EFFECTS OFAQUEOUS STEM BARK EXTRACT OF *ANOGEISSUS LEIOCARPUS*ON THE HISTOLOGY AND FUNCTION OF THE OVARIES OF ADULT WISTAR RATS

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M.Sc/MED/9768/2009-2010

NOVEMBER, 2020

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DEPARTMENT OF HUMAN ANATOMY,

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ZARIA, NIGERIA

NOVEMBER, 2020

DECLARATION

I, MUHAMMAD JIBRIL,MURTALA, declare that work in the thesis entitled **"Effects Of Aqueous Stem Bark Extract Of *Anogeissus Leiocarpus*On The Histology And Function Of The Ovaries Of Adult Wistar Rats"** has been performed by me in the department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria, under the supervision of Prof. S. A. Ojo, Dr. W. O. Hamman, and Prof. E. K. Bawa. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any University.

MUHAMMAD JIBRIL MURTALA 13/11/2020

Name of student Signature Date

CERTIFICATION

This thesis entitled **"Effects of Aqueous Stem Bark Extract of Anogeissus Leiocarpus on the Histology and Function of the Ovaries of Adult Wistar Rats"** by Muhammad, Jibril Murtala meets the regulation governing the award of the Master of Science of Ahmadu Bello University for its contribution to scientific knowledge and literary presentation.

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DEDICATION

To

My Late Grandmother

Hajiya Rahmatu Jibril (Turai)

A mother and trainer like no other

Rest InPeace.

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ABSTRACT

This study evaluates the effect of aqueous stem bark extract of *Anogeissus leiocarpus* on the ovaries and ovarian function through investigating its effect on the histomorphologic changes in the ovaries using Hematoxylin and eosin for light microscopy, and the associated changes in the serum levels of estradiol and progestin hormones using the enzyme-linked-immunosorbent assay (ELISA). Twenty-four young adult Wistar rats were used for the study. The experimental animals were synchronized using 0.5ml injection of prostaglandin PGF2α to ensure uniformity in the estrous cycle before extract administration. Four experimental groups were assigned and received an extract concentration of 600mg/kg, 400mg/kg, and 200mg/kg for groups I, II and III, while the control group (IV) received distilled water for the period of six (6) weeks respectively. The extract has no effect on the microscopic slides of the ovaries prepared at the end of the experiment.There was an increase in the concentration of progesterone in groups II, and group III following the extract administration. The progesterone level in the orally treated group was 19% higher, and statistically significant ($p< 0.05$), and an increase in the level of serum estradiol in groups II, and III was also observed after extract administration ($p< 0.05$).These results suggest that *Anogeissus leiocarpus* has a potential effect on the ovarian function indirectly via its inhibitory effect on PDEs; an effect which is established to promotes developmental competence of mice oocytes retrieved from small antral follicles.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

*Anogeissus leiocarpus*is referred to by many vernacular names in different languages across Africa.It is referred to as *Orin-odan*in Yoruba,*Kojoli* in Fulbe, and*Marke*in Hausa, is a common plant distributed across the African savanna region, mostly across the West and Central Africa (Takahashi, 1978). It is mostly employed for agricultural purposes for woods, charcoal, and production of agricultural implements.

A. leiocarpus has many applications locally in Nigeria. It is used medicinally for the treatment of ascaris, gonorrhea, pain, blood clotting, and coughing (Mann *et al.,* 2003*).* The Yoruba's of South-Western Nigeria illustrated that the plant is also used as an antimicrobial agent to treat bacterial infection (Dweek, 1996).

A. leiocarpus is also locally used for problems associated with reproduction and to enhance fertility in many parts of Northern Nigeria.The effect of *A. leiocarpus* on reproduction and fertility has not been investigated, and was the motivating factor for this study.

Phytochemical studies conducted on *A. leiocarpus* revealed that the stem bark extracts has various chemical constitutions such as alkaloids, glycosides, steroid, anthraquinone, phenol, tannins, saponins, calcium, and fluorides (Adekunle *et al.,* 2006).

The World Health Organization (WHO) in 2008 estimated that 80% of the world population; particularly in Asian and African countries depend on traditional medicine for primary health care. The use of herbal medicine in developed countries has expanded sharply in the latter half of the twentieth century, and the WHO produce monographs on selected herbs and their sources (WHO, 1999).

The problem of infertility has been one of the major concerns affecting most couples all over the world. In Africa, impaired fertility is a well-known public health issue (Larsen, 1994). Despite advancement in medicine and the accessibility of reproductive health clinic, the problem of infertility continues to affect many couples thereby serving as a common source of marital problem and disharmony, a problem more aggravated in the African society.

The International Family Planning perspectives in 2005 declared that a substantial proportion of women suffer from infertility in Nigeria, making about 33% of women aged 20-44yrs.

Several studies indicate that infertility is the most frequent reason for gynecological consultation in Nigeria (Okonofua, 2003).More than 50% of gynecological case loads are as a result of infertility (Otuba,1990), and over 80% of laparoscopic investigations are as a result of infertility (Okoye, 2002).

1.2 Statement of research problem

Anogeissus leiocarpus extract has been used locally for a long time to treat different kind of ailment generally and infertility problem. Clinical studies regarding its use are few; therefore a clear understanding through which it exert its effect has not been established.

1.3 Justification of the study

Various studies conducted (invitro and in vivo) using crude and refined extract of *A. leiocarpus* have shown it to have some antimicrobial activities. The clinical significance of *A. leiocarpus* and its use for problems associated with reproduction and fertility is yet to be determined.

The present study is aimed at determining the effect of consuming *A. leiocarpus*extracts on the microscopic structure of ovary, and the associated changes in the serum levels of female reproductive hormones in mature female adult wistar rats.

1.4 Significance of the study

The result of this study could confirm the effectiveness of *A. leiocarpus* in the treatment of some problems associated with reproduction and infertility through its effect on the ovary.

1.5 Research Hypothesis

Aqueous extract of *Anogeissus leiocarpus* improves the function of the ovaries and the output of ovarian hormones

1.6 Aim and objectives

The aim the study was: -

To study the effect of aqueous stem bark extract of *Anogeissus leiocarpus* onthe ovaries and ovarian function.

The objectives of the study were:-

1. To investigate the effect of aqueous stem bark extract of *A. leiocarpus* on the histomorphologic changes in the ovaries of the adult Wistar rats exposed, using Hematoxylin and eosin for light microscopy.
2. To determine the associated changes on the serum levels of estradiol and progestin in the ovaries of the mature Wistar rats exposed, using the enzyme-linked-immunosorbent assay (ELISA).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 Medicinal Plants

In most cultures from ancient times to the present day, plants were used as a source of medicine by man to control diseases in humans and animals (Schultes *et al.,* 1992). These plant-based systems continue to play an essential role in health care, and it has been estimated by the World Health Organization that approximately 80% of the world's inhabitants rely mainly on traditional medicine for their primary health care (Fernsworth *et al.,* 1985; Arvigo *et al.,* 1993). World Health Organization described plant with one or more organs which contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (WHO, 1992).

Many of the plant materials used in traditional medicine are readily available in rural areas and this has made traditional medicine relatively cheaper than modern medicine (Apulu *et al.,* 1994). Over Sixty percent of Nigeria rural populations depend on traditional medicine for their healthcare need (Apulu *et al.,* 1994). Medicinal properties of plants are normally dependent on the presence of certain phytochemical principles such as alkaloids, anthraquinones, cardiac glycosides, saponins, tannins and polyphenols which are the bioactive bases responsible for the antimicrobial property (Ebana *et al.,* 1993). Plant products also play an important role in the health care systems of the remaining 20% of the population, mainly residing in developed countries.

The Nigerian flora has over seven thousand, three hundred and forty-nine species of higher plants that had made serious impact on the health and wealth of Nigerians and could be an enormous source of foreign exchange for the country (Mann *et al.,* 2003), the least explored in terms of available untapped resources.

Extensive and in-exhaustive list of plants have been screened for their various chemical constituents that served both economical and medicinal purposes (Faraz *et al.,* 2003). Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities. A substantial number of drugs currently being used are discovered as a result of chemical studies directed at the isolation of the active substances from plants used in traditional medicine (Grifo *et al.,* 1997).

2.2.0 The Plant *Anogeissus Leiocarpus*

Anogeissus leiocarpus (DC) Guill and Perr(family: *Combretaceae*) is a tall evergreen tree native to the savannas of West Africa (Steentoft, 1988). It is the sole West African species of the genus *Anogeissus*, a genus otherwise distributed from Tropical,Central and East Africa through tropical Southeast Asia (Steentoft, 1988). *A. leiocarpus* germinates in the soil produced by seasonal wetlands and grows at the edges of the rainforest, although not in the rainforest, in the savanna, and along riverbanks forming gallery forests. The tree flowers in the rainy season, from June to October. The seeds, winged samaras, are dispersed by ants(Steentoft, 1988).

The inner bark of the tree is used as a human and livestock anthelmintic for treating worms, and for treatment of a couple of protozoan diseases in animals, nagana an animal trypanosomiasis, and Babesiosis (Bizimana *et al.*,1994). The inner bark used as a chewing stick in Nigeria and extracts of the bark show antibacterial properties (Mann *et al.*, 2008). The stem barks contains castalagin (Shuaibu *et al.*, 2008).

2.2.1 Scientific classification of *Anogeissus leiocarpus*

The plant belongs to: -

Kingdom- Plantae

Division- Magnoliophyta

Class- Magnoliopsida

Order- Myrtales

Family- Combretaceae

Genus- *Anogeissus*

Specie- *A. leiocarpus*

Binomial name: - ***Anogeissus leiocarpus***(DC) Guill. & Perr.

Plate I. ***Anogeissus leiocarpus*** **(DC) Guill and Perr(Steentoft, 1988)**

2.2.2 Properties of *Anogeissus leiocarpus*

The leaves of *Anogeissus leiocarpus* contain ellagic, gallic and gentisic acids, derivatives of gallic and ellagic acid and several flavonoids (derivatives of quercetin and kaempferol). The high concentration (up to 17%, based on dry matter) of hydrolysable tannins (Gallic and ellagic acid derivatives) explains the usefulness of *Anogeissus leiocarpus* in the bogolan technique (Arbonnier,2004).

According to Kerharo *et al.*, 1974, the majority of the medicinal uses of *Anogeissus leiocarpus* are likely to be based on its tannin content. No experimental data on their non-toxicity appears to have been published but the popular use of giving the bark decoction to drink to new-born infants is of interest in this connection. The bark contains nearly no flavonoids but is rich in derivatives of ellagic acid (2.5–5% of the dry matter) and contains the polyalcohol sorbitol, terpenoids (α-amyrin, β-amyrin and β-sitosterol) and traces of alkaloids. Six molecules of ellagic acid derivatives were observed and four of them were isolated and characterized (Kerharo *et al.*, 1974).

These molecules are 3,3',4'-tri-O-methylflavellagic acid, 3,3'-di-O-methylellagic acid, tri-O-methylellagic acid and 3, 3'-di-O-methyl-4-β-O-xylopyranosyl-ellagic acid. These derivatives are good antioxidants acting as scavengers of free radical oxygen and as protectors of DNA from injury by alkylating agents(Smart *et al.*, 1976).These are anti-inflammatory and anti-allergic agents and have anticarcinogenic and antimutagenic activities. Research has shown that the ellagic acid derivatives show an inhibition of certain enzymes of the metalloproteinase type in several types of cellular skin cultures and retard the degradation of collagen.

Chewing sticks made of *Anogeissus leiocarpus* showed strong activity against a wide spectrum of bacteria, including some contributing to tooth-deterioration. *Anogeissus leiocarpus* extracts exhibited in-vitro activity against chloroquine-resistant *Plasmodium falciparum* strains (Ouattara *et al.*, 2003).

2.2.3 Phytochemical analysis of *Anogeissus leiocarpus*

The phytochemical screening of the extracts of *A. leiocarpus* indicated presence active chemical compounds. The extracts were analyzed using the procedure of Harbone and Evans (1998). Mann, *et al.*,in2008, reported the following chemical compositions; Alkaloids, Glycosides, Phenols, Steroids, Tannins, Ellagic acids, Anthraquinone, Saponins and Flavonoid, are found to be presence in the stem and leaves extract of the plant. The gum of *Anogeissus leiocarpus* contains amino acids (glutamic acid, aspartic acid, alanine, and glycine) as well as 20% of a polysaccharide. On hydrolysis the polysaccharide gives 12% D-xylose, 32%L-arabinose, 5% D-galactose, 2% D-mannose and 20% oligosaccharides (Saunois 2001).

2.2.4 Plant extracts and active principles

The efficiency of plant extract is due to the presence of one of more of the biologically active principles. Pharmacological assays have shown that the activity is not due to the main components, but the minor ones, or even to the synergism of all the active principles (Galeffi. 1980). With modern advances in the techniques for isolation and structure determination of the active principles, even a minute amount of them can be isolated and their structures determined (Salemink, 1980).

2.2.5 Phytochemical constituent of plants

Phytochemical screening is the method employed to determine or detect the presence of and isolation of accumulated natural plant products or secondary metabolites of plants such as alkaloids, tannins, steroids, terpenes, glycosides, flavonoids, carbohydrates, resins, gums, and other volatile substances, in plant extract through simple chemical tests. These secondary metabolites give plants their therapeutic properties and thus are referred to as active constituents (Farnsworth, 1966).

2.2.5.1 Alkaloids

These are basic, nitrogenous compounds (usually in a heterocyclic ring) which usually have marked physiological actions on man and animals. The nitrogen can be primary (mascaline), secondary (ephedrine), tertiary (atropine) or quaternary (tubacurarine). The present of alkaloids in an extracts can be tested using Dragendoff's reagent, Wagner's reagent or Mayer's reagent. They are of high medical values (Trease and Evans, 2001).

2.2.5.2 Cardiac glycosides

These are C23 Steroidal glycosides which exert slowing and strengthening effect on a failing heart. The heart arresting properties of these glycosides also render them the most effective, and a number of tropical plants are better known in this respect than their medicinal use (Trease and Evans, 2001).

2.2.5.3 Tannins

Tannins are substances present in plant extracts which are able to combine with protein of animal hides to prevent their putrefaction and convert them to leather. They act as antidiarrhea, and have been employed as antidote in poisoning by heavy metals, alkaloids, glycosides. Also used as protective of inflamed surfaces of mouth and throat internally (Treas and Evans, 2001). Tannins are considered to have anticancer properties (Lambert and Yang, 2003; Keil *et al.,* 2004).

2.2.5.4 Saponins

Saponins are glycosides with high molecular weight and a high polarity characterized by their property of producing frothing aqueous solution. They also have hemolytic properties. In the blood stream, saponins are highly toxic but comparatively harmless when taken orally. They are classified into steroidal and pentacyclic triterpenoids saponins (Trease *et al*., 2001).

2.2.5.5 Flavonoids

Flavonoids are present in different plant parts including the leaves, stems, roots, flowers, and seeds (Amin *et al.,* 2007). They are the largest group of naturally occurring phenols occurring both in the free state and as glycosides. Flavonoidic derivatives have a wide range of biological activities (Amin *et al.*, 2007).

2.2.5.6 Phytoestrogen

Phytoestrogens are substances present in plant that imitate the effects of steroidal estrogens when consumed by animals. Many phytoestrogenic plants are known to cause reproductive disorders (Farnsworth *et al.*, 1975). Phytoestrogenic effect may be found in grasses, but the most important plants shown to contain high amounts of these compounds are legumes (Linder, 1976). However, their estrogenic concentration varies according to the species and variety of the plants, as well as the environment (Davies, 1987). Phytoestrogens are known to imitate animal estrogens by binding to estrogen receptors, effecting changes known as nuclear translocation and manifesting tissue effects consistent with estrogenic activity (Hughers, 1988; Adams, 1989). They also cause typical estrogenic changes in the mammary glands, uterus, cervix, vagina, and influence mammary gland development (Barberan *et al.*, 1990).

The biological actions of Phytoestrogens are believed to have health implications due to the populations that regularly consumed them (Brown and Setchell, 2001). According to Liyou *et al.*, (2001), accelerated vaginal openings and alterations in the length of stages of estrous cycle towards persistent estrus were observed on pubertal rats exposed to phytoestrogen. It has been reported that, phytoestrogens may interfere with the actions of physiological estrogens (Marieletizia *et al.*, 2007). It was also reported by Kuiper *et al.*, 1998. that Genistein, an endocrine-active phytoestrogen which alters sexual and reproductive development of exposed male and female rats has been shown to interact with estrogen-receptor ER-alpha and ER-beta, although it preferentially activate the latter (Kuiper *et al.*, 1998).

Many phytoestrogen are believed to act endocrine disruptors in human and animals. These endocrine disruptors can alter normal hormone regulation via several mechanisms, such as binding to hormone receptors, mimicking hormones, or inhibition of enzymes that play essential roles in hormone synthesis such as tyrosine kinase, which is believed to be important in transducing the estrogenic signals (Koroma and De Juan, 1997). Some of these chemicals are known to posses estrogen-like activities and can bind to estrogen receptors and induce or modulate estrogen-receptor mediated responses (Dang *et al.*, 2007).

The end points used to determine the estrogenic effect of plant extracts on laboratory animals include: uterine wet weight, degree of vaginal cornification and quantal vaginal opening (Paul *et al.*, 1998). It is known that administration of estrogen has uterotrophic effects in several animal species, including rats and mice, and such effects are also associated with growth and proliferation of the endometrial cell number, vaginal opening and cornification (Paul *et al.*, 1998).

2.2.6 Toxicological Studies

The result of the intraperitoneal acute toxicity study showed that LD_{50} of the extract is 1400mg/kg, indicating that the extract is of low toxicity (Agaie *et al.*, 2003). Another study conducted by Clarke and Clarke (1977) reported that any substance with an i/p $LD_{50}$of above 1000mg/kg should be regarded as safe.

Agaie *et al.,* reported the absence of gross and histopathological lesions in the liver, kidney, intestines and testicles,which further buttress the level of safety of the extract on these organs except the lungs where extensive lesions were observed as the dose increases (Agaie *et al.,* 2003).It was therefore concluded that the high LD_{50} obtained following i/p administration of the extract and lack of mortality when orally administered may be an indication that the aqueous extract of leaf and stem bark of *A. leiocarpus* could be use with some degree of safety especially when administered by oral route.

2.2.7 Medicinal Properties and Uses Of*anogeissus Leiocarpus*

Anogeissus leiocarpus is used medically for the treatment of ascariasis, gonorrhea, general body pain, blood clots, asthma, coughing and tuberculosis (Mann *et al*., 2003). It is also used as antimicrobial agent for the treatment of bacterial infections (Dweek, 1996). *A. leiocarpus* has been reported to have a large number of therapeutic properties and pharmacological activities. These include: -

2.2.7.1 Antihypertensive activity

Anogeissus leiocarpus has been shown to have antihypertensive activity, and is used traditionally to treat Arterial Hypertension (HTA) (Ouedraogo *et al.*, 2008).

Aqueous stem bark extracts of *Anogeissus leiocarpus* were reported to have an effect on the porcine coronary artery through phosphodiesterases (PDE) inhibition (Ouedraogo *et al.*, 2008). The result of the study showed concentration-dependent and significant inhibition of PDEs (1-5) with better activity on the PDE1, activated by calmoduline (IC_{50} = 7.5 ± 4µg/ml).The same effect was obtained in the presence of N-nitro-L-arginine (LNA, 300µM, an inhibitor of endothelial NO-synthase).The study concluded that *AEAL* induced endothelium-dependent relaxations on porcine arteries, which involve a NO-mediated component but also partially an EDHF- mediated component.

2.2.7.2 Antibacterial activity

The ethanolic extract of the leaf, stem and root bark of *Anogeissus leiocarpus* were investigated for invitro antibacterial activity against clinical isolates of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* using agar diffusion techniques (Mann *et al.*, 2008). The result showed that the extracts had exhibited antibacterial activity against *S. aureus, E. coli* and *P. aeruginosa*. It was also revealed that the antibacterial activity of the extract was enhanced by an increase in the extracts concentration (Mann *et al.*, 2008).

2.2.7.3 Antihelminthic activity

Kabore *et al.*, 2009, evaluate the effect of the leaf extract of *Anogeissus leiocarpus*and found it to be effective remedy for gastrointestinal parasites. The antihelminthic activity of these extracts on eggs, first stage larvae and adults of *Haemonchus contortus* was examined by in vitro tests. The result shows that *H. contortus* eggs were inhibited from hatching by the leaves extract of *A. leiocarpus.*

2.2.7.4 Antiplasmodial activity

Methanolic extracts of *Anogeissus leiocarpus* has been considered locally to have the same antimalarial activities as artemisinin derivatives (Akanbi *et al*., 2011). The work studied the in vivo antiplasmodial activity of methanolic extracts of *A. leiocarpus* and its effect on oxidative stress and lipid profile in mice infected with *Plasmodium bergheii*.

The study shows that the methanolic extract of*A. leiocarpus* has high antimalarial activities, high antioxidant property, and capable of boosting HDL level in malaria-infected organisms.

2.2.7.5 Hypoglycemic effect

The effect of the methanolic leaves extract of *Anogeissus leiocarpus* which is widely used in Sudan for the treatment of diabetes mellitus was studied on blood glucose and liver glycogen content in normal Wistar albino rats (Mahgoub *et al*., 2007).

The result of the study shows that there is a significant reduction in blood glucose level which is dose dependent. At a dose of 200mg/kg body weight the plant extract showed no significant change in blood glucose(Mahgoub *et al*., 2007).

2.2.7.6 Antifungal activity

Aqueous, ethanolic, methanolic, chloroform and ethyl acetate extracts of *Anogeissus leiocarpus* and *Terminalia avicenniodes* exhibited antifungal activity against *A.niger, A.fumigatus, Penicillium species, M. audouinii* and *T.rubrum*according to Mann *et al.,* 2008.The study conclude that the Ethanolic, methanolic, ethyl acetate, chloroform and aqueous root extracts of *Anogeissus leiocarpus* and *Terminalia avicenniodes* used in the study exhibit antifungal effect on the test organisms thereby making the extracts useful in the treatment of fungal infections.

2.3.0 The Ovaries

The ovaries are paired organs situated close to the wall on either side of the pelvis minor, a little below the brim. Each measures 2.5–5 cm in length, 1.5–3 cm in breadth, and 0.7–1.5 cm in width, weighing about 4–8 g. The ovary has 2 surfaces, medial and lateral; 2 borders, anterior or mesovarian and posterior or free; and 2 poles, upper or tubal and lower or uterine.When the uterus and adnexa are in the normal position, the long axis of the ovary is nearly vertical, but it bends somewhat medially and forward at the lower end so that the lower pole tends to point toward the uterus.The medial surface is rounded and, posteriorly, may have numerous scars or elevations that mark the position of developing follicles and sites of ruptured ones (Alan *et al.*, 2003).

2.3.1 Development of the ovary

The ovary develops from the paramesonephric ridge of the intermediate cell mass in the same way as the testis. Its site of origin lies in the peritoneum of the posterior abdominal wall. It descends, preceded by the gubernaculums (Chummy, 2005).

The gubernaculums proceeds through the inguinal canal, as in the male, and becomes attached to the labium majus. The ovary does not follow its gubernaculum so far, and its descend is arrested in the pelvis as the gubernaculum becomes attached to the uterus and persists as the ligament of the ovary and the round ligament of the uterus (Chummy, 2005).The mesonephric tubules and mesonephric ducts normally disappear in the female. Should they persist their remnant are to be found between the layers of the broad ligament. The *epoophoron* consist of a number of tubules joining at right angles a persistent part of the mesonephric duct. It lies in the mesosalpinx between ovary and tube(Chummy, 2005).

2.3.2 Relationships of the ovary

The upper portion of this surface is overhung by the fimbriated end of the uterine tube, and the remainder lies in relation to coils of intestine. The lateral surface is similar in shape and faces the pelvic wall, where it forms a distinct depression, the fossa ovarica.This fossa is lined by peritoneum and is bounded above by the external iliac vessels and below by the obturator vessels and nerve; its posterior boundary is formed by the ureter and uterine artery and vein, and the pelvic attachment of the broad ligament is located anteriorly (Alan *et al.,* 2003).

Because the vessels, nerves, and lymphatics enter the ovary through this border, it is referred to as the hilum of the ovary. Anterior to the hilum are embryonic remnants of the male and female germ cell ducts. The posterior or free border is more convex and broader and is directed freely into the rectouterine pouch. The upper or tubal pole is large and rounded. It is overhung closely by the infundibulum of the uterine tube and is connected with the pelvic brim by the suspensory ligament of the ovary, a peritoneal fold. The lower or uterine pole is smaller and directed toward the uterus. It serves as the attachment of the ligament of the ovary proper (Alan *et al.,* 2003).

2.3.3 Mesovarium

The ovary is suspended by means of the mesovarium, the suspensory ligament of the ovary, and the ovarian ligament. The mesovarium consists of 2 layers of peritoneum, continuous with both the epithelial coat of the ovary and the posterosuperior layer of the broad ligament. It is short and wide and contains branches of the ovarian and uterine arteries, with plexuses of nerves, the pampiniform plexus of veins, and the lateral end of the ovarian ligament (Alan *et al.,* 2003).The suspensory ligament of the ovary is a triangular fold of peritoneum and is actually the upper lateral corner of the broad ligament, which becomes confluent with the parietal peritoneum at the pelvic brim. It attaches to the mesovarium as well as to the peritoneal coat of the infundibulum medially, thus suspending both the ovary and the tube.It contains the ovarian artery, veins, and nerves after they pass over the pelvic brim and before they enter the mesovarium (Alan *et al.,* 2003).

The ovarian ligament is a band of connective tissue, with numerous small muscle fibers, that lies between the 2 layers of the broad ligament on the boundary line between the mesosalpinx and the mesometrium, connecting the lower (uterine) pole of the ovary with the lateral wall of the uterus. It is attached just below the uterine tube and above the attachment of the round ligament of the uterus and is continuous with the latter (Alan *et al*., 2003).

2.3.4 Histological Structure of the Ovary

The ovary consist of an inner vascular medulla and an outer cortex (containing the ovarian follicles) encapsulated by a fibrous connective tissue layer, the tunica albuginea, covered by a layer of cubical cells, the superficial epithelium (Chummy, 2005).

During early fetal development primitive germ cells (oogonia) derived from endodermal cells of the yolk sac migrate into the developing ovarian cortex, where they multiply and grow to become primary oocyte, which are surrounded by a single layer of follicular cells to form primordial follicles (Chummy, 2005).

There are about 1 million primordial follicles at birth which gradually reduce to about 40,000 by puberty. After puberty, during each ovarian cycle a relatively small number of primordial follicle undergo a series of development changes and of these usually only one from either ovary comes to full maturity and releases its oocyte (ovulation) into the peritoneal cavity for transport into the uterine tube, potentially for fertilization (Chummy, 2005).This development involves oocyte enlargement, follicle (granulosa) cell proliferation and fluid (liquor folliculi) accumulation as the primordial follicle is transformed successively into a primary, a secondary and a tertiary (Graafian) follicle, the surrounding stromal cells forming the theca of these follicle (Chummy, 2005).Before ovulation the primary oocyte undergoes meiosis to form the secondary oocyte. At ovulation this is discharged, the liquor folliculi escapes and hemorrhage occur into the collapsed follicle. The granulosa cells and some of the thecal cells now develop into a corpus luteum. This persists for a week if pregnancy does not occur or for 9 months if it does. At the end of either time it atrophies and becomes replaced by a fibrous scar, the corpus albicans (Chummy, 2005).

2.3.5 Blood supply of the ovary

The ovarian artery is the chief source of blood for the ovary. Though both arteries may originate as branches of the abdominal aorta, the left frequently originates from the left renal artery; the right, less frequently.

The vessels diverge from each other as they descend. Upon reaching the level of the common iliac artery, they turn medially over that vessel and ureter to descend tortuously into the pelvis on each side between the folds of the suspensory ligament of the ovary into the mesovarium.An additional blood supply is formed from anastomosis with the ovarian branch of the uterine artery, which courses along the attached border of the ovary. Blood vessels that enter the hilum send out capillary branches centrifugally (Alan *et al.,* 2003).

The veins follow the course of the arteries and, as they emerge from the hilum, form a well-developed plexus (the pampiniform plexus) between the layers of the mesovarium.Smooth muscle fibers occur in the meshes of the plexus, giving the whole structure the appearance of erectile tissue (Alan *et al.,* 2003).

2.3.6 Lymphatic drainage

Lymphatic channels drain retroperitoneally, together with those of the tubes and part of those from the uterus, to the lumbar nodes along the aorta inferior to the kidney.The distribution of lymph channels in the ovary is so extensive that it suggests the system may also provide additional fluid to the ovary during periods of preovulatory follicular swelling (Alan *et al.,* 2003).

2.3.7 Nerve supply of the ovary

The nerve supply of the ovaries arises from the lumbosacral sympathetic chain and passes to the gonad along with the ovarian artery (Alan *et al.,* 2003).Sympathetic (vasoconstrictor) fibers reach the ovary from the aortic plexus along its blood vessels; the pre-ganglionic cell bodies are in T_{10} and $_{11}$segments of the cord (Chummy, 2005).

Some parasympathetic fibers may reach the ovary from the inferior hypogastric plexus via the uterine artery and are presumably vasodilator. Autonomic fibers do not reach the ovarian follicles; an intact nerve supply is not required for ovulation. Sensory fibers accompany the sympathetic nerves, so that ovarian pain may be periumbilical, like appendicular pain (Chummy, 2005).

2.4.0 The Oocyte

2.4.1 Origin and development

The primordial germ cells are known to originate from the endoderm of the yolk sac (at the caudal end of the embryo), at this site, they can be identified as early as the end of the third week of gestation by alkaline phosphates staining. Migration of the germ cells toward the genital ridge, occurred by amoeboid movements, with the aid of pseudopodia (Witschi, 1963).The route of migration along the dorsal mesentery of the hind gut is interrupted only by the required lateral crossing of the coelomid angle at the level of the genital ridge. Whereas some chemotaxis is clearly operational, the precise cellular mechanisms underlying the guidance of germ cells to the genital ridge remain uncertain. Importantly, germ cells appear unable to persist outside the genital ridge, which may thus be viewed as the only region competent to sustain gonadal development (Witschi, 1963).

On arrival at the genital ridge by the fifth week of gestation, the premeiotic germ cells are referred to as oogonia (Baker *et al.*,1967). During the subsequent 2weeks of intrauterine life, the primordial gonadal structure constitutes no more than a bulge on the medial aspect of the urogenital ridge.

This protuberance is created by proliferation of surface (coelomic) germinal epithelium, by growth of the underlying mesenchyme, and by oogonial multiplication, the oogonia total 10,000 by around 6 to 7 weeks of intrauterine life.Because meiosis and oogonial atresia are not operational, the actual number of germ cells is dictated by mitotic division at this stage (Baker *et al.,* 1967).

2.4.2 Oocyte growth and differentiation

Primary follicle development is accompanied by striking changes in the Oocyte. During the prenatal period, the Oocyte increases in diameter from ~25 µm to ~120 µm and develops its surrounding extracellular matrix, the zona pellucida (ZP). This enormous growth occurs as a consequence of the reactivation of the Oocyte genome (Bachvarova *et al.,* 1989). During the growth phase, the Oocyte is highly transcriptionally active because it must generate sufficient proteins and mRNA transcripts to support its own growth as well as future critical processes of Oocyte maturation, fertilization and early embryo development. Some Oocyte transcripts are immediately translated and the resulting proteins contribute to ongoing oocyte growth and differentiation, while others required for future developmental processes are stored for later translation.

Joshi *et al.,* in 2007, identifies several Oocyte-specific transcription factorsusing mouse models that are important for Oocyte growth. Factor in the germ line alpha (FIGLA) is a basic helix-loop-helix transcription factor that serves as a central regulator of Oocyte-specific genes including the expression of zona pellucida (ZP) proteins according to this finding. Another factor identified by Choi 2006, is the NOBOX-factor. This is a homeobox transcription factor that regulates expression of GDF-9 (Choi 2006).

How these and other Oocyte-specific transcription factors regulate the Oocyte developmental program is an active area of research.Important growth factors expressed in growing Oocyte include GDF-9 and BMP-15 (Elvin *et al.,* 2000; Moore *et al.,* 2005). GDF-9 is of particular interest because studies in rodents have demonstrated that GDF-9 plays an important role in stimulating granulosa cell proliferation and theca development (Shimasaki *et al.,* 2004).

The general concept to emerge is that novel growth factors produced by the oocyte play a crucial role in regulating preantral folliculogenesis via effects on the surrounding granulosa and theca cells (Vanderhyden 2002; Gilchrist *et al.,* 2008). Human oocytes express high levels of GDF-9 and BMP-15, and GDF-9 has been found to stimulate growth of human preantral follicles in vitro (Teixera *et al.,* 2002; Hreinsson *et al.,* 2002). It follows, therefore, that preantral folliculogenesis will likely be determined by these oocyte growth factors.Interestingly, a dysregulation of oocyte GDF-9 expression has been implicated in polycystic ovarian syndrome (PCOS) in women (Teixera *et al.,* 2002).

The kit ligand-c-kit tyrosine kinase receptor signaling pathway is critically important for oocyte growth and follicle development. Kit ligand generated by granulosa cells is required for oocyte growth (Packer *et al.,* 1994). In addition, kit ligand is important for organizing theca cells around the growing follicle. Several growth factors appear to influence follicular growth by stimulating granulosa cells to increase expression of kit ligand. Kit ligand then stimulates oocyte growth and resulting additional production of growth factors.This results in a "feed-forward" mechanism of paracrine interactions supporting further development of follicles that have initiated the process.

2.4.3 Oocyte-granulosa cell connections

An important event in primary follicle development is the development of intimate intercellular connections between the oocyte and granulosa cells (Eppig 1994; Albertini *et al.,* 2001). Both the oocyte and granulosa cells elaborate numerous cytoplasmic projections and microvilli that interdigitate with each other to create an extremely large surface area for diffusion (Makabe *et al.,* 2006). In addition, some of the follicle cell microvilli and cytoplasmic projections physically penetrate deeply into the oocyte via invagination of the oocyte plasma membrane, occasionally reaching close to the nuclear membrane. Cell-cell contacts comprised of adhesive junctions and gap junctions are established in these regions. Gap junctions, which are intercellular channels composed of proteins called connexins, directly couple adjacent cells allowing the diffusion of ions, metabolites, and signaling molecules (Bruzzone *et al.,* 1996).

Simon and Raynolds 1997, identifies Connexin 37 (Cx37) as the predominant gap junction protein synthesized by the oocyte after follicle recruitment, whereas granulosa cells mainly synthesize Cx43 (Simon *et al.,* 1997; Raynolds *et al.,* 1997). Therefore heterotypic gap junctions comprised of Cx37 and Cx43 are formed between the oocyte and granulosa cells, whereas granulosa cells form homotypic Cx43 gap junctions between each other. The importance of oocyte-granulosa cell gap junctions was documented in rodents by the demonstration that ovaries of Cx37 deficient mice display a profound defect in oocyte and follicle growth resulting in failed folliculogenesis and female infertility (Simon *et al.,* 1997). The underlying mechanism for these findings is that regulatory and nutrient molecules required for oocyte growth and acquisition of the potential to resume meiosis pass through these gap junctions from the granulosa cells to the oocyte (Eppig 1994; Carabatsos *et al.,* 2000).

2.4.4 The Secondary Follicle

The major changes to occur during secondary follicle development include the accumulation of increased numbers of granulosa cells that form multiple layers around the oocyte, and the acquisition of a theca. The development of a primary to a fully grown secondary follicle results from an active autocrine/paracrine regulatory process that involves growth factors produced by the oocyte.Secondary follicle development begins with the acquisition of a second layer of granulosa cells.This step is termed the primary-to-secondary follicle transition. It involves a change in the arrangement of the granulosa cells from a simple cuboidal epithelium to a stratified or pseudostratified columnar epithelium (Carmen *et al.*, 2008).

Chang *et al.*, 2002, have established that the primary/secondary stage is a critical regulated step in the process of folliculogenesis, e.g., follicle growth and development stop at the primary stage in mice and sheep in the absence of GDF-9 and BMP-15, respectively. There work have led to the concept that oocyte-derived GDF-9 and BMP-15 are obligatory for the primary/secondary transition, presumably through their ability to stimulate granulosa cell proliferation and/or their pattern of arrangement (Chang *et al.*, 2002).Homotypic Cx43 gap junctions continue to develop between the granulosa cells as multiple layers form, resulting in an integrated and functional electrophysiological syncytium of communicating cells.Interestingly, folliculogenesis arrests at the primary/secondary transition in Cx43-deficient mice (Barr *et al.*, 1999). By implication it can be said that Cx43 coupling plays an indispensable role in the mechanisms controlling the formation of a secondary follicle.

2.4.5 Theca Development

According to Erickson *et al.,* 2003, Secondary follicle development is also characterized by thecal development.They also opined that at or about the time of the primary/secondary transition, several layers of stromal-like cells appear around the basal lamina. In the rat, some of these cells express a novel functional marker for differentiated theca cells (BMP-4), indicating that the theca develops very early in folliculogenesis.

Erickson *et al.,* 2003, opined that further development of the secondary follicle gives rise to the appearance of two primary theca cells; an inner theca interna that differentiates in the theca interstitial cells and an outer theca externa that differentiates into smooth muscle cells.Theca development is also accompanied by the neo-formation of numerous small blood vessels, presumably through angiogenesis. Consequently, blood now circulates around the follicle, bringing nutrients and gonadotropins to, and waste and secretory products from, the developing follicle. At the completion of the preantral phase of folliculogenesis, a fully grown secondary follicle contains five distinct but interacting structural units: a fully grown oocyte surrounded by a zona pellucida, approximately 9 layers of granulosa cells, a basal lamina, a theca interna, a theca externa and a capillary net in the theca tissue (Erickson *et al.,* 2003).

2.4.6 Oocyte Meiotic Competence

When the oocyte completes its growth during preantral folliculogenesis, it will spontaneously resume meiosis if removed from the follicle environment (Pincus *et al.*, 1935). However, fully grown oocytes rarely resume meiosis during folliculogenesis. This has led to the concept that there exists a meiotic inhibiting mechanism controlled locally by the follicle cells. The study carried out by Conti *et al.*, 2002 provides extensive evidence that cyclic AMP has a critical role in inhibiting meiosis resumption (Conti *et al.*, 2002). For many years it was thought that cAMP entered oocytes from granulosa cells via gap junctions and that this process was disrupted when the oocyte was removed from the follicle, allowing meiotic maturation to occur. However, recent experiments in both rodent and human suggest that meiotic arrest is maintained by cAMP generated within the oocyte itself, and that transit of cAMP into the oocyte via gap junctions is not required (Mehlmann, 2005; DiLuigi *et al.*, 2008).The new model is that a constitutively active G protein-coupled receptor, GPR3, persistently stimulates Gs protein to activate oocyte transmembrane adenylyl cyclases to generate high levels of cAMP within the oocyte (Mehlmann, 2005; DiLuigi *et al.*, 2008).

2.4.7 The Antral Follicle

Edward 1974 characterized the cavity of the follicle to be filled with follicular fluid. He also describes the follicular fluid as a plasma exudate conditioned by secretory products from the oocyte and granulosa cells. It is the medium in which the granulosa cells and oocyte reside and through which regulatory molecules must pass on their way to and from this microenvironment. This onset of antrum development is characterized by the appearance of a fluid filled cavity at one pole of the oocyte.

2.4.8 Histological Architecture Of The Graafian Follicle

The basic plan of the antral follicle, according to Erickson, is established after the formation of the antrum, and all the various cell types are present in their proper position awaiting the stimuli that lead to gradual growth and development. An antral follicle is a member of the heterogeneous family of relatively large follicles that in human ovaries measure 0.4 to ~25 mm in diameter (Erickson *et al.,* 2000).The structure and organization of antral follicles remains essentially the same despite enormous growth and regardless of the stage of the menstrual cycle. The overall size of an antral follicle is determined largely by the size of the antrum, which in turn is determined by the volume of follicular fluid. Depending on follicle size, the volume of follicular fluid varies between 0.02 to 7 ml. (Erickson *et al.,* 2000).

The proliferation of the follicle cells also contributes to follicle size, as studied by Erickson *et al.,* 2000. In a dominant follicle, the granulosa and theca cells proliferate extensively (as much as 100-fold) concomitant with the antrum becoming filled with follicular fluid. Thus, increased follicular fluid accumulation and cell proliferation are responsible for the tremendous growth of the dominant follicle during the follicular phase of the cycle. It is the cessation of follicular fluid formation and mitosis that limits the size of the atretic follicle. An atretic follicle usually fails to develop beyond the small to the medium stage (1-10 mm) (Erickson *et al.,* 2000).

Hendricks*et al.,* 2005, established that the relative abundance of antral follicles and their sizes vary as a function of age and the menstrual cycle, implying that the total number of antral follicles present in a woman's ovaries early in the menstrual cycle appears to be an indicator of her ovarian reserve (Hendricks *et al*., 2005).

The theca externa consists of concentrically arranged smooth muscle cells, which are innervated by autonomic nerves (Erickson *et al.,* 2000). The theca interna contains a population of large epithelioid cells termed the theca interstitial cells. They have the ultrastructural characteristics typical of active steroid producing cells, i.e., the cytoplasm is filled with lipid droplets, smooth endoplasmic reticulum and mitochondria with tubular cristae. The theca interstitial cells possess receptors for LH and insulin. In response to LH and insulin stimulation, they produce high levels of androgens, most notably androstenedione. The theca interna is richly vascularized by a loose capillary network that surrounds the antral follicle during its growth (Hendricks *et al.*, 2005).

In the antral follicle, the granulosa cells and oocyte are distributed as a mass of precisely shaped and precisely positioned cells. This spatial organization gives rise to distinct subtypes of granulosa cells: the membranal, the periantral area, and the cumulus oophorus. All the granulosa cells express FSH receptors during antral follicle development; however, each group of granulosa cells is influenced by its position to express a specific differentiated state in response to FSH stimulation. For example, the membranal granulosa cells express P450AROM and LH receptor whereas the periantral and cumulus cells do not (Erickson *et al.*, 2000).

2.4.9 Follicular Atresia

According to Daniel *et al.,* 2004, in mammals, 99.9% of the follicles (oocytes) die by atresia. Also the study describes the fundamental property of atresia to be the activation of apoptosis in the oocyte and granulosa cells. This is a complex process involving signaling pathways coupled to program cell death.It can be initiated externally (extrinsic pathway) by ligand binding to cell surface “death receptor” signaling such as that induced by tumor necrosis factor (TNF) or Fas ligand.

Intrinsic (intracellular) cell death pathways are mediated by alterations in mitochondrial outer membrane permeability that cause release of pro-apoptotic factors into the cytoplasm, and are typically controlled by B cell/lymphoma-2 (Bcl-2) family proteins. Both pathways result in activation of caspases, a family of cysteine-aspartate-specific proteases, as the final mediators of programmed cell death (Danial *et al.,* 2004).

Another study by Hussein in 2005, suggest that follicle atresia is controlled by a balance between pro-survival factors that promote cell proliferation, follicle growth and differentiation and pro-apoptotic factors that promote cell death. Follicle atresia and oocyte loss in adults appears to be initiated by apoptosis in the granulosa cells, unlike the massive loss of oocytes during fetal development that occurs via apoptosis within the oocyte (Hussein 2005). Both extrinsic and intrinsic cell death pathways appear to control apoptosis in granulosa cells.

Craig *et al.,* 2007 established the importance ofFSH in supporting follicle growth after antrum formation and in preventing apoptosis which has led to the concept that FSH is a survival factor for antral follicles. Aspects of the downstream signaling pathway induced by FSH that are important for follicle survival have been determined by this study. FSH activates the PI3K signaling pathway in granulosa cells, causing phosphorylation of Akt (protein kinase B) that leads to an increase in cell survival proteins including members of the IAP (inhibitor of apoptosis) family and resulting in inhibition of the intrinsic cell death pathway. Oocyte-secreted factors also inhibit granulosa cell apoptosis (Craig *et al.,* 2007).

Another study by Orisaka *et al.,* 2007, establishes evidence from studies with rats that GDF-9 serves as a pro-survival factor in preantral follicles by activating the PI3K signaling pathway. Also in the bovine, oocyte-secreted BMP-15 and BMP-6 are important for maintaining cumulus cell survival (Hussein 2005).

At least three ligands of the tumor necrosis factor (TNF) family have roles in ovarian follicle atresia: TNF-alpha, Fas ligand, and TRAIL (TNF-related apoptosis-inducing ligand) (Craig *et al.,* 2007). Other intra-ovarian pro-apoptotic factors include Apaf-1 (apoptotic protease-activating factor-1), nodal, a TGFβ family member found in granulosa cells of apoptotic follicles, and the p53 stress response gene (Robles *et al.,* 1999; Wang *et al.,* 2006). Prohibitin is a ubiquitous mitochondrial membrane protein that may mediate p53-induced apoptosis in granulosa cells (Thompson *et al.*2004*;* Chowdhury *et al.* 2007).

2.5.0 Ovulation

On or about the fourteenth day of an idealized 28 day menstrual cycle, the preovulatory follicle releases a mature egg enclosed within a cumulus complex for possible fertilization.This process, termed ovulation, requires the collective actions of the endocrine system, immune signals, and intraovarian paracrine factors.The distinct cellular compartments in the preovulatory follicle — the oocyte, cumulus granulosa cells, mural granulosa cells, and theca cells — have dramatically different but strictly coordinated responses to the hormonal and other signals controlling ovulation (Carmen *et al.,* 2008).

2.6.0 Reproductive System of the Female Rat

The female reproductive system consists of the two ovaries and the female genital tract. The genital tract includes the oviducts, uterus, cervix and vagina. The female genital tract in mammals arises from the Mullerian ducts, commencing with the ostium of the oviduct. In the rat, this ostium forms a complete capsule called the ovarian bursa, which envelops the ovary. The oviducts are small, highly coiled tubes. The uterus consists of two separated uterine horns, enabling the rat to have multiple offspring. The vagina of the rat opens directly to the exterior (Kent and Carr, 2001).

2.6.1 Estrous Cycle

Marcondes *et al.*, 2001, describe the reproductive cycle of female rats is called the estrous cycle and characterized it as proestrus, estrus, metestrus (diestrus I) and diestrus (diestrus II). The cyclic change in the female rat commences from the onset of maturity up to the age of 12 months as established by Marcondes, and has a mean cycle length of 4 days. This also confirms other studies conducted before him by Yong *et al*., 1941, and Schwartz, 1964.

The rat estrous cycle is short, lasting four to five days. It occurs throughout the year, with no seasonal effect. The first regular estrous cycle occurs about one week after the opening of the vaginal orifice, usually 33 to 42 days after birth (Maeda *et al*., 2000). The cycle length increases slightly with age and lasts about 6 days near the end of the reproductive life span (Lu *et al*., 1979).

2.6.2 Phases of the Estrous Cycle

The characterization of each phase of the estrous cycle is based on the proportion of cell types observed in the vaginal smear. These are; round and nucleated epithelial cells, cornified cells of irregular shape and without nucleus, and leucocytes which are small and round. These cells are identified based on the vaginal smear cytology (Long & Evans, 1922; Hoar & Hickman, 1975). The phases of the estrous cycle classified according to Marcondes *et al.*, 2001, are proestrus, estrus, metestrus (diestrus I) and diestrus (diestrus II).

2.6.3 Proestrus Phase

The proestrus stage consists of predominance of nucleated epithelial cells. This period is accompanied by rapid follicular growth under gonadotropic stimulation. There is progressive increase in the level of estrogen secreted by the developing follicles, and progressive declining in the level of progesterone due to the regression of corpus luteum from preceding cycle, and it lasts only for 12 hours (Marcondes *et al.*, 2001).

2.6.4 Metestrus Phase (diestrus I)

Metestrus stage consists of the same proportion among leucocytes, cornified, and nucleated epithelial cells. This stage marks the transition period between ovulation and full development of the corpus luteum (Marcondes *et al.*, 2001).

2.6.5 Diestrus

Diestrus describe the stage in which the corpus luteum is fully develops and the predominant hormone at the stage is the progesterone. Diestrus smear primarily consists of predominant leucocytes (Long & Evans, 1922; Hoar & Hickman, 1975).

2.6.6 Characteristics of the Estrus Cycle In the Rats

The estrous cycle in the rat consists of four stages knownas proestrus, estrus, metestrus and diestrus. Proestrus lasts approximately 12 hours; estrus, 9 to15 hours; metestrus, 21 hours; and diestrus (the longest phase), over 57 hours (Lohmillerand Swing, 2006).

The changes in the cyclicity in the reproductive tract of the female rats is directly controlled and influenced by the fluctuation of the hormones of the reproductive system in the female rat.Hormones play critical roles in the estrous cycle.Gonadotropins, which are secreted by the anterior pituitary, regulate the estrous cycle through luteinizing hormone (LH) and follicle stimulating hormone (FSH). Hormonal fluctuations result in ovarian and follicular changes, as well as changes in vaginal cytology (Freeman, 1988).

FSH stimulates follicle growth, while LH stimulates the follicles to ovulate and form the corpus luteum. Progesterone is secreted by the corpus luteum during metestrus and declines during diestrus. During follicular development, the level of estradiol-17β increases. The cycle ends when estrogen peaks during proestrus, stimulatinggonadotropin release to trigger ovulation (Freeman, 1988).

2.7.0 Hormones of the Gonads

Two classes of hormones produced by the ovaries are estrogens and progestin. Chemically, estrogen and progestin are classified as steroids and have cholesterol as a common precursor (Bearden and John, 1997). Estrogens represent a group of steroids with similar physiological activity, and are produced by specific cells of the Graafian follicle. The thecal cells of the follicle are stimulated by LH to produced androgens which diffuse across the basement membrane, where they are converted to estrogens by the aromatase activity in the granulosa cells, which are under the influence of FSH.

The estrogen of greatest importance in quantitative and physiological term is the estradiol. Others of importance include estriol and estrone. The principal actions of estrogens are the manifestation of mating behavior during estrus; cyclic changes in the female reproductive tract, duct development in the mammary gland and development of secondary sex characteristics. Estrogens have been called the female sex hormone, and are known to be luteolytic in the cows and ewes but are luteotropic in the sows (Hafez, 2000).

2.7.1 Effect of Progestins on the uterus

Progestins are another group of hormones of the gonads, the most important being progesterone. they are secreted by the corpus luteum. The larger luteal cells of granulose origin are the principal progesterone secreting cells in the corpus luteum, but small luteal cells of the thecal origin also secrete progesterone when stimulated by LH. The important function of this hormone are inhibition of sexual behavior, maintenance of pregnancy by inhibiting uterine contractions and promoting glandular development in the endometrium, promotion of alveolar development

of the mammary gland. The synergistic actions of estrogens and progestins are notable in preparing the uterus for pregnancy and the mammary gland for lactation (Moss *et al.,* 1981).

2.7.2 Effect of estrogen on the uterus

Serum estrogen level rises immediately before ovulation, and in response to this surge the uterus exhibits well characterized physiological and biochemical responses (Clark and Mani, 1994). Among the morphological and physiological changes induced in the uterus after the estrogen surge include an increase in water imbibitions in the uterus endometrium as a result of hyperemia and vasodilation of uterine capillaries. This process leads to swelling of the uterus with a consequent significant decompaction of stromal cells. This water then moves from the epithelium into the lumen, leading to uterine wet weight.

The secretory capacity of the uterus is highly increased by estrogen stimulation, mainly due to the development of the epithelial layer into columnar secretory epithelial cells. At the same time there is an increase in the vascularisation of the uterus, which increases the amount of blood in this organ (hyperaemia), accompanied by infiltration of immune system cells such as macrophages and eosinophils. This event mimics an acute inflammatory response. There is also an increase in the number and size of specific cell types (hypertrophy and hyperplasia), which also contributes to the large increase in uterine weight that can be detected after estrogen stimulation (Jorge *et al.,* 2007).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Experimental Animals

Twenty-four young adult Wistar rats weighing between 135 - 150g per group wereobtained from the department of pharmacology of Ahmadu Bello University Zaria, Kaduna state, Nigeria. They were kept in plastic cages and maintained under laboratory condition at room temperature and humidity. Waterandstandard pellet diet were provided to the rats ad libitum.The rats were pre-conditioned to this new environment for two weeks before the commencement of the study. All animals received care in compliance with the guidelines of the ethical committee of medical research, ABU Zaria.Thereafter, the animals were divided into four groups of six animals each.

3.2 Collection and Identification of Plant Material

The fresh stem bark of *A. leiocarpus*, readily available within the Ahmadu Bello University Zaria, were collected and authenticated in the herbarium of Biological Sciences Department of ABU Zaria by U. S. Gallah with a voucher specimen number 1738 for future reference.

3.2.1 Preparation of plant extract

Two kilogram of*A. leiocarpus*stem bark was air dried, minced and powdered using a clean, sterilelaboratory mortar. 50g of the resulting powder was dissolved in 0.5 liters of distilled water using a soxhlet extractor.The solution was allowed to settle for twenty-four hours and filtered with Whartmann's filter paper. The resultant solute was allowed to settle for one hour, and placed in water bath under reduced pressure at 35℃ for evaporation.

The extract was weighed and dissolved in distilled water to prepare the stock solution. The percentage yield was calculated to be 25%, and it was calculated as follows:

$$\frac{\text{Average weight of dried aqueous extract (50g) x 100}}{\text{Weight of dried coarse powdered sample (200g)}}$$

3.2.2 Choice of solvent for extract administration

The suitable solvent for the dilution of the extract used in thestudies was determined by trying to dissolve the extract in propylene glycol and distilled water. The extract was only partially soluble in propylene glycol but was completely soluble in water after rigorous stirring with a glass rod. Therefore water was chosen as solvent of choice for the extract administration.

3.2.3 Determination of concentration and volume of extract

The maximum convenient concentration (MCC), that is the maximum amount of the extract dissolved in 0.1ml of solvent, such that the solution can be delivered through an 18gauge needle at room temperature, was determine by measuring 1.0g of the extract and adding 0.05ml of solvent at a time, until the solution could be easily delivered through the needle (Ibrahim, 1984). From the volume of solvent used, the MCC of the extract in mg/ml was determined as 50mg/ml and stock solution was made and refrigerated until required. The maximum convenient volume (MCV) that can be administered to rats by the oral route is 5ml/kg (Loomis, 1978).

3.2.3 Route of administration of extract

The extract was given orally using an insulin syringe (1ml) fitted to curved 18 – gauge needle with blunt end.

3.3 Chemical Reagents

1. Lutalyse (Dinoprost tromethamine) used for synchronization of animals was supplied by Upjohn Ltd, animal health division, Crawley, west Sussex, RH10 2NJ, UK; ELISA Kit
2. Estradiol and progestin assay kits supplied by WKEA Med Supplies Corp, Changchun, China.

3.4 Experimental Design

Twenty-four adult female Wistar rats were used for this study. The animals were randomly assigned into four groups I, II, III, and IV, each group consisting of six animals selected at random. Each group was placed in a separate cage. The experimental groupswere given rat pellets and various concentrations of aqueous extract of *A. leiocarpus* of varying concentrations of the extract gavage, corresponding to 600mg/kg (½ LD_{50}) for group VI, 400mg/kg (⅓LD_{50}) for group III, and 200mg/kg (¼ LD_{50}) for group II, the control group I, were given food and distilled water for the period of six weeks.

Table 3.1: Experimental groups and their respective treatments

Group	Treatment dose/ daily for 6 weeks
I	Distilled water
II	200mg/kg
III	400mg/kg
IV	600mg/kg

3.5 Synchronization Of Experimental Animals

Experimental animals in all four groups were synchronized using (Dinoprost tromethamine), supplied under the trade mark name of **Lutalyse**. Dinoprost tromethamine is a synthetic, naturally occurring prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) in the product in the form of its crystalline THAM salt, its structural formula is represented as below:-

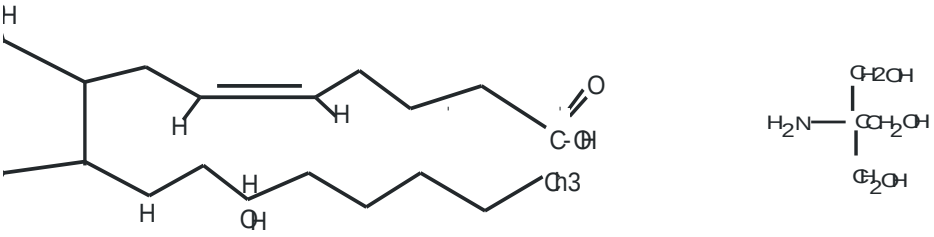


Figure1. The molecular weight of Dinoprost is 354.5 and that of its tromethamine salt is 475.6 in the form of THAM salt.

Synchronizing estrus utilizing progestogen plus prostaglandin F2α Prostaglandin F2α (PGF2α) and its analogs cause luteolysis and a return to estrus in cattle when given during the luteal phase of the estrous cycleand the fertility of the induced estrus is normal (Lauderdale,1975). Research has shown that a higher percentage of cattle treated with PGF during the late luteal phase (Days 10 to 17) exhibited estrus than those treated during the early luteal phase (Days 5 to 9) (Tanabe *et. al.,* 1984). It has also been shown that the closest synchrony of estrus occurs when mammals are at a similar stage of the estrous cycle when PGF2α is administered. Based on the results of these research trials, a system that initially synchronizes heifers by feeding MGA and then administers PGF2α during the late luteal phase of the subsequent cycle should produce a high percentage of heifers displaying closely synchronized estrus and with improved fertility (Tanabe *et. al.,* 1984).

Specifically, $PGF_{2\alpha}$ is used for synchronization in this study because it has been shown according to the studies outlined to have the following effect:-

1) Causes the release of pituitary tropic hormones involved in the process of ovulation and gamete transportation (Rowson*et. al.,* 1984).
2) It is used to more effectively control the time of estrous and ovulation.
3) Causes increase in the uterus and blood to the levels similar to exogenous hormones levels required for eliciting luteolysis (Rowson*et. al.,* 1984).

0.5 mg/kg of Dinoprost tromethamine containing prostaglandin $PGF_{2\alpha}$was administered twice intramuscularly at two day interval, according to the manufacturer's instruction.

Following injection, daily vaginal smears of the animals were evaluated to monitor the estrous cycle phases of the animals.

3.6 Method of Taking Vaginal Smear

Vaginal secretion from the experimental animals was collected according to the method described by Marcondes *et al.,* 2001. Every morning between 8:00 and 9:00 a.m. rats were allowed to remain undisturbed for an hour before sampling. The genital area of each rat was cleaned with cotton wool soaked in methylated spirit. The tip of a pasture pipette filled with 10µls of normal saline (Nacl 0.9%) was inserted into the rat vagina, but not deeply. Vaginal fluid obtained was used to make a smear on a clean glass slides. One drop was collected with a clean tip from each rat. Unstained material was observed under a light microscope with 10 and 40× objective lenses. Estrous stage was then evaluated according to the method of Long and Evans, 1922; Mandl, 1951.

The phases of the estrous cycle of the rat were identified as follows: -

Proestrus: - Smears with prominence of epithelial cells indicated rats in proestrus phase of the cycle. Plate II (A).

Estrus: - Smears with prominence of epithelial cells indicated rats in proestrus phase of the cycle (B).

Metestrus: - Smears with near equal proportions of epithelial, cornified and leucocytes indicated rats in metestrus phase of the cycle (C).

Diestrus: - Smears with prominences of leucocytes cells indicated rats in diestrus phase of the cycle (D).

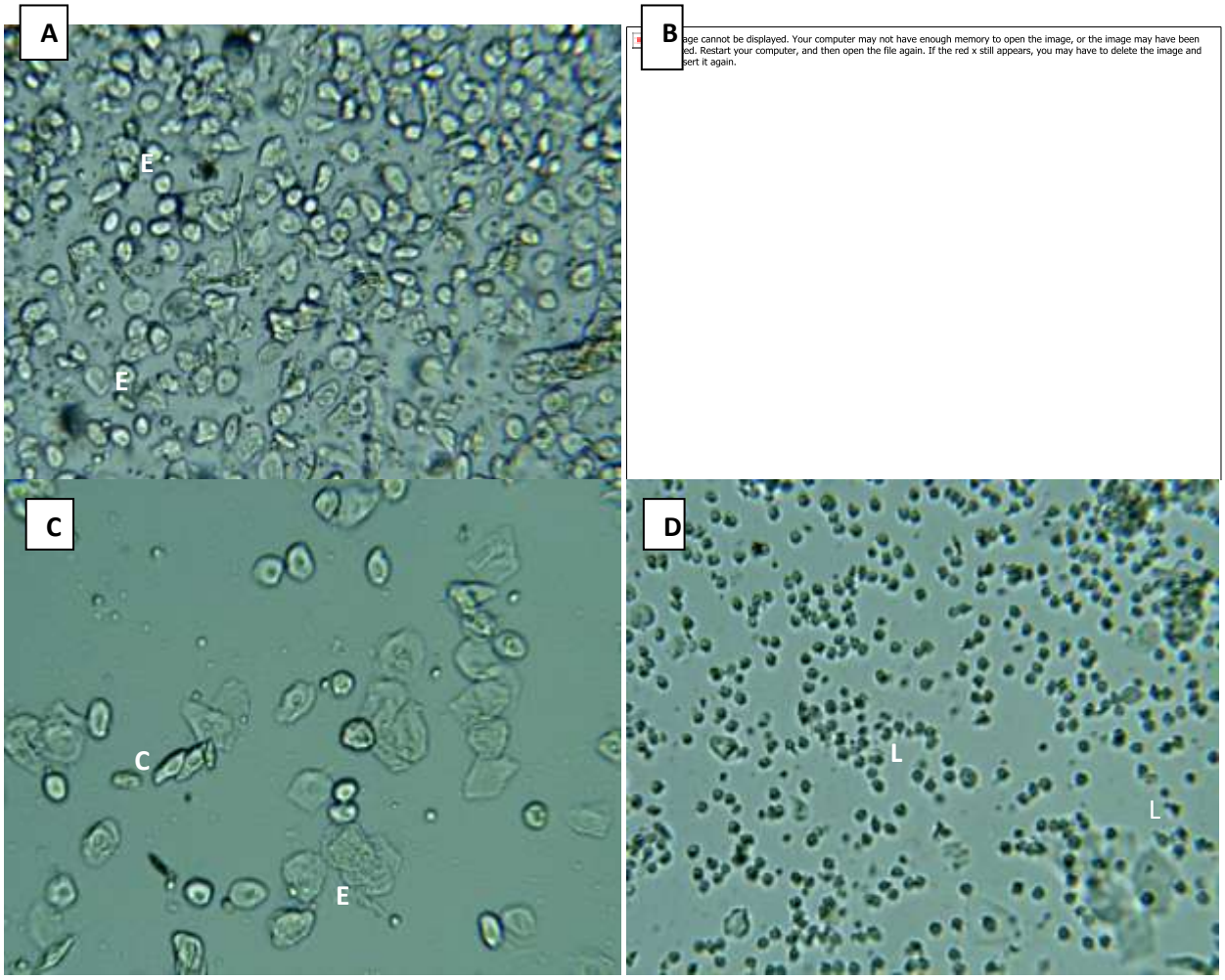


Plate II: Photomicrograph showing the vaginal smear of the experimental rats at different estrous stages. (A) Proestrus**:** round and nucleated ones are epithelial cells. **(B) Estrus:** irregular ones without nucleus are the cornified cells. **(C)Metestrus:** the same proportion of cells in proestrus, estrus and diestrus. **(D)Diestrus:** and the little round ones are the leukocytes. (x 200)

3.7 Hormonal Assay

Blood samples were collected from the rats before the extract administrationfrom the infraorbital vein using a microhematocrit blood tube. 2mls of blood was collectedand centrifuged at 2000rmp (revolution per minute) to separate the serum for hormonal analysis.

On the last day of the experiment, blood sample was collectedthrough cardiac puncture in a 5ml tube under deep anesthesia using chloroform. It is then centrifuged to obtain serum for the analysis of ovarian hormones (estradiol and progestin).

Serum for hormonal analysis was obtained in two phases: -

1) Pre-administration of extract, following synchronization of the estrous cycle of the experimental animals.
2) Post-administration of extract at the end of the experiment.

The assay for the determination of estradiol and progesterone level in the serum was carried out using the Estradiol (E2), and Progestin ELISA kits, and the procedure is conducted according to the manufacturers' instructions in the kits manual.

3.8 Histological Studies

The following reproductive tract were harvestedimmediately after the rats were sacrificed, weighed in analytical balance,and fixed in 10% Bouin's fixative solution. Tissue sections were prepared and stained using Hematoxylin & Eosin stain for light microscopy. Photomicrographs were obtained using a microscope eyepiece attached to a computer.

3.8.1 Tissue processing procedure

The fixed tissues were fixed in Bouin's solution and dehydrated using graded alcohol concentrations. This method involved dehydration of tissue in 70% alcohol, 90% alcohol, 95% alcohol, and absolute alcohol, each stage lasting for 30minutes. The use of ascending concentration of alcohol is to prevent the rapid dehydration of tissue thereby causing structural damage to the tissue. The dehydrated tissue is the cleared in two changes of chloroform for 120minutes each. The clearing is to remove the opacity caused by the dehydrating agent, and make the tissue transparent. The tissue is then infiltrated by immersion into molten paraffin for a period of 30minutes. The tissue were then embedded into molten paraffin wax and allowed to solidify. The embedded tissue were blocked in a rectangular block and sectioned using the rotary microtome at 5µm per section.

The tissue sections were allowed to float in water bath at 30^{o}C to help the spreading of the tissue ribbons. Clean slides were used to pick the tissues from the warm water bath. The slides were left to dry and later stained using H&E, and PAS stains.

3.9 Histometrical Studies

Histometrical study was conducted using the method of Plowchalk *et al*., 1993. Paraffin sections of 3µm thickness were cut on a microtome and stained with Hematoxylin and eosin (H&E) for morphometry and enumeration of ovarian follicles. Homologous cross sections of the entire ovary showing a better area of vision were chosen. The ovarian section was traced around the tissue boundaries with the computerized software and a sampling grid was superimposed over the section. Each square of the sampling grid represent a sampled area measuring 6400µm.

A counting frame was then used to sample a pre-determined area within each square of the sampling grid. Section area was calculated by integrating the area inside the traced perimeter and volume calculated by multiplying the section thickness. This is to count for the number of sections skipped. The ovarian volume in this study was 7.9µm.

3.10 Statistical Analysis

The results obtained were analyzed using Statistics 5.0 software and Microsoft Excel 2007.All results were expressed a mean value ±SEM.The variance of the data and the confidence interval were determined using the analysis of variance (ANOVA) and a value of $P<0.05$ was considered as statistically significant. Dunnet's post hoc tests were used to determine where the level of significance lies.

CHAPTER FOUR

4.0 RESULTS

4.1 Physical Observations

The animals in all the experimental groups did not show any signs of distress throughout the experimental period. Therewasan increase in activity in both the treated and the control groups. There was also an increase in defecation immediately following the extract administration, which was more in the high dose group that received 600mg/kg concentration of the extract.

All treated groups showed an increase in urination after the administration of extract, and a gradual decreased in food consumption, particularly in the groupsthat received extract concentration of 600mg/kg, and 400mg/kg.

4.2 Synchronization of Experimental Rats

The result obtained from the vaginal smear of the experimental rats injected with 0.5ml of $PGF_{2\alpha}$, showed that 96% of the rats were synchronized within 6 cycles.

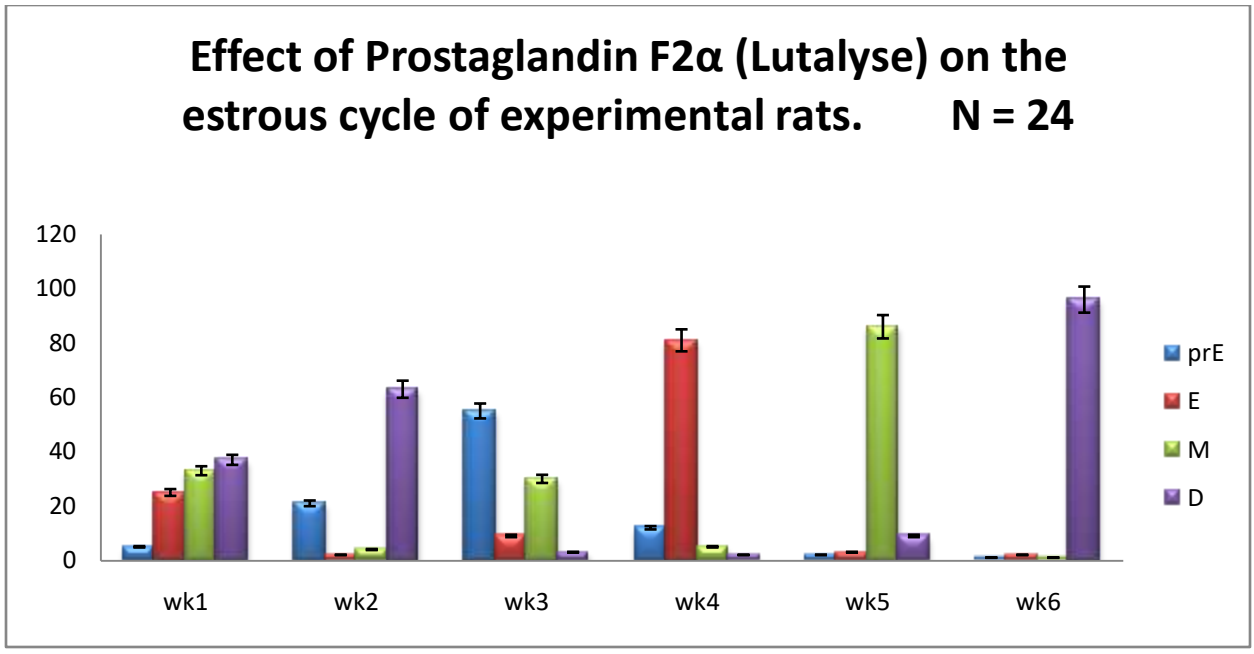


Figure 4.1: **Effect of Prostaglandin F2α (Lutalyse) on the estrous cycle of experimental rats.** **N = 24**

prE - Proestrus
E - Estrus
M - Metestrus
D - Diestrus

4.3 Effect of *Anogeissus leiocarpus* extract on body weight of the animals

The result of the effects of the extract on body weight showedan increase in weight of the animals across the experimental groups compared to the control.The increase was significant between the high dose group (600mg/kg), however not statistically significant ($P \leq 0.05$). and the control. It is non-significant compared with experimental groups II and III ($P \leq 0.05$).

Table 4.1 Effect of oral administration of *Anogeissus leiocarpus* extract on body weight of animals

Group	N	Mean weight (g) ± SEM	Mean weight (g) ± SEM	Mean weight(g) ± SEM	Mean weight (g) ± SEM	Mean weight(g) ± SEM	Mean weight(g) ± SEM	Mean weight(g) ± SEM	P-value
		0week	1st	2nd	3rd	4th	5th	6th	
I- Control	6	146 ± 5.1	145 ± 5.0	146 ± 5.1	146 ± 5.1	147 ± 5.3	146 ± 5.1	147 ± 4.0	0.47
II- 200mg/kg	6	142 ± 4.2	142 ± 4.2	143 ± 4.3	145 ± 4.8	144 ± 4.5	146 ± 4.7	146 ± 4.9	0.46
III- 400mg/kg	6	137 ± 4.1	137 ± 4.1	138 ± 4.2	139 ± 4.3	140 ± 5.3	141 ± 3.9	141 ± 5.1	0.46
IV- 600mg/kg	6	135 ± 4.5	134 ± 4.7	135 ± 4.5	137 ± 4.1	137 ± 3.9	138 ±4.0	141 ± 5.3	0.49

Keys
P> 0.5
SEM = Standard Error in Mean

4.4 Effect of administration of *Anogeissus leiocarpus* extract of the Estrus Cycle of rats at different doses.

Group	Percentage (Mean ± S.E.M) of rats at different Phases of Estrus Cycle			
	Proestrus	**Estrus**	**Metestrus**	**Diestrus**
I - Control	**15.46±3.2**	**11.73±1.8**	**51.07±3.2**	**23.38±2.8**
II - 200mg/kg	**5.30±1.8**	**35.03±4.7**	**21.30±4.7**	**39.05±3.7**
III - 400mg/kg	**15.26±3.2**	**33.43±3.6**	**5.35±4.6**	**47.31±5.4**
IV - 600mg/kg	**23.32±3.6**	**40.03±5.4**	**31.06±3.2**	**6.83±3.6**

Table 4.4 the mean ± S.E.M of the percentage of rats at different phases of Estrus Cycle on different doses of the extract.

4.5 Effect of different doses of extract on the weight of the ovaries, uterus, and fallopian tubes.

No significant difference ($P \leq 0.05$) was observed in the weight of the ovaries, uterus, and the fallopian tubes of rats that were treated with the extract when compared with the control group (table 4.3).

Table 4.3 Weight of the Uterus, fallopian tubes, and Ovaries across the experimental groups

Group	Weight of Uterus, fallopian tubes, and Ovaries Mean weight (g) ± SEM	Body weight/Organ ratio Mean weight (g) ± SEM	P- value
I- Control	0.54 ± 0.04	242 ± 5.3	0.043
II- 200mg/kg	0.56 ± 0.04	260 ± 5.1	0.047
III- 400mg/kg	0.56 ± 0.04	251 ± 4.9	0.046
IV– 600mg/kg	0.57 ± 0.06	272 ± 4.0	0.057*

Keys

P< 0.05
SEM =Standard Error in Mean
***= Statistically significant**

Table 4.4 Number of follicles across each experimental group

Group	Follicles		
	Primary follicles Mean ± SEM	**Secondary follicles Mean ± SEM**	**Tertiary follicles Mean ± SEM**
I- Control	**96.3±3.70***	**39.8± 2.90**	**13.6± 1.47**
II- 200mg/kg	**94.2± 3.70**	**37.1 ± 2.90**	**14.2± 1.47***
III- 400mg/kg	**98.7± 3.70**	**47.1 ± 2.90***	**12.5± 1.47**
IV- 600mg/kg	**87.1± 3.70**	**34.1± 2.90**	**2.8± 1.47**

Keys

P<0.5
SEM = Standard error in mean
***= Not statistically different**
****= Statistically significant**

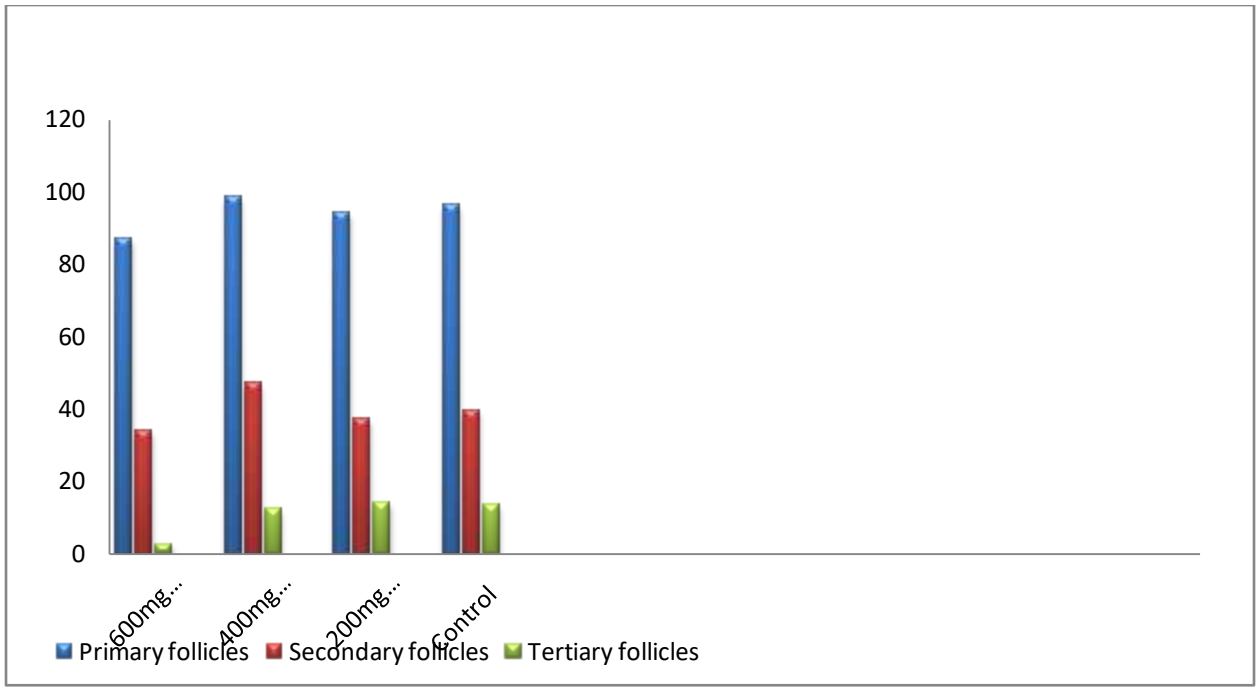


Figure 4.3 Number of follicular cells across each experimental group

Table 4.5 Mean serum level of Progesterone of rats pre and post- administration of Anogeissus leiocarpus extract by 42 days

	Progesterone Pre administration Mean(pg/ml) ± SEM	Progesterone Post-administration Mean(pg/ml) ± SEM
I- Control	8.13 ± 0.12*	6.31± 0.13*
II- 200mg/kg	4.50 ±0.12	5.61± 0.09
III- 400mg/kg	5.10 ± 0.12	6.20± 0.10
IV- 600mg/kg	8.25±0.13*	6.46± 0.14*

Keys

P <0.05
SEM = Standard Error in Mean
***= Statistically Significant**

Table 4.6 Mean serum level of Estradiol of rats pre and post- administration of Anogeissus leiocarpus extract by 42 days

	Estradiol Pre administration Mean(pg/ml) ± SEM	Estradiol Post-administration Mean(pg/ml) ± SEM
I- Control	**6.33 ± 0.14***	**7.91± 0.13***
II- 200mg/kg	**5.80 ±0.13**	**6.41± 0.14**
III- 400mg/kg	**5.30 ± 0.14**	**6.70± 0.13**
IV- 600mg/kg	**6.75±0.14***	**5.76± 0.14***

Keys

P <0.05
SEM = Standard Error in Mean
***= Not statistically significant**
****= Statistically Significant**

Figure 4.4

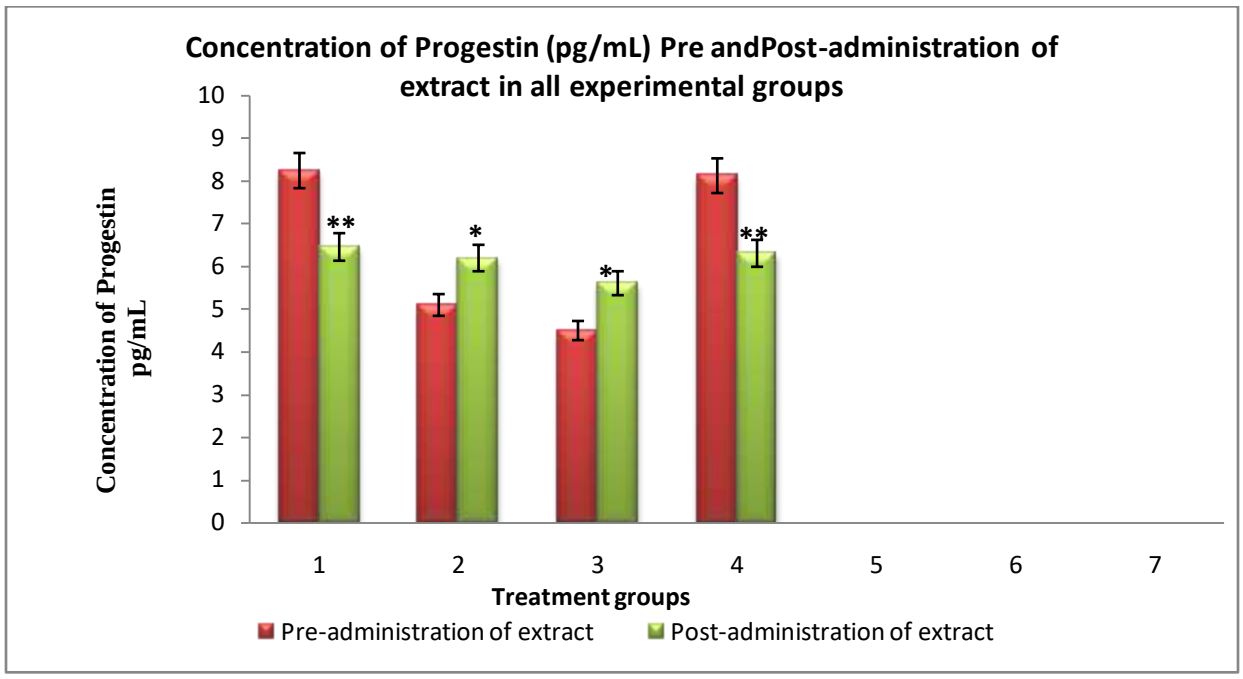
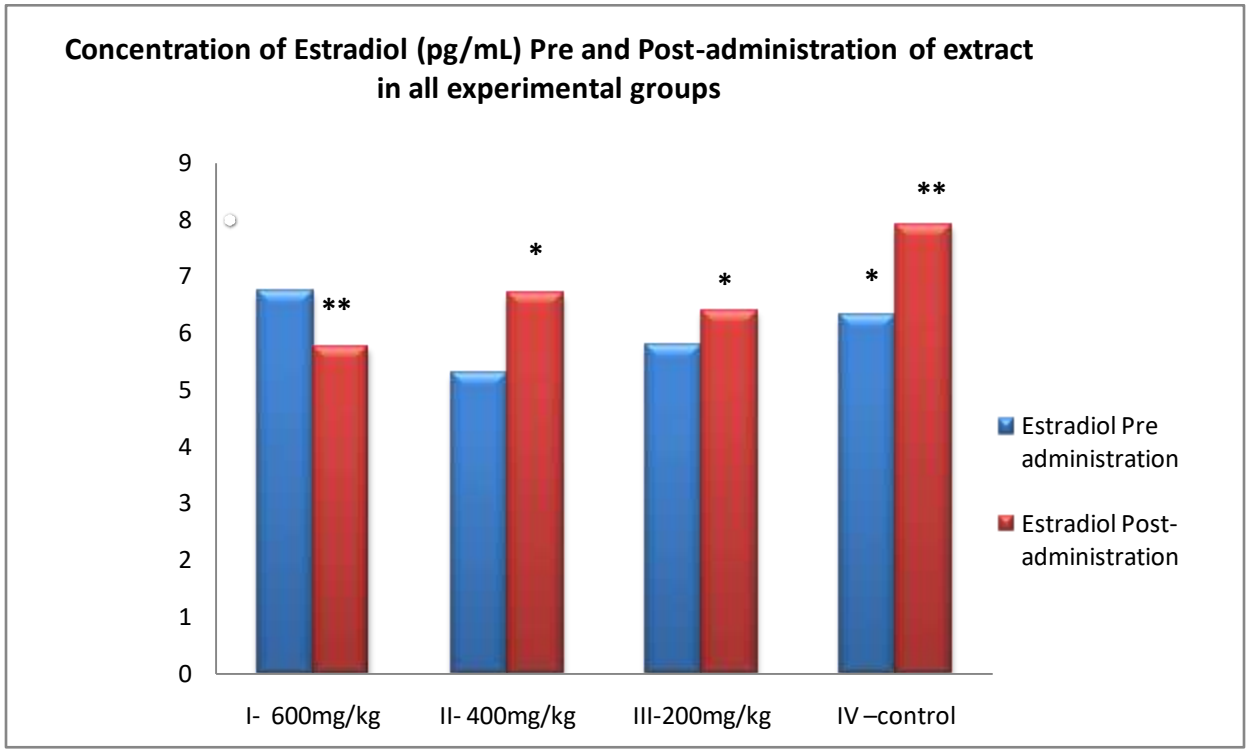

Concentration of Progestin (pg/mL) Pre andPost-administration of extract in all experimental groups
Concentration of Progestin pg/mL
10
9
8
7
6
5
4
3
2
1
0
**
*
*
**
1
2
3
4
5
6
7
Treatment groups
Pre-administration of extract
Post-administration of extract

Figure 4.5


Concentration of Estradiol (pg/mL) Pre and Post-administration of extract in all experimental groups
9
8
7
6
5
4
3
2
1
0
**
*
*
*
**
I- 600mg/kg
II- 400mg/kg
III-200mg/kg
IV –control
Estradiol Pre administration
Estradiol Post-administration

4.6 Effect of Extract administration on the Histology of the Ovaries

The histological features of the ovaries in the control group (I) showed normal features of the ovary as evidence by the presence of follicles in different stages of development (plate1).

The dose treatment of the extract did not affect the ovarian structure in terms of number of developing follicle, tertiary folliclesas observed in histological sections (table 4.3). However there was increased folliculogenesis, which shows an increase in the number of primary follicles in the treatment group that received 400mg/kg (group III). An increase in secondary follicles in the group II that received extract concentration of 200mg/kg was also observed.

4.4 Hormonal assay for Estradiol and Progestin Pre and Post-administration of Extract

The mean progesterone concentration inbefore the extract administration was significantly higher in groups IV and the control (group I), (8.25 ± 0.13; and 8.13 ± 0.12) pg/ml in the pre-treatment group, and (6.46 ± 0.16; and 6.13 ± 0.13) pg/ml in the post treatment group. This was about 23% less and statistically significant ($p< 0.05$) after the extract administration shown in table 4.4. There was an increase in the concentration of progesterone in groups III, and group IVfollowing the extract administration. The progesterone level in the orally treated group was 19% higher, and statistically significant ($p< 0.05$) when compared to the concentration of serum progesterone in the pre-treated blood. Table 4.4

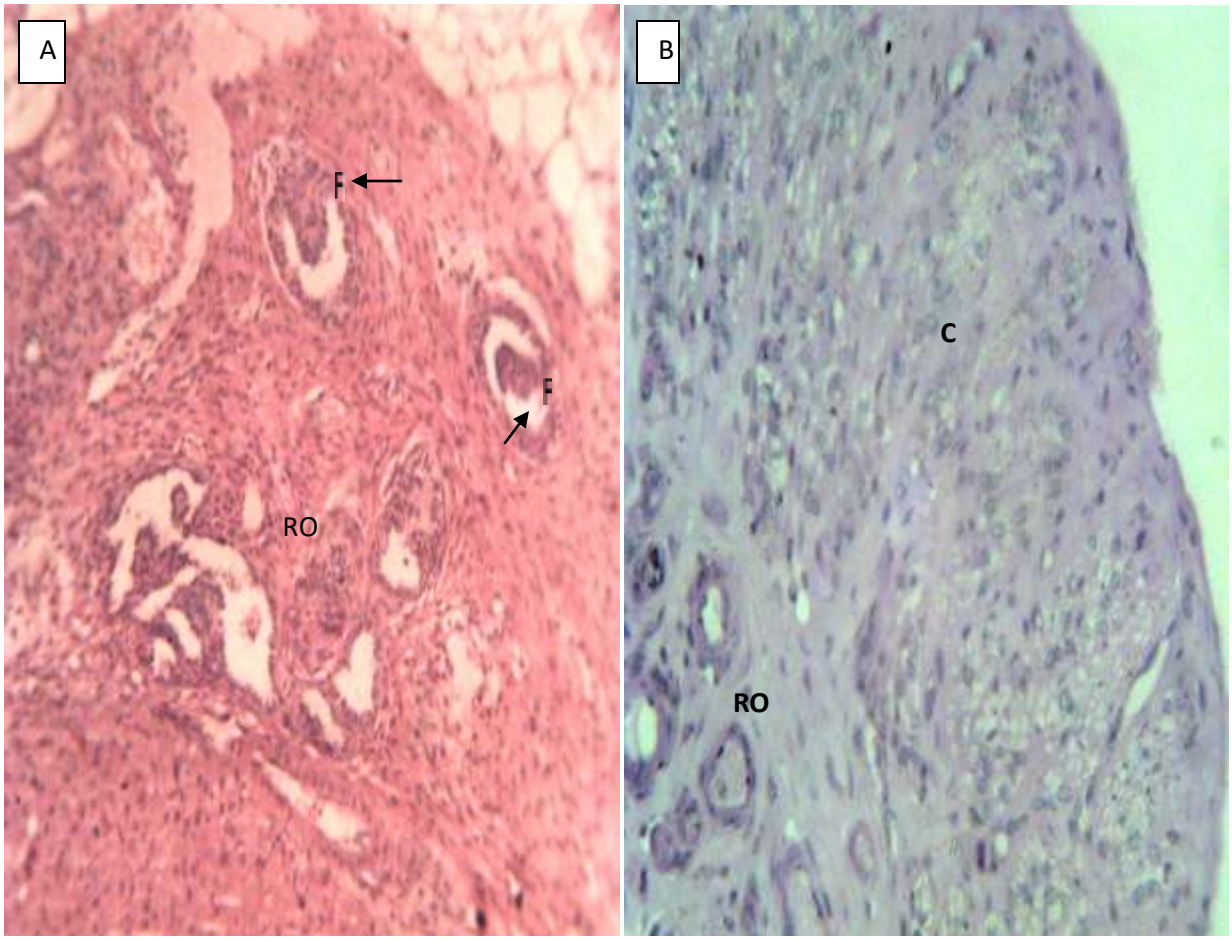


Plate 1: **Photomicrograph showing the cross section of the medulla of the ovary from (A)Group treated with 200mg/kg body weight, (B) Control group showing some atretic follicles(F); Cortex (C) and rete ovaries (RO). (H&E x 100)**

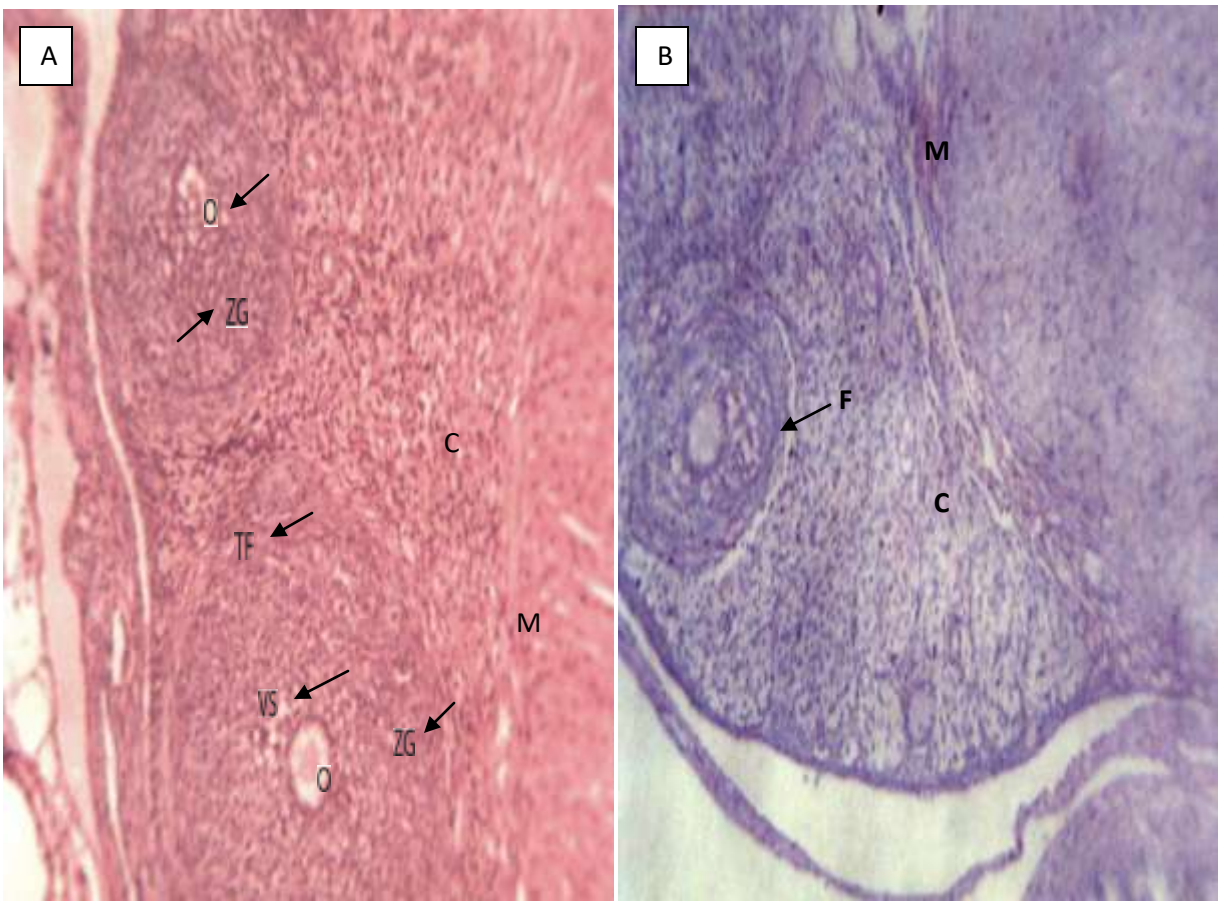


Plate 2: **Photomicrograph showing a cross section of the ovary of (A) Group that received 200mg/kg body weight, (B) Control group, showing normal architecture of the ovary with primary oocyte (O); theca folliculi (TF); vesicular spaces (VS); zona granulosa (ZG); ovarian cortex (C); ovarian medulla (M). (H&E x 100)**

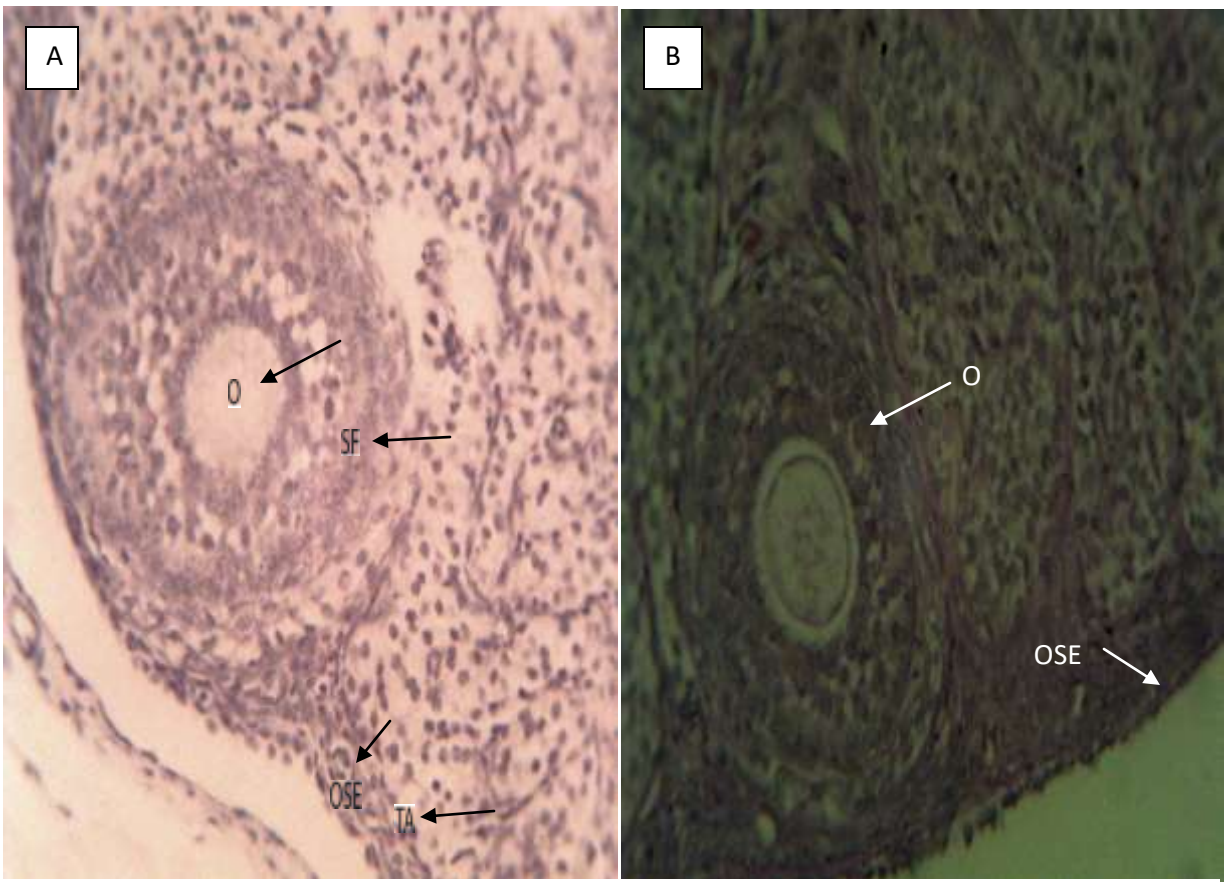


Plate 3: **Photomicrograph showing a cross section of the ovary of (A)GroupIII received 400mg/kg body weight, and (B) Control group,both showing normal architecture of the ovary with primary oocyte (O); outer surface epithelium (OSE); tunica albuginea (TA). (Pas x 250)**

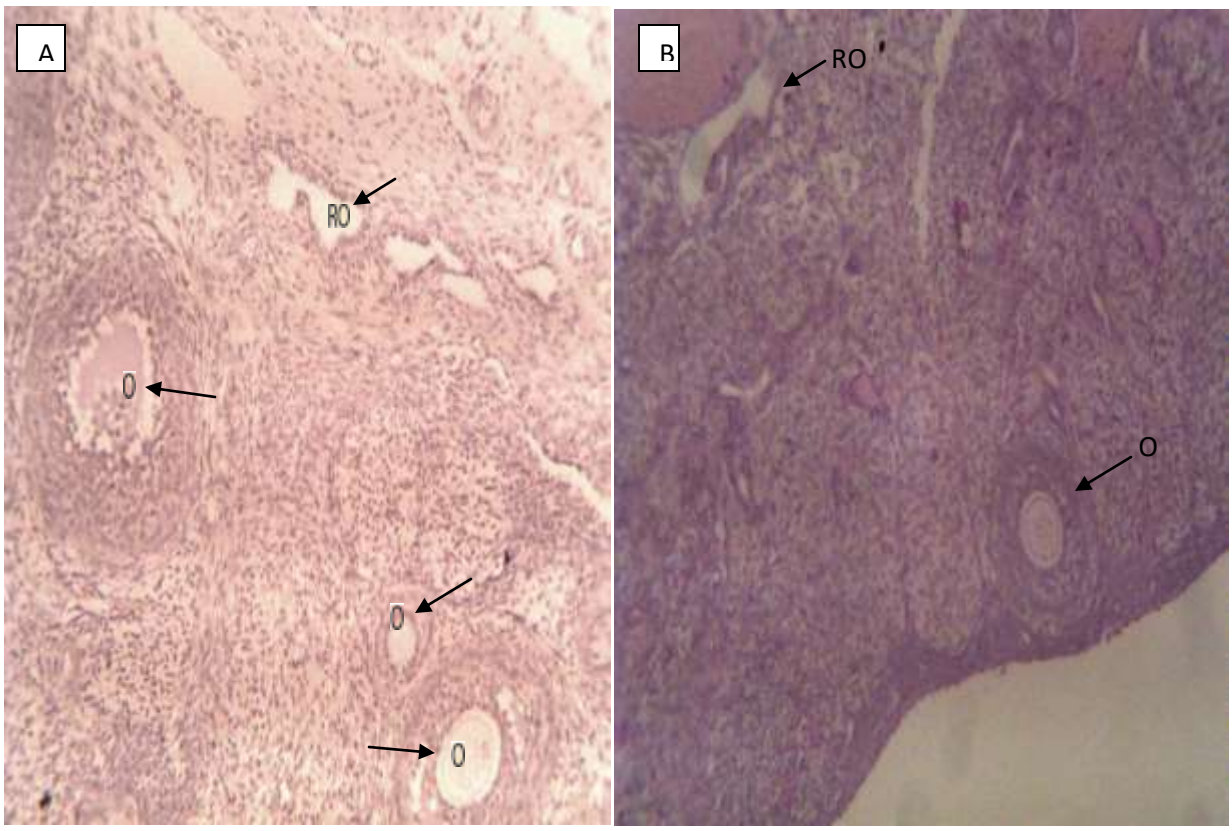


Plate 4: **Photomicrograph showing the cross section of the ovaries of (A) Group II, treated with 200mg/kg body weight, and (B) Control group, showing normal architecture of the ovary with oocytes at different stages of development (O); and rete ovaries (R). (Pas x 250)**

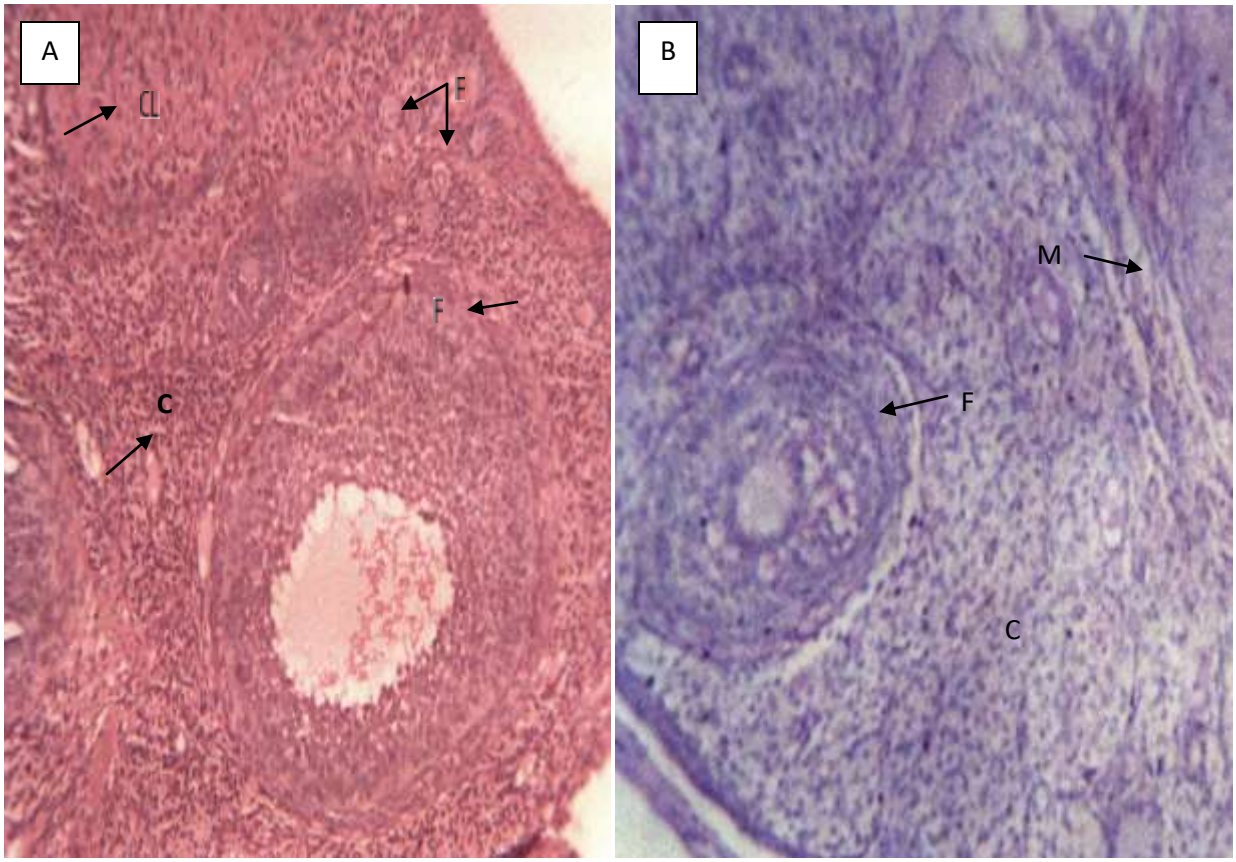


Plate 5: **Photomicrograph showing the cross section of the ovaries of (A) Group III treated with 400mg/kg body weight; and (B) Control group, showing normal architecture of the ovary with secondary oocytes and developing follicles (F) at different stages of development follicle (F); Cortex (C); Medulla (M)(H&E x 250)**

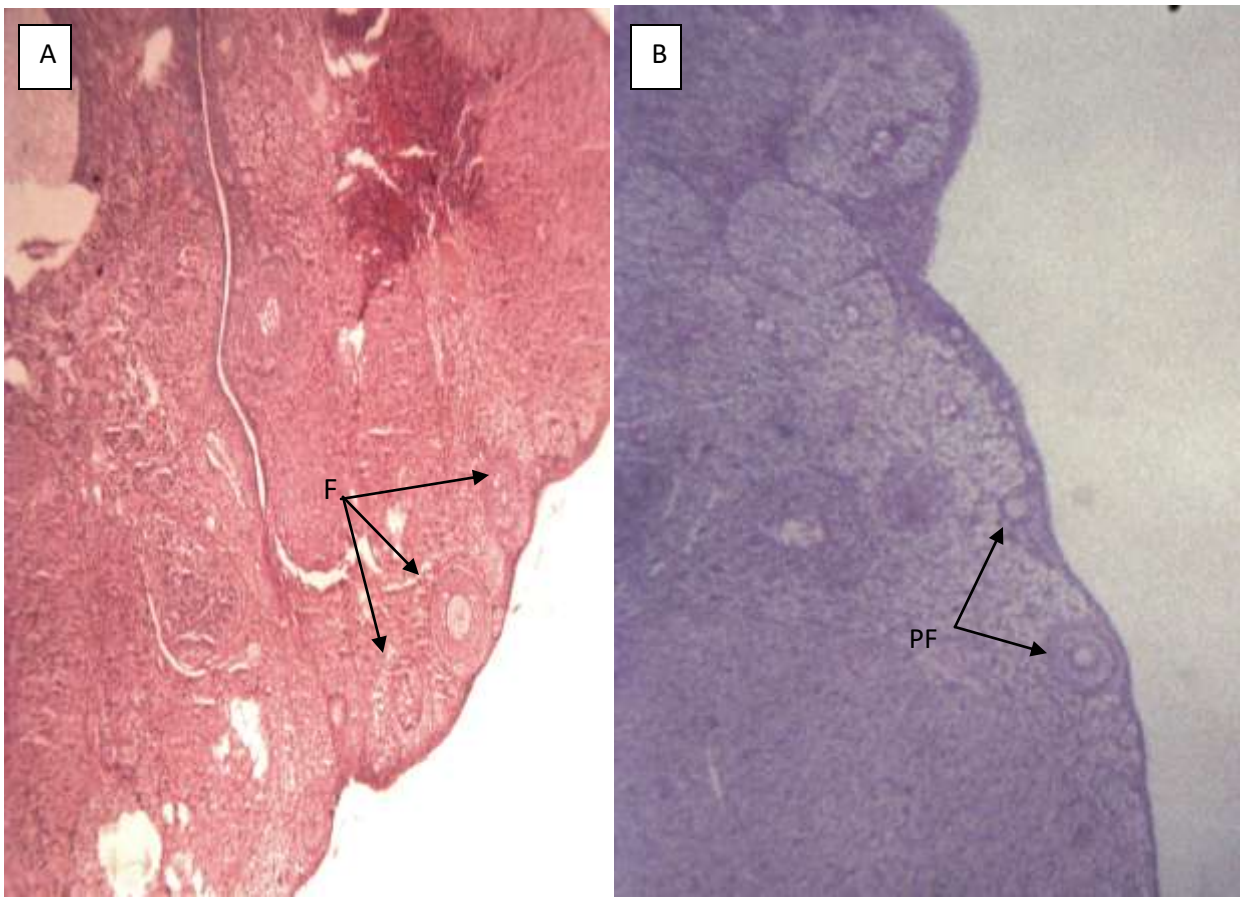


Plate 6: **Photomicrograph showing the cross section of the ovary (A) Group III, treated with 400mg/kg showing developing follicles (F), and (B) Control group, showing normal architecture developing primordialfollicles (PF) at different stages of development. (H&E x 100)**

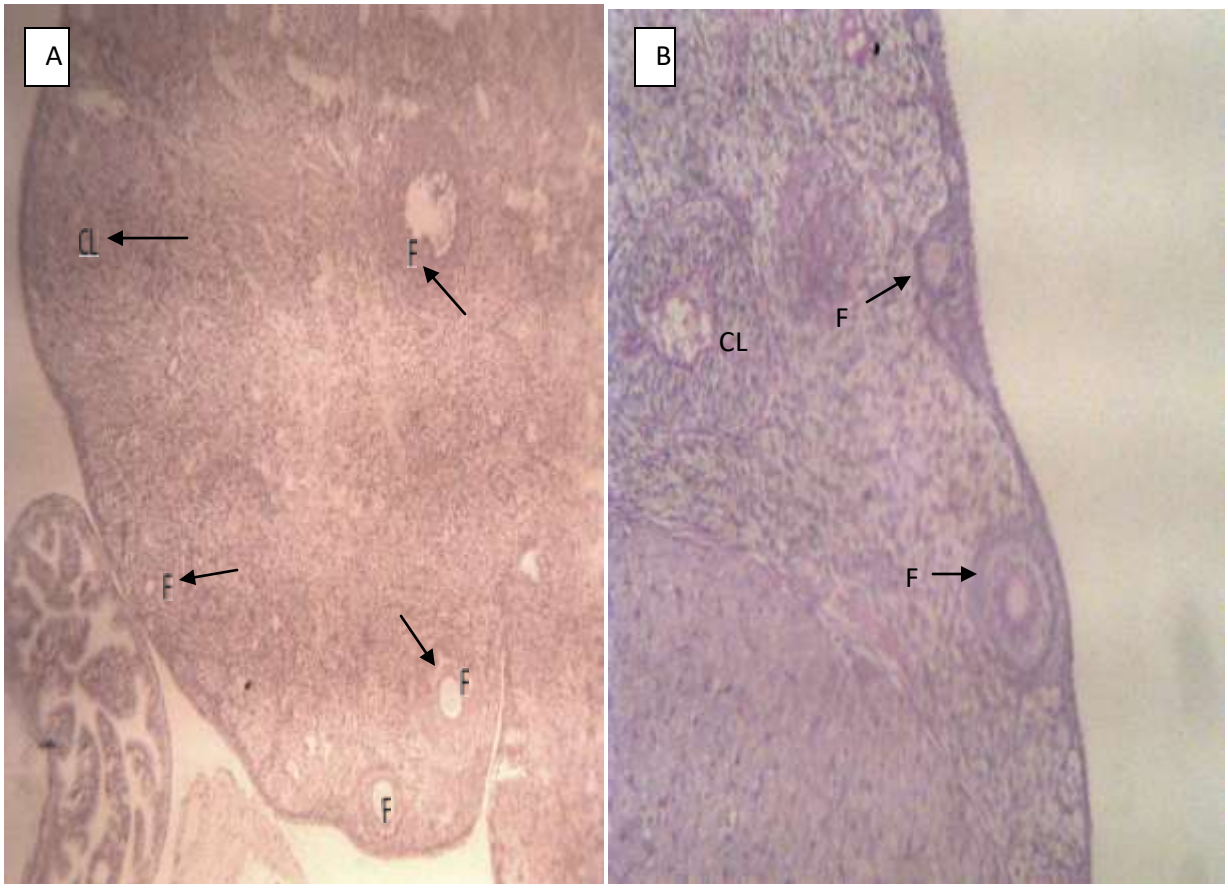


Plate 7: **Photomicrograph showing the cross section of the ovary (A). Group III treated with 400mg/kg body weight, (B). Control group, both shows a normal architecture with follicles at different stages of development, corpus luteum (CL) (PAS x 100).**

