

# **CHAPTER ONE**

## **INTRODUCTION**

### **1.1 BACKGROUND**

#### **1.1.1 Infertility**

Infertility primarily refers to the biological inability to achieve and carry a live pregnancy to full term after one year of non-contraceptive, regular up to 3 to 4 times per week, unprotected and active sexual intercourse (Makar and Toth, 2002; Cooper *et al.*, 2010). Infertility is a global health issue that is of a major concern for health authorities and has been reported to affect approximately 14% of couples worldwide (Reproductive Health Outlook, 2005). However, incidence varies from one region of the world to the other, being highest in Africa due to limited resources for investigation and treatment (Okonofua, 2003). In Nigeria, various studies have shown incidence rate ranging from 20% - 30% (Otubu, 1998; Bello, 2004). Primary infertility occurs when couples have never been able to conceive, while the inability to conceive following a previous pregnancy in the absence of contraception, breastfeeding or postpartum amenorrhea for a period of two years is termed secondary infertility (Kumar, 2005). The causes of infertility are categorized into four main factors: male factor when it is principally due to poor semen parameters, female factor when infertility is due to factors such as occlusion of fallopian tube, uterine, endometrial and cervical abnormalities and anovulation; combined factor which is established when both the male and female are infertile and unexplained factor when the cause of abnormality leading to infertility is present but is not detected (Ekwere *et al.*, 2007; Altmae *et al.*, 2010).

### **1.1.2 Female infertility**

It is a common assumption that infertility is primarily related to women. This is because the female reproductive system is a highly dynamic system which undergoes numerous sequential morphological changes than those of the male. Hence it is more vulnerable to anatomical and physiological factors that could induce infertility (Barbara and Paul, 1998; Obuna *et al.*, 2012). In Africa, women are often blamed for infertility while men are assumed innocent. In some culture, it is an abomination to declare a man infertile while women are often charged with the consequences of infertility (Ojiyi *et al.*, 2012). Furthermore, several studies have shown that females are responsible for more than half of the causes of infertility in Nigeria; 51.8% female, 26.8% male and 21.4% combined (Olatunji and Sule-Odu 2003; Obuna *et al.*, 2012). A study in south-eastern part of Nigeria reported that the causes of infertility are attributed to female in 32.1%, male in 26.1%, combination of male/female factors in 29.4% and unknown factors in 12.4% (Ifeanyi *et al.*, 2014).

The majority of the causes of female infertility such as; anovulation, endometriosis, polycystic ovary syndrome and galactorrhea have been associated with imbalances in reproductive hormones (Filicori *et al.*, 1994; Karimi *et al.*, 2013; Patel, *et al.*, 2014). The processes involved in procreation, starting with sexual intimacy to parturition are under hormonal influence (Frankowski, 2004). More so, ovulation, conception and maintenance of pregnancy are complicated processes that depend on the fact that the reproductive organs must be active and there must be balances in the endocrine system (Barbara and Paul, 1998).

### **1.1.3 Endocrine-induced infertility in female**

There is a rising prevalence in endocrine-induced infertility as a result of industrialization. This leads to the release of synthetic compounds and by-products of production into the air, soil, water and food (Saalu and Osinubi 2009). It has been reported that industrial waste and environmental pollutants act as endocrine disruptors (Evanthia *et al.*, 2009). Extensive detection of industrial chemicals in human serum, seminal plasma and follicular fluid has been established to disrupt hormonal homeostasis (Diamanti-Kandarakis *et al.*, 2009). Additionally, neurological diseases, some medications, alcohol and cigarettes have been shown to disrupt the endocrine system (Burger *et al.*, 2001; Mark, 2005; Jesse *et al.*, 2012). Hyperprolactinaemia is a common endocrine cause of infertility in female. It is defined as the presence of consistently high level of prolactin in the blood. An excessive prolactin secretion decreases the pulsatile release of gonadotropin releasing hormone which consequently impairs the production of follicle stimulating hormone and luteinizing hormone (Davis, 2004). Hyperprolactinaemia is essentially associated with anovulation which is a major factor in female infertility (Evanthia *et al.*, 2009).

### **1.1.4 Clinical Management of hyperprolactinaemia**

The clinical suppression of high prolactin is primarily with dopamine agonist (DA) drugs. The DA, bromocriptine mesylate is often the initial drug of choice and may require high doses to achieve quantifiable improvement. It has been reported to present side effects in patient that were undergoing treatment. Most frequent side effects are nausea, orthostatic hypotension, headaches and vomiting through the stimulation of brainstem vomiting centre. Vasospasms with serious consequences such as myocardial infarction and stroke have also been reported (Smith, 1992; Webster *et al.*, 1994; Pascal-Vigneron *et al.*, 1995; Bolko *et al.*, 2003; Vilar *et al.*, 2008). Agents

other than bromocriptine have also been used; cabergoline is more effective and causes fewer adverse effects than bromocriptine. It is often used in patients who cannot tolerate the adverse effects of bromocriptine or in those who do not respond to bromocriptine management. However, it is much more expensive and there is the need for a long term therapy. More so, hyperprolactinemia has been reported to reoccur following cabergoline withdrawal (Kharlip *et al.*, 2009; Nachtigall and Valassi, 2010). Trans-sphenoidal surgical therapy can be offered as a definitive procedure for prolactinomas. It has been reported that there is a recurrence rate of 21% within the first year following surgery. Complications such as cerebrospinal fluid rhinorrhea and transient diabetes insipidus have also been reported (Asa and Ezzat, 1998). The option of treatment with radiation is rare, its usage usually occurs when medication and surgery have not been effective. This is done by focusing high-intensity radiation at the prolactinoma to destroy the abnormal cells. However, data of its success are limited which makes the treatment less common and the risk of hypopituitarism has been reported (Molitch, 1999).

#### **1.1.5 Medicinal plants**

For many centuries before the arrival of modern medicine, widely available herbal remedies were used to prevent illness and restore natural healthy function including fertility. In developing countries, nearly 80% of the populations have used medicinal plant products in handling medical problems as they are readily available and affordable (Sofowora, 1993). The World Health Organization estimated that in many developed countries, 70% to 80% of the populations have used some form of alternative or complementary medicine. Moreover above 50% of all modern chemical drugs are of natural origin (W.H.O., 2005). There is a large volume of information from folk reports that indicate the existence of medicinal plants which are alleged to have effects on

virtually all steps in the reproductive process. There are variety of plants that have traditionally been used to treat fertility problems but just a few of them have been scientifically tested for such effects. The aqueous extract of *Justicia insularis* has been reported to significantly induce ovarian folliculogenesis and fertility in female rats (Telefo *et al.*, 2002). The reproductive effect of *Ficus asperifolia* in female rats show pro-implantation, pro-development and uterotrophic-like activities. These results give value to the popular use of *Ficus asperifolia* in handling some women's sterility problems (Baker *et al.*, 1999).

Coconut fruit is widely consumed in the world especially in tropical countries and Africa (FAOUN, 2007). It has long been believed that the water from a green or immature coconut is useful in treating many illnesses including reproductive-related problems such as combating nausea and fatigue during pregnancy, treating irregular or painful menstruation and also taken during pregnancy to give the unborn baby strength and vitality (Pragya, 2010). Its usage in the treatment of many disease conditions is justified by its unique chemical compositions. