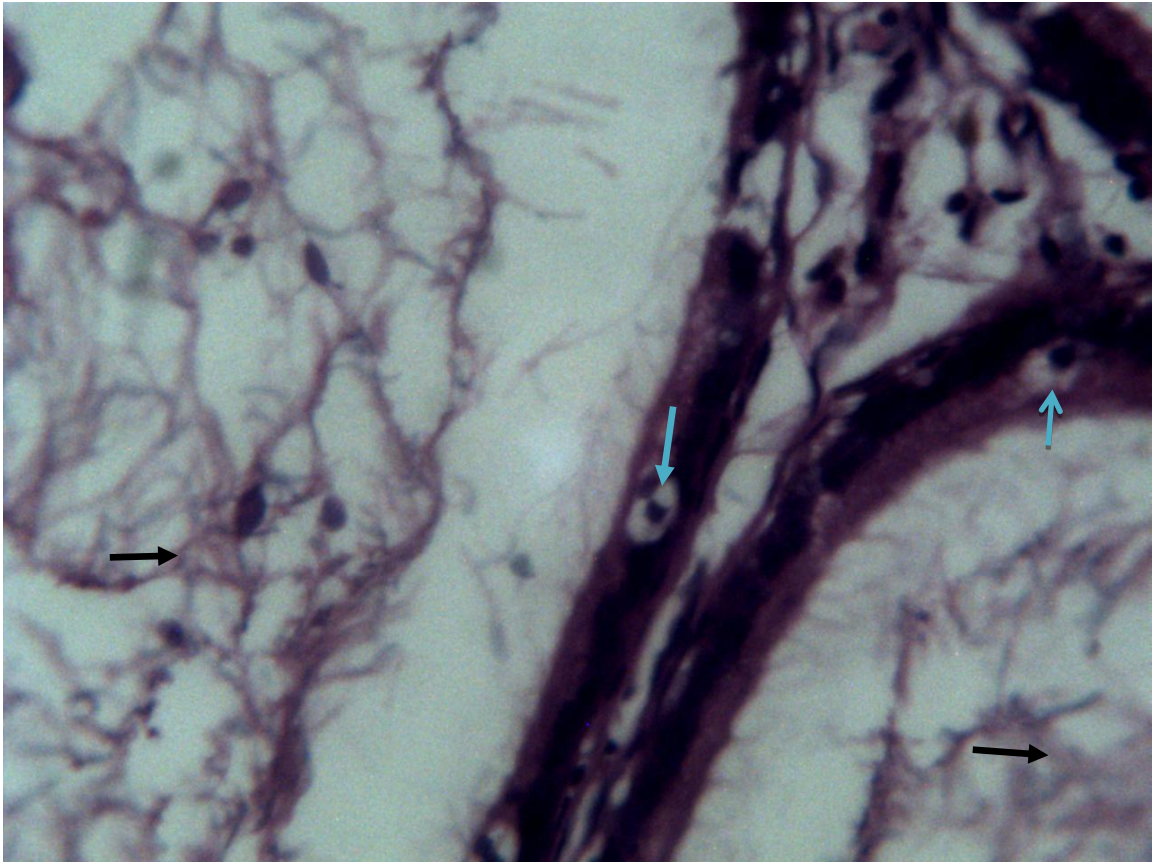


Plate 4.11: Photomicrograph of transverse section of epididymis of rats treated with aqueous extract of *Phoenix dactylifera* at 1000mg/kg; showing distorted and scanty sperm cells(black arrows),and epithelium with vacuolated cells (Light blue arrow)H and E x400.

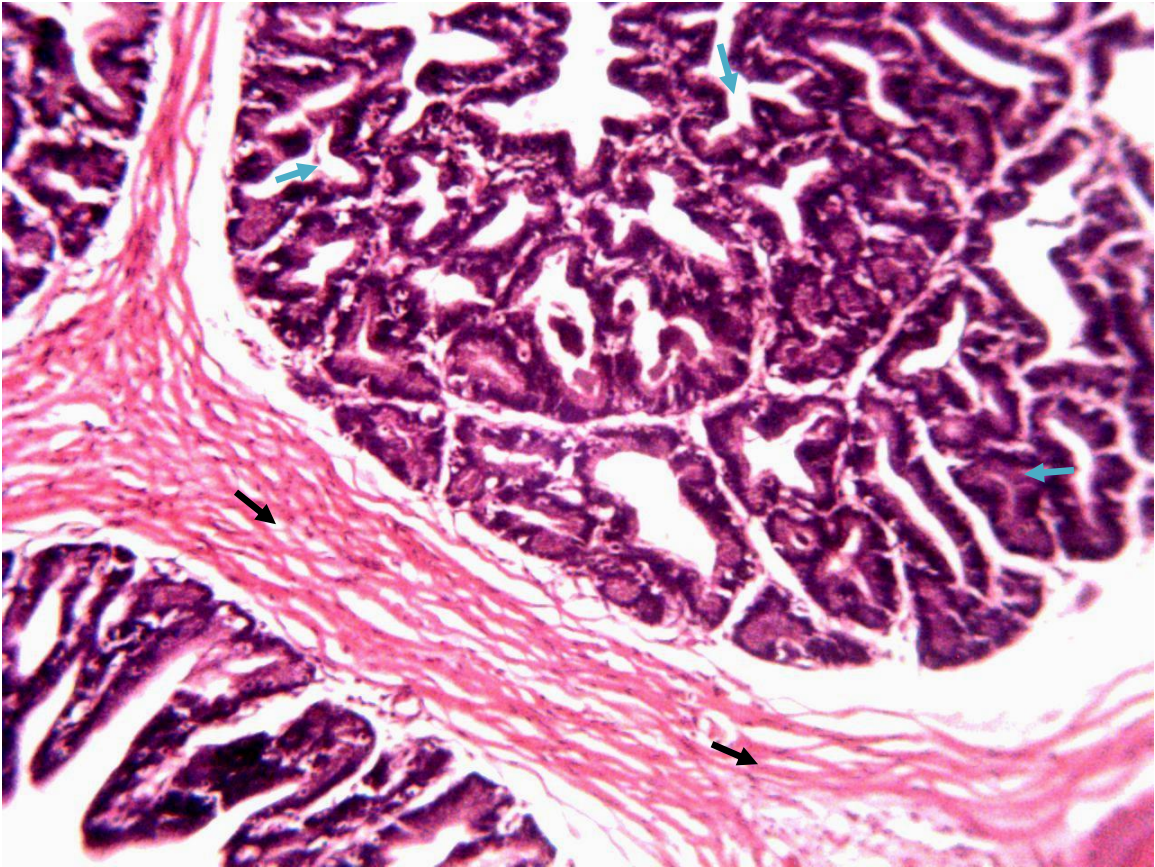


Plate 4.12: Photomicrograph of transverse section of seminal vesicle of control rats; illustrating the typical structure of the seminal vesicle showing the glands (light blue arrows) and smooth muscle (black arrows) H and E x100.

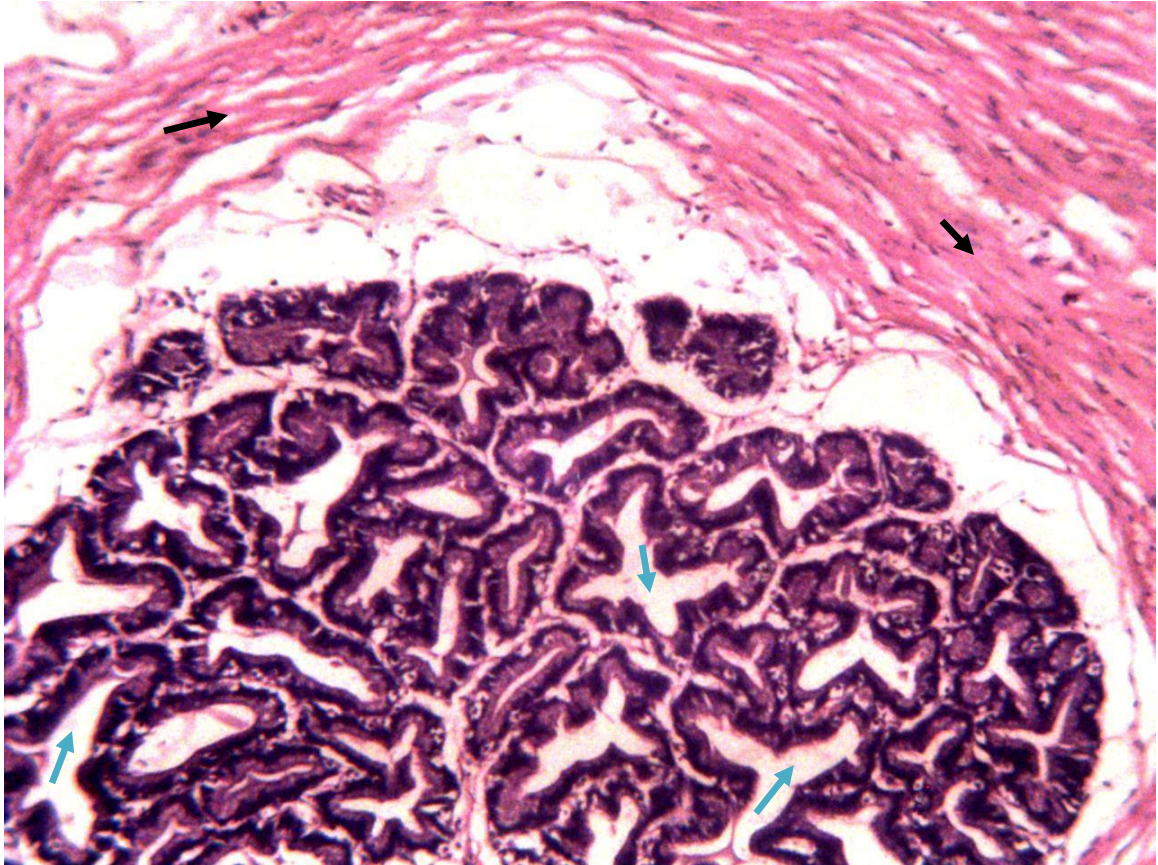


Plate 4.13: Photomicrograph of transverse section of seminal vesicle of rats treated with aqueous extract of *Phoenix dactylifera* at 250mg/kg; showing the normal architecture with normal glands (light blue arrows) and smooth muscle layer (black arrows) H and E x100.

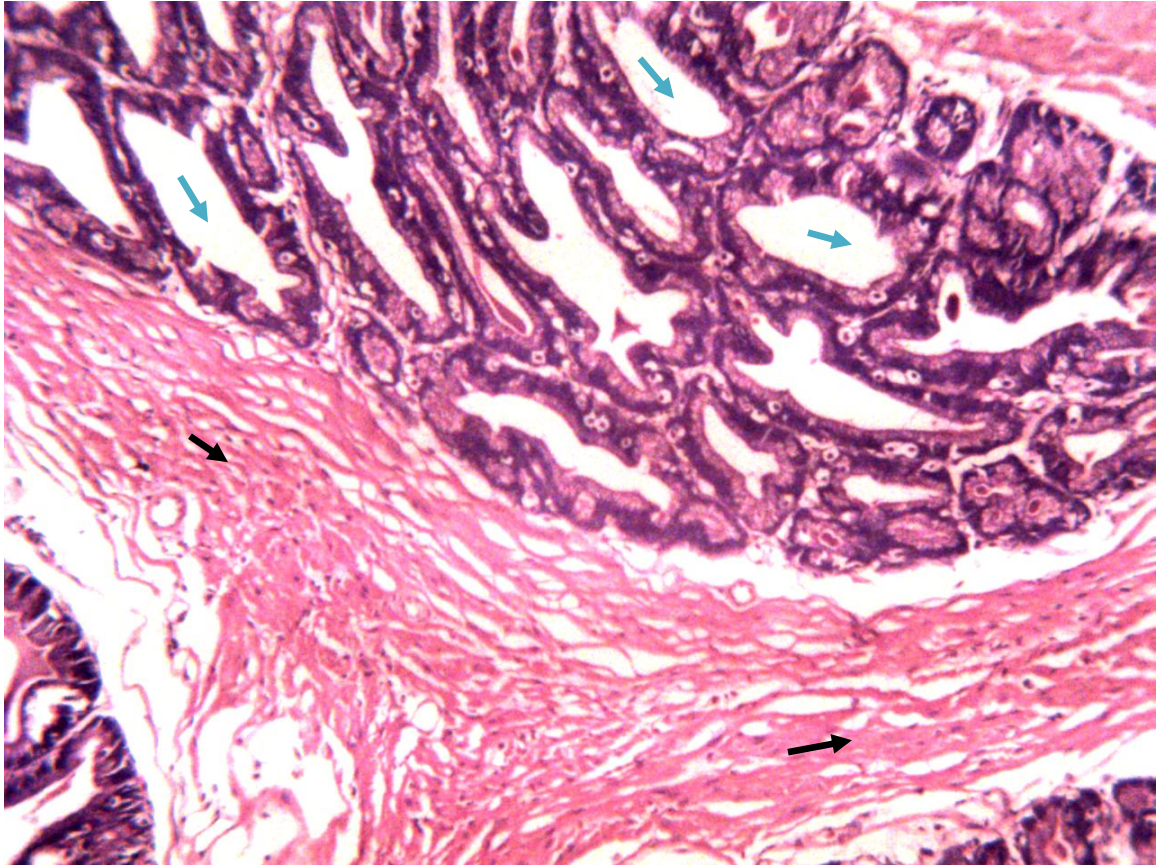


Plate 4.14: Photomicrograph of transverse section of seminal vesicle of rats treated with aqueous extract of *Phoenix dactylifera* at 500mg/kg showing normal glands (light blue arrows) and smooth muscles (black arrows) H and E x100.

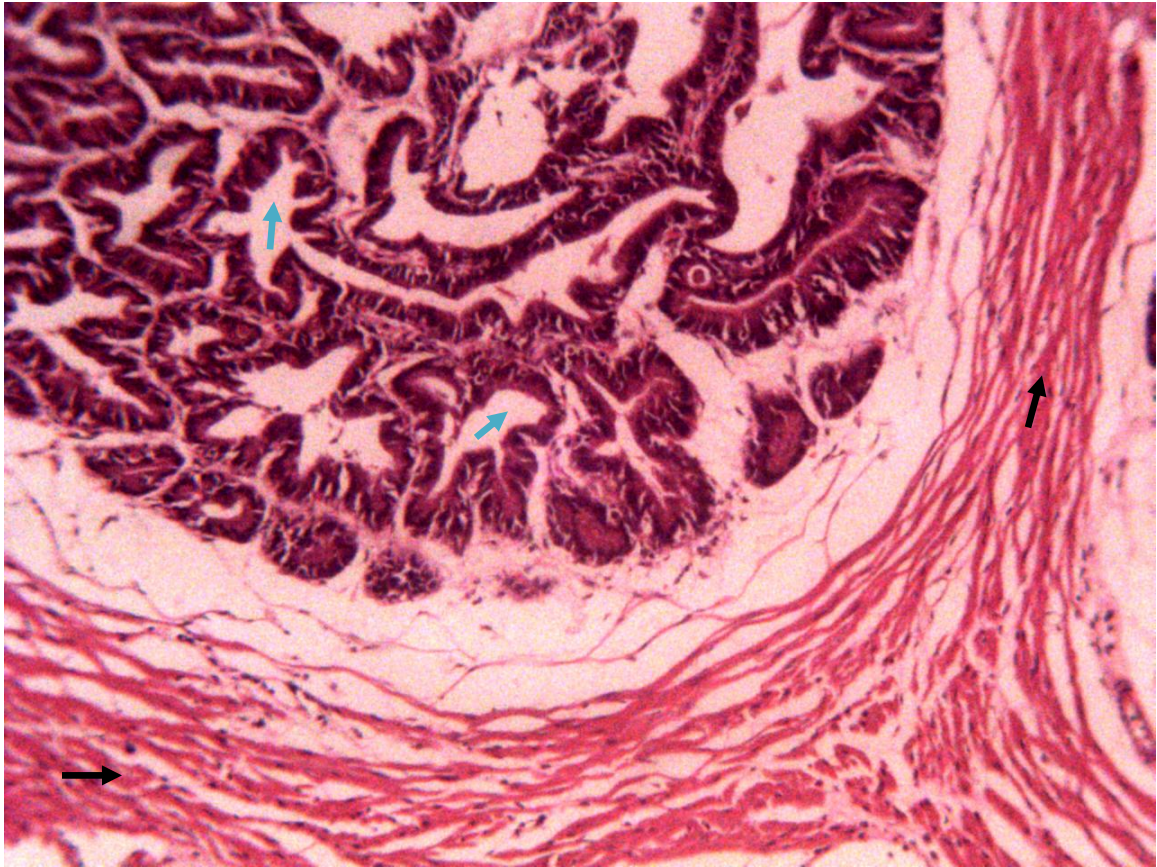


Plate 4.15: Photomicrograph of transverse section of seminal vesicle of rats treated with aqueous extract of *Phoenix dactylifera* at 1000mg/kg; showing normal glands (light blue arrows) and smooth muscles (black arrows) H and E x100.

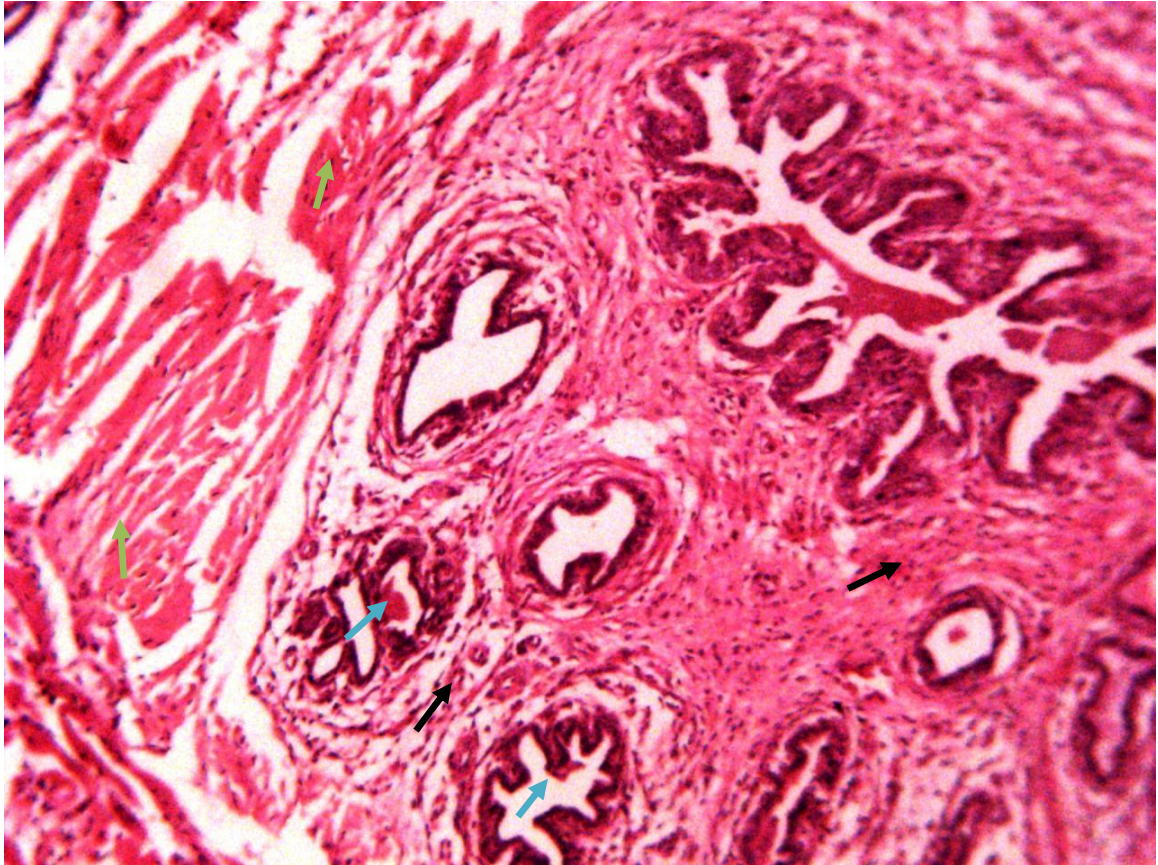


Plate 4.16: Photomicrograph of prostate gland of control rats; illustrating the typical structure with normal glands (light blue arrows), connective tissues (black arrows) and smooth muscles (green arrows) H and E x100.

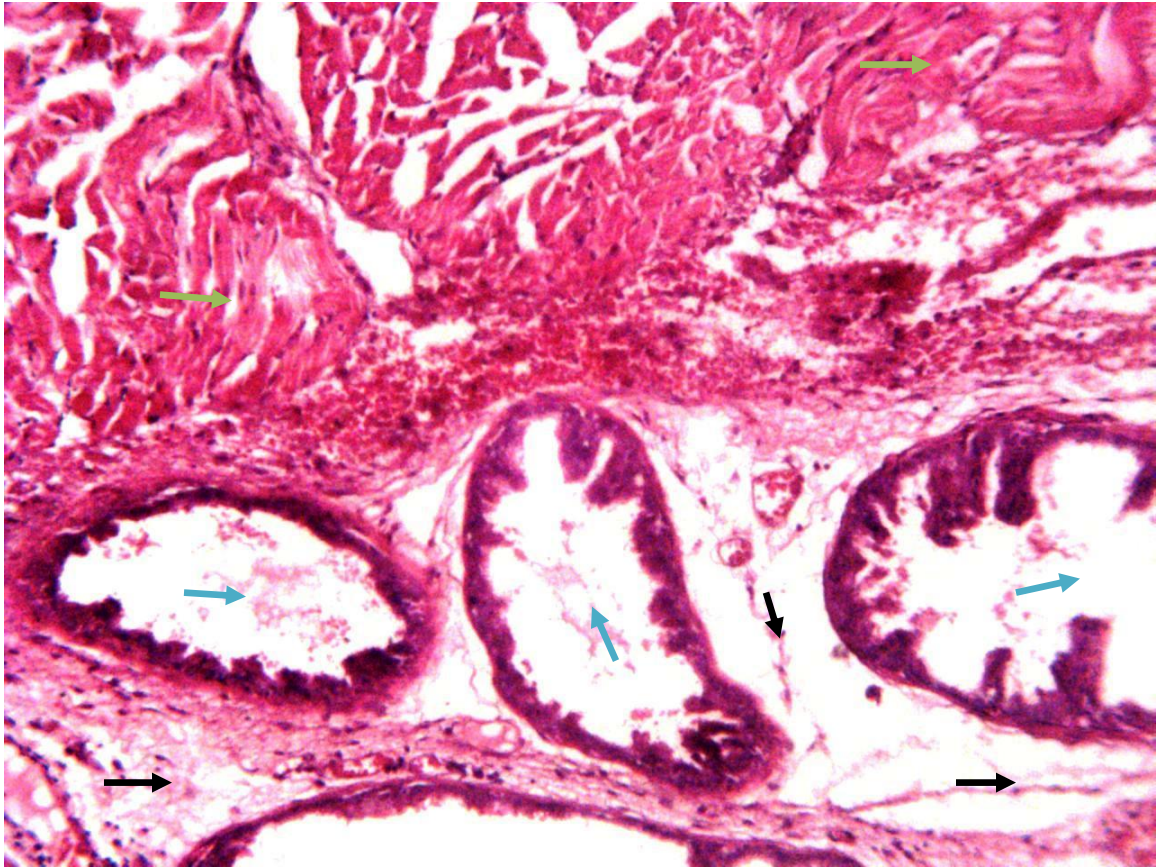


Plate 4.17: Photomicrograph of prostate gland of rats treated with aqueous extract of *Phoenix dactylifera* at 250mg/kg showing normal smooth muscles (green arrows), degenerated connective tissues (black arrows) and distorted glands (light blue arrows) H and E x100.

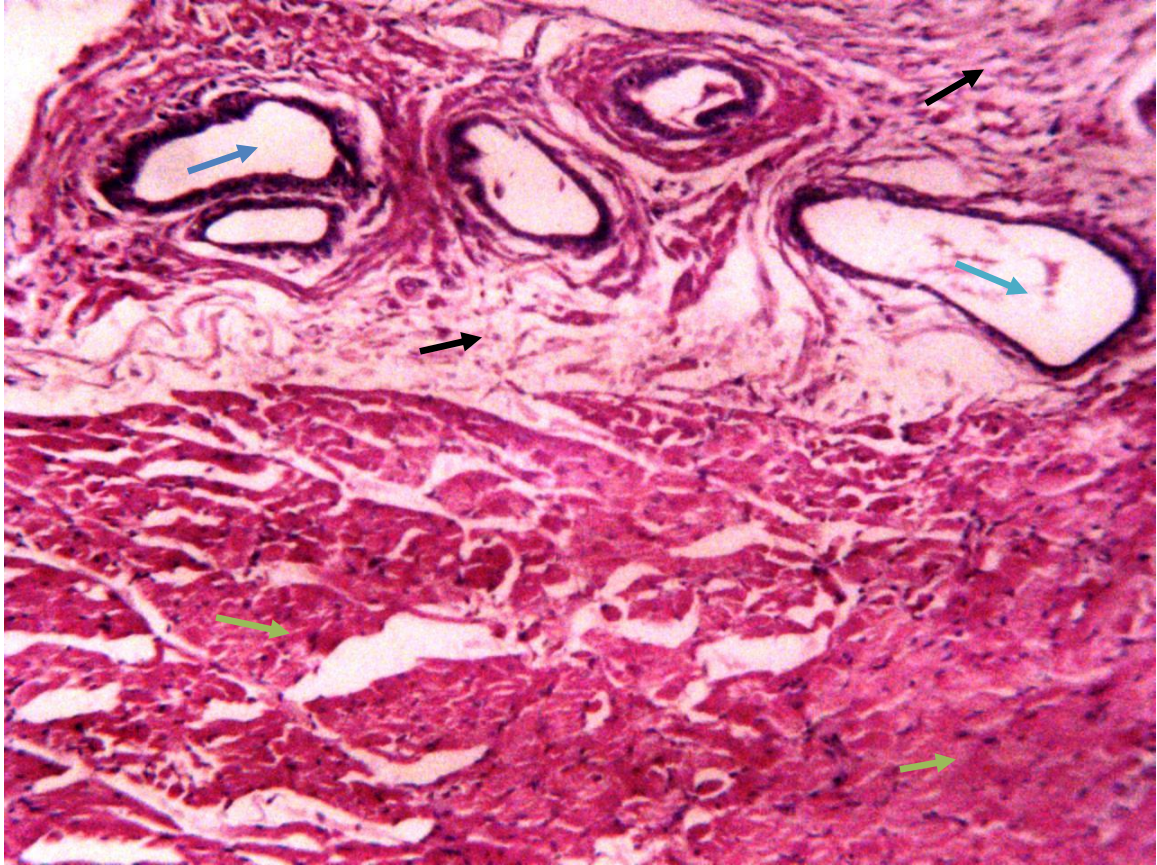


Plate 4.18: Photomicrograph of prostate gland of rats treated with aqueous extract of *Phoenix dactylifera* at 500mg/kg showing normal smooth muscles (green arrows), normal connective tissues (black arrows) and distorted glands (light blue arrows) H and E x100.

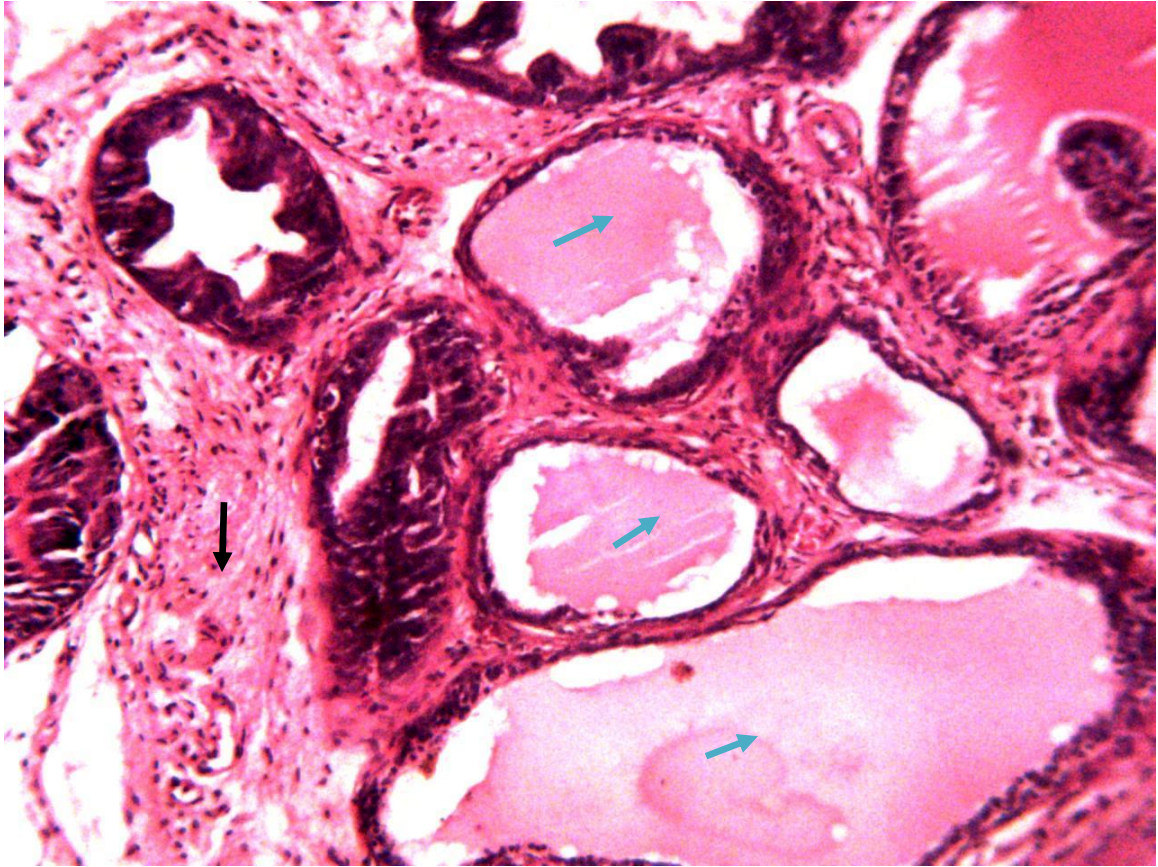


Plate 4.19: Photomicrograph of Prostate gland of rats treated with aqueous extract of *Phoenix dactylifera* at 1000mg/kg showing normal connective tissues (black arrow) and distorted glands (light blue arrows) H and E x100.

#### 4.6 Effect of Aqueous Extract of *Phoenix dactylifera* on Morphometric Parameters of some Reproductive Organs of Male Wistar Rats.

Table 4.3 showed a significant increase in seminiferous tubular diameter between the control ( $23.35 \pm 0.07 \mu\text{m}$ ) and the groups that received the extract at 250mg/kg ( $25.72 \pm 0.46 \mu\text{m}$ ) and 1000mg/kg ( $25.14 \pm 0.55 \mu\text{m}$ ) ( $P \leq 0.05$ ). For the size of interstices, there was a significant decrease in the size of interstices between the control ( $36.50 \pm 2.18 \mu\text{m}$ ) and the groups that received the extract at 250mg/kg ( $27.30 \pm 1.38 \mu\text{m}$ ), 500mg/kg ( $26.60 \pm 1.98 \mu\text{m}$ ) and 1000mg/kg ( $27.30 \pm 1.38 \mu\text{m}$ ) at  $P \leq 0.05$ , while for the epididymis, there was also significant decrease in the epithelial thickness between the control ( $10.06 \pm 0.09 \mu\text{m}$ ) and groups treated with the extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight ( $7.20 \pm 0.22 \mu\text{m}$ ,  $6.64 \pm 0.25 \mu\text{m}$  and  $6.12 \pm 0.12 \mu\text{m}$ , respectively) at  $P \leq 0.05$ .

**Table 4.3: Morphometric Parameters of Testis and Epididymis of Rats treated with Aqueous Extract of *Phoenix dactylifera*.**

<b>Groups</b>	<b>Seminiferous Tubular</b>	<b>Size of Interstices</b>	<b>Epididymal Epithelial</b>
---------------	-----------------------------	----------------------------	------------------------------

	<b>Diameter (<math>\mu\text{m}</math>) x100</b>	<b><math>\mu\text{m}</math> x400</b>	<b>Thickness <math>\mu\text{m}</math> x400</b>
<b>Control</b>	23.35 $\pm$ 0.07 <sup>a</sup>	36.50 $\pm$ 2.18 <sup>a</sup>	10.06 $\pm$ 0.09 <sup>a</sup>
<b>250 mg/kg</b>	25.72 $\pm$ 0.46 <sup>b</sup>	27.30 $\pm$ 1.38 <sup>b</sup>	7.20 $\pm$ 0.22 <sup>b</sup>
<b>500 mg/kg</b>	24.83 $\pm$ 0.56	26.60 $\pm$ 1.98 <sup>b</sup>	6.64 $\pm$ 0.25 <sup>b</sup>
<b>1000 mg/kg</b>	25.14 $\pm$ 0.55 <sup>b</sup>	27.30 $\pm$ 1.38 <sup>b</sup>	6.12 $\pm$ 0.12 <sup>b</sup>

All values expressed as Mean $\pm$ SEM. Values in the same column with different superscript are significantly different at P $\leq$ 0.05 using one-way Analysis of Variance (ANOVA) (SEM =standard error of the mean)

## CHAPTER FIVE

## DISCUSSION

Aqueous extract of *Phoenix dactylifera* did not result in death of any Wistar rat at 2500mg/kg and 5000mg/kg after 72 hours of treatment, this suggests that the extract is relatively safe and the lethal dose (LD<sub>50</sub>) is greater than 5000mg/kg. The relative safety observed may have been responsible for the widespread use of the fruit both as food and for treatment of various ailments/illnesses and an important part of the diet of people in the Arab countries where they are consumed fresh, dried, or in various processed forms (Kader and Hussein, 2009; Mallhi *et al.*, 2014).

There was no significant change in the serum levels of FSH and LH in rats treated with the extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg compared to that of the control, this showed that the extract may not have effect on the hypothalamus and pituitary gland which are the glands responsible for production of FSH and LH (Hiller-Sturmhofel and Bartke, 1998). This is in line with the study conducted by Ismail and Radzi (2013) which showed that *Phoenix dactylifera* extract improve brain function and has neuroprotective effects. The significant decrease in serum testosterone level of rats treated with the extract compared to that of the control is an indication that the extract could affect spermatogenesis as testosterone is necessary for normal development of the cells of the spermatogenic lineage (Junqueiro and Cameiro, 2005). The steroid and Flavonoid contents of the extract might be the cause of decrease in serum testosterone levels as steroids are reported to decrease testosterone levels (Karila, 2003). Flavonoids are also reported to decrease plasma testosterone levels in rodents (Weber *et al.*, 2001; Cline *et al.*, 2004), and cause malformation in male reproductive system in pups of rats exposed to flavonoids during gestation (Musameh *et al.*, 2014). The decrease in testosterone level

might be the cause of decrease in sperm count, sperm motility and sperm morphology because testosterone is the principal hormone responsible for development of the spermatogonial stem cells to mature sperm cells (Joensen *et al.*, 2008).

Animals treated with the *Phoenix dactylifera* extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg did not show any significant change in body/organ weight compared to that of the control, this shows that the extract may not affect metabolism. This is in agreement with earlier studies by Bahmanpour *et al.*, (2006) and Bahmanpour *et al.*, (2013) that there was no significant change in the prostate, seminal vesicular, testicular and epididymal weight following the administration of date palm gemules/pollen to Rats.

The significant decrease in epididymal sperm count, motility and morphology in rats treated with *Phoenix dactylifera* extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg compared to that of the control suggested that the extract could affect spermatogenesis or had the potentials of destroying sperm cells. The extract contained high content of steroids, flavonoids and saponins (Anjum *et al.*, 2012), steroids are believed to affect male fertility by reduction in sperm quality and serum testosterone levels (Torres-Calleja *et al.*, 2000; Leme de Souza and Hallak, 2011). Saponins are reported to have antifertility, abortifacient and anti-implantation properties in rodents (Chou *et al.*, 1971; Dande and Patil, 2012; Dande *et al.*, 2014). Flavonoids and saponins have tumor suppressive activities and are used as anticancer agents (Ren *et al.*, 2003; Dia and Mumper, 2010) and any substance/compound with tumor suppressive activity inhibits proliferation of stem cells (Sadri-Ardekani and Atala, 2014); therefore, the steroids, flavonoids and saponin content of *Phoenix dactylifera* extract might be the cause of decrease sperm count and sperm morphology.

The compaction of interstices of rats treated with *Phoenix dactylifera* extract at 250 mg/kg and 500 mg/kg and destruction of Leydig cells at 1000 mg/kg might be the cause of decrease serum testosterone as impaired/damaged Leydig cells could affect steroidogenesis (testosterone production), (Zirkin and Chen, 2000; Joensen *et al.*, 2008; Goluzza *et al.*, 2014), leading to scanty and distorted sperm cells in the lumen of seminiferous tubules, vacuolation and distortion of spermatogenic cells which were noticed in the rats treated with the extract at 250 mg/kg and 500 mg/kg with destruction of basement/cell membrane and capsule of seminiferous tubules at 1000 mg/kg as compared to that of the control rats. The destruction of basement/cell membrane showed that the extract can affect membrane of some cells, saponin content of the extract might be the cause of basement/cell membrane destruction as saponins are reported to affect cell membranes by forming pores, they also exert a lytic action on erythrocytes membranes (Authi *et al.*, 1988; El Izza *et al.*, 1992; Francis *et al.*, 2002).

The epididymis of rats treated with *Phoenix dactylifera* extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg showed scanty and distorted sperm cells with vacuolated epithelium, indicating decrease in sperm count, sperm motility and sperm morphology. This shows that the extract could impair epididymal function by either affecting the stored sperm cells or altering the maturation of spermatozoa because the epididymal duct produces the morphological, biochemical, physiological and functional changes in the structures of the spermatozoa through a process known as epididymal maturation, which converts the spermatozoa into fertilization-competent cells (Toshimori, 2003; Gatti *et al.*, 2004). There were no changes in the structure of the seminal vesicle of rats treated with *Phoenix dactylifera* extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg compared to that of the

control signifying that the extract had no negative effect on the structure and function of seminal vesicles.

The prostate glands of the rats treated with the extract showed destruction of connective tissues and distorted glands; this is an indication that the extract could affect prostate function by reducing sperm quality/viability as secretions from the prostate and seminal vesicles constitute the bulk of the semen and helps in the nourishment of sperm cells (Mann, 1974).

The significant increase in seminiferous tubular diameter of rats treated with the extract at 250mg/kg and 1000mg/kg, compared to that of the control might be as a result of inflammation of the spermatogenic cells. The destruction of the cell/basement membrane might be the cause of increased cell sizes as basement membranes are thought to play some roles in filtration and passage of substances/fluid in and out of the cell (Miner, 1999). The decrease in epididymal epithelial thickness of rats treated with *Phoenix dactylifera* extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg compared to that of the control showed that the extract might have potentials of destroying epididymal epithelium. The decrease in the size of interstices of rats treated with the extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg compared to that of the control might be the cause of decrease serum testosterone as the primary function of interstitial cells of Leydig is testosterone production (Opuwari and Mousees, 2015).

Administration of aqueous extract of *Phoenix dactylifera* at 250mg/kg, 500mg/kg and 1000 mg/kg to male Wistar rats might affect fertility by degeneration of Leydig and distortion of spermatogenic cells and decrease in serum testosterone levels with reduction

in sperm count, sperm motility and sperm morphology. This might be the cause of declining fertility in Middle East and North Africa as reported by Roudi-Fahimi and Kent, 2007; Macfarquhar, 2009; Eberstadt and Shah, 2011, where date palm is used as a staple food (Forbes, 1971).

## **CHAPTER SIX**

### **CONCLUSION AND RECOMMENDATION**

#### **6.1 Conclusion**

From this study, it can be concluded that,administration of aqueous extract of *Phoenix dactylifera* at 250mg/kg, 500mg/kg and 1000 mg/kg for (35 days) might affect fertility in male Wistar rats through the following ways.

- i. Degeneration of Leydig cells and spermatogenic cells with distorted epididymal sperm cells, vacuolated epididymal epithelium and distorted prostate glands.
- ii. Decrease in sizes of interstices and epididymal epithelial thickness.
- iii. Decrease in serum testosterone levels and
- iv. Decrease in sperm count, sperm motility and sperm morphology.

Therefore, caution should be taken in the consumption of *Phoenix dactylifera* fruit, even though it has numerous therapeutic effects.

## **6.2 Recommendations**

Based on the findings from this study, the following are recommended

- i. Further studies should be conducted on the effects of the various phytochemical components of *Phoenix dactylifera* fruit on male reproductive system of Wistar rats.
- ii. Further studies should be carried out to ascertain the exact mechanism that results in decrease sperm count and testosterone levels.
- iii. Further studies should be conducted on DNA cytological staining of sperm cells in Wistar rats.

## **6.3 Contributions to knowledge**

- i. Administration of aqueous extract of *Phoenix dactylifera* to male wistar rats at 250 mg/kg, 500 mg/kg and 1000 mg/kg for 35 days caused significant decreases

in serum testosterone levels ( $1.14\pm 0.09$  ng/ml,  $1.00\pm 0.11$  ng/ml and  $1.14\pm 0.09$  ng/ml, respectively) compared to that of the control rats ( $2.20\pm 0.07$  ng/ml).

- ii. There was significant decrease in size of interstices ( $27.30\pm 1.38$   $\mu\text{m}$ ,  $26.60\pm 1.98$   $\mu\text{m}$  and  $27.30\pm 1.38$   $\mu\text{m}$ ) following the administration of aqueous extract of *Phoenix dactylifera* to male wistar rats at 250 mg/kg, 500 mg/kg and 1000 mg/kg, respectively for 35 days compared to that of the control rats ( $36.50\pm 2.18$   $\mu\text{m}$ ).

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## APPENDIX I

Growers feed contains the following;

- i. Cereals/grains
- ii. Vegetable protein
- iii. Premix (vitamins/minerals)
- iv. Essential Amino acids
- v. Salt
- vi. Antioxidants
- vii. Antitoxins
- viii. Prebiotic
- ix. Enzymes

i. Crude protein	13 % (min)
ii. Fat	8 % (Max)
iii. Crude fibre	15 % (Max)
iv. Calcium	0.9 % (Min)
v. Available phosphorus	0.35 % (Min)
vi. Metabolisable energy	2600 Kcal/kg(Min)