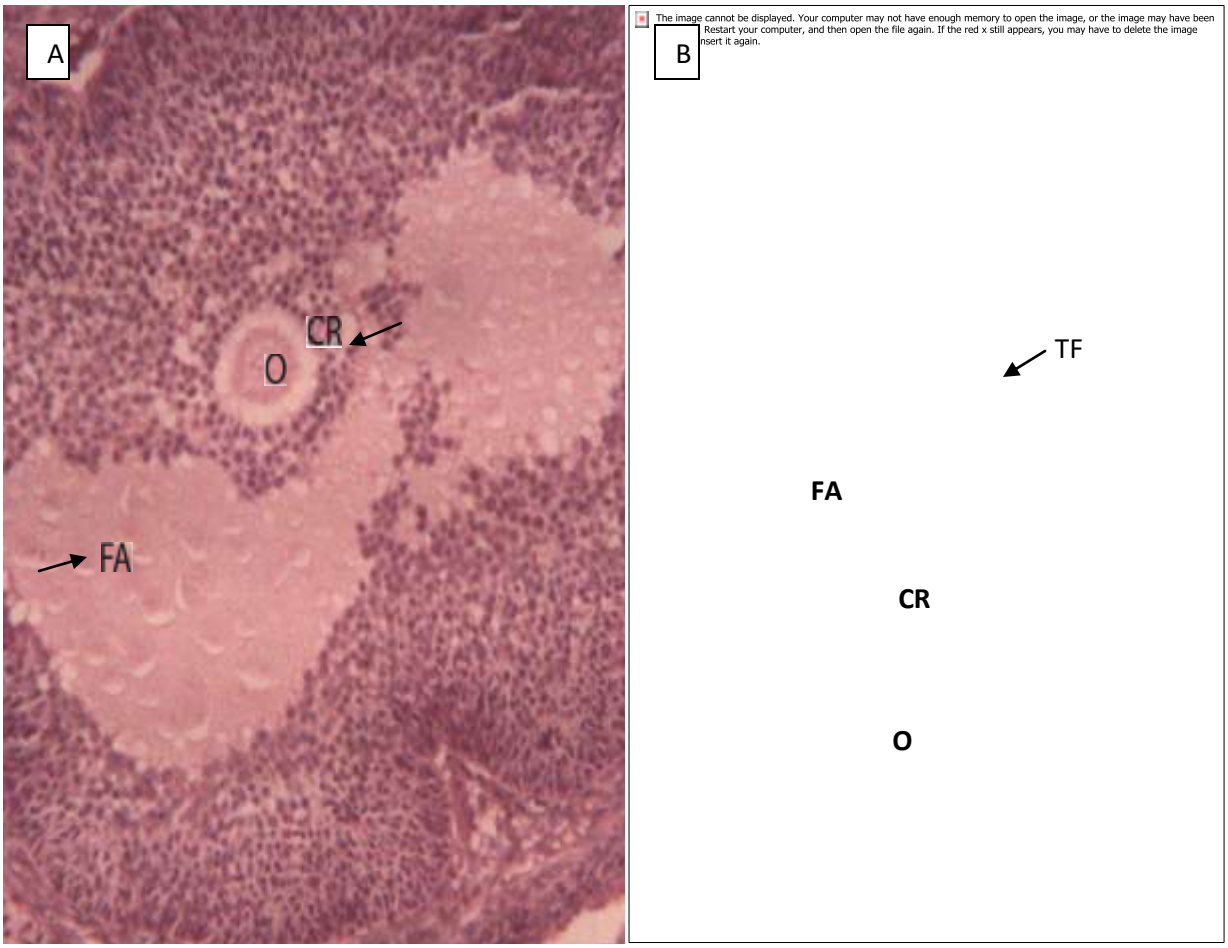


Plate 8: **Photomicrograph showing the cross section of a tertiary follicle, with large follicular antrum (FA), corona radiata (CR); and a centrally situated ovary from (A) Group IV received 600mg/kg, and (B) Control group. (H&E x 100)**

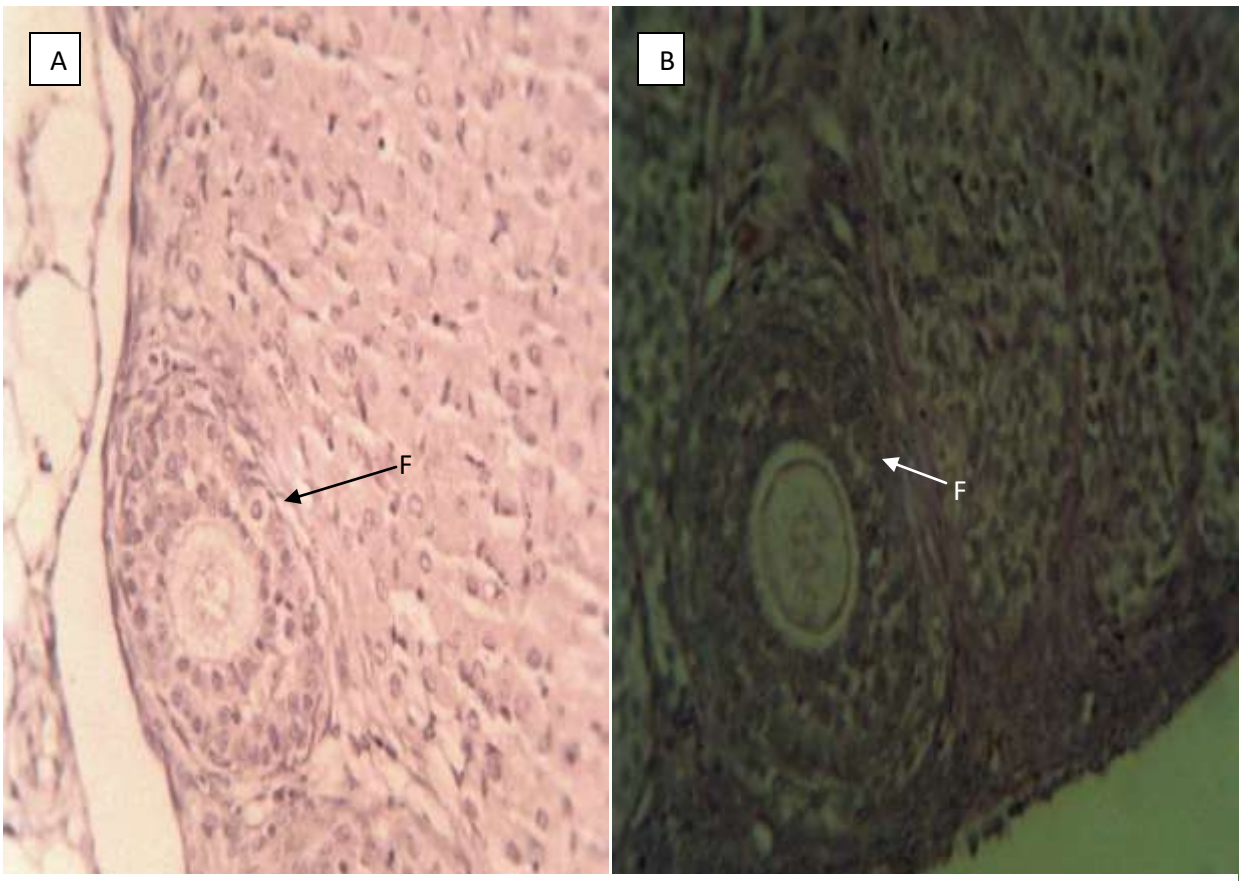


Plate 9: **Photomicrograph showing the cross section of a secondary follicle (A). Group IV, treated with 600mg/kg, and (B). Control group. (PAS x 250).**

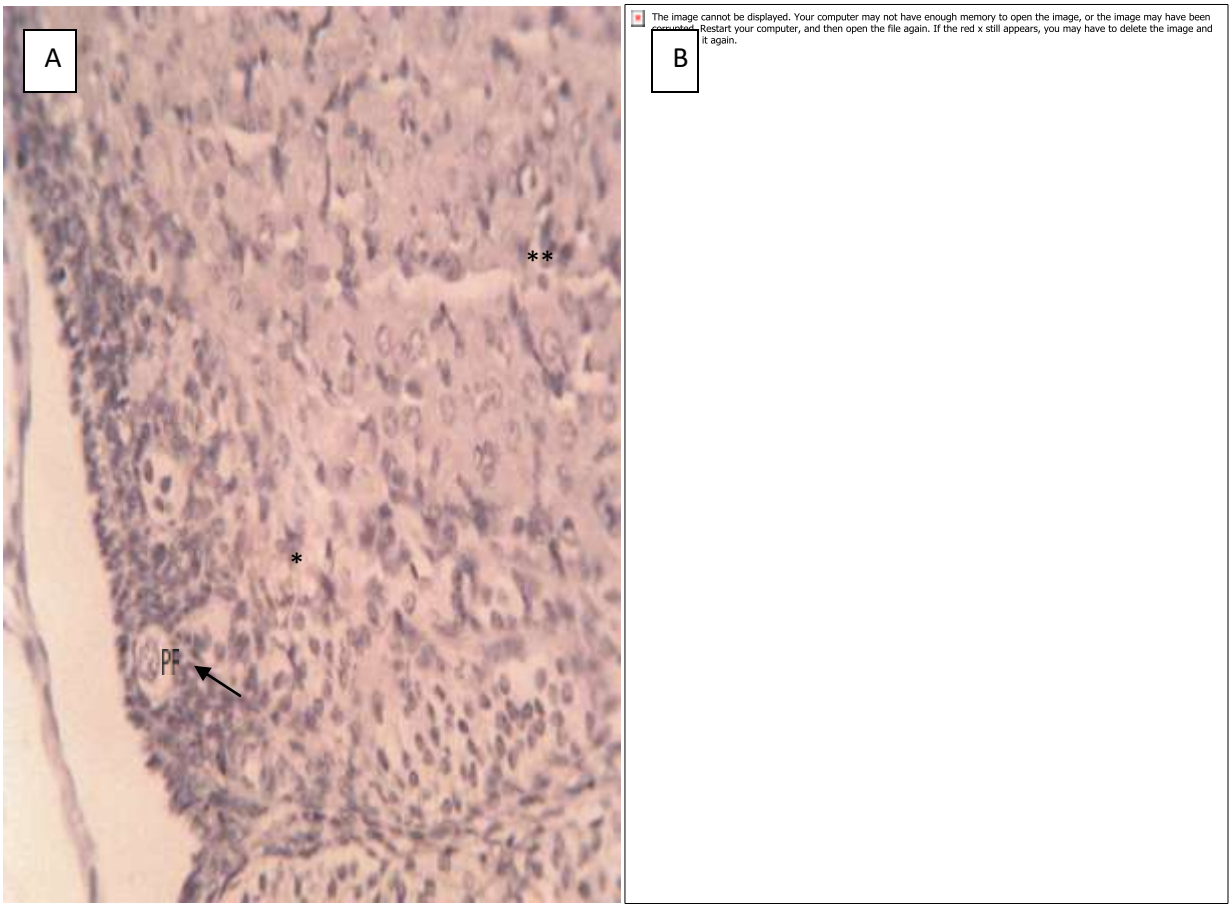


Plate 10: **Photomicrograph showing the cross section of the ovary with a primary follicle from (A) Group II that received 200mg/kg, and (B)Control group; (*) positive PAS reaction. (PAS x 250).**

CHAPTER FIVE

5.0 DISCUSSION

Changes in body weight were observed across all the experimental groups. This increase was more pronounced in the treatment groups (I, II, and III). The increase in the weight of the control group was non significant. This suggests that the extract didhave an effect on the rate of food intake and utilization, thus confirming the studies conducted by Mahgoub *et al*., 2007, on the hypoglycemic effect of the leaf extract of *A. leiocarpus* on blood glucose utilization in wistar rats, which has more effect at a dose of 400mg/kg.

The histological slides of the ovaries showed no gross or pathological changes as a result of the extract administration. There was simple cuboidal epithelium with typical features throughout its extent. The medulla was well formed and contains blood vessels and abundant amount of fibro-connective tissue. The medulla was well formed and shows normal histological structure of the wistar rat ovary. A precise border between the cortex and the medulla cannot be distinguished, characteristics of the normal structure of the mouse ovary.

The number of follicles shown by the microscopic sections of the ovaries indicated that there was an effect of the extract administration with the number of follicles seen in the cross sections. This effect was particularly seen in the group that received high dose of the extract (600mg/kg). In this group there was a significant decrease in the number of primary and tertiary follicles (2.8 ± 1.47; 87.1±3.70), as compared with the control group (96.8 ± 3.70). Also, there was a significant increase in the number of primary follicle in group II (400mg/kg), when compared with the control group, (98.7 ± 3.70), and (96.8 ± 3.70).

This finding to the best of my knowledge is the first reported on the effect of the stem bark extract of Anogeissus leiocarpus on follicular dynamics.

The essential prerequisite in the process of follicular development and ovulation is a complex sequence of hormonal events. It is important however to emphasized that estradiol and progesterone concentrations vary during different phases of the estrous cycle, however, in this study it was expected that the hormone concentration would not be significantly affected by the estrous phases as a result of the synchronization process of the animals prior to the extract administration.

Within the period of six(6) estrus cycle all the experimental animal were 96% synchronized, following the injection of 0.5 ml of $PGF_{2\alpha}$ (Lutalyse) regardless of the estrous stage of the animal, to induce luteal regression, thereby synchronizing the onset of a follicular phase. The vaginal smear taken daily showed a gradual change in phases of the estrus cycle;at the end of the second week following injection of $PGF_{2\alpha,}$ 63% of animals were in diestrus phase, by the end of the fourth week 81% of animals are in estrus phase, and by week six 96% of animals were in diestrus phase of their estrus cycle.

After the commencement of extract administration, there was a gradual change of estrus cycle pattern observed in each group. The variation in the phases of the estrus cycle in the treated rats has shown a dose dependent pattern with a prolonged diestrus in the group that received 400mg/kg, and prolonged estrus in the group that received 600mg/kg. The prolongation of the same phase of estrus cycle is considered to be irregular (Marcondes *et al.*, 2002). The presence of saponins and alkaloids in the extract of *A. leiocarpus*, as indicated from the phytochemical analysis may be responsible for the disruption of regular cyclicity in the rats. Singh *et al*.,

2007,suggested that when female rats are exposed to plant steroidal saponins, the elevation of circulating estrogen is resulted in the female rats.This prolonged the estrus and proestrus phases, as seen in the high dose group (600mg/kg) of this study.

There was an increase in the mean serum level of progesterone in group II (400mg/kg), and III (200mg/kg) following extract administration, and about the same percentage decrease in the high dose treated group (600mg/kg), and also in the control group. At the end of extract administration however, the mean serum concentration of progesterone did not show any significant change in all the groups.

Dichloromethane fraction from *A. leiocarpus* stem bark has been identified as the most active inhibitor on cyclic nucleotide phosphodiesterases (PDEs) and characterized its specificity towards purified vascular PDE1 to PDE5 isoenzymes. It was potently and preferentially inhibits (IC50=1.6 ± 0.6 µg/ml), the calmoduline-dependent phosphodiesterase PDE1(Lazare*et al.* 2008). Phosphodiesterases (PDE) are responsible for the breakdown and concomitant inactivation of the cyclic nucleotides cAMP and cGMP and are implicated in the regulation of oocyte meiotic maturation. Selective inhibitors of phosphodiesterase type 3 (PDE3) prevent meiotic resumption of mammalian oocytes.It was suggested that the use of arresters of meiosis could improve cytoplasmic maturation of immature oocytes by controlling the period of prophase I. (Nogueira*et al.*2003).The results of the studies conducted by Lazare *et al.,* 2008, suggest that a temporal block of meiosis by PDE3 inhibitor promotes developmental competence of mice oocytes retrieved from small antral follicles.

The inhibitory effect of *Anogeissus leiocarpus* extract to phosphodiesterase as reported by the studies conducted by Lazare *et al*., 2008, has probably resulted in the increase in mean serum concentration of progesterone in the experimental group 1 and II, through negative feedback effect, where the decreasing level of the steroid hormone from the ovary result in excess release of progesterone. Also the hormone level in the control in the control group of the pre administration phase is higher when compared to the post administration phase in the animals. This is in agreement with the normal reproductive physiological mechanism.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

Anogeissus leiocarpus stem bark extract have shown a dose dependent effect on the ovary in the following manner:

a) An increase in the number of primary follicle at a dose of 400mg/kg, while there was a decrease in the number of matured follicle at a dose concentration of 600mg/kg
b) There was no effect on the histological architecture of the ovaries at the end of extract administration
c) There was an increase in the serum level of both estradiol and progesterone at a dose of 200mg/kg, and 400mg/kg;

6.2 Conclussion

Anogeissus leiocarpus has a potential effect on the ovarian function indirectly via its inhibitory effect on PDEs; an effect which is established to promote developmental competence of mice oocytes retrieved from small antral follicles. This has confirmed earlier report (Lazare *et al.*, 2008).

6.3 Recommendations

a) There is need for further studies to evaluate the effect of *Anogeissus leiocarpus* on the ovaries and reproductive function, using different isozymes of PDEs which plays an important role in oocyte maturation and competency.

b) There is also need for a more elaborate study on the effect of *Anogeissus leiocarpus* on all the hormones that plays a role in the ovarian circle.

c) Further study on the hypophysis cerebri in relation to the hormonal functions of the ovary is recommended.

d) There is the need to determine the exact mechanism of interaction between *Anogeissus leiocarpus* and the ovarian function other than the inhibitory effect of its extracts.

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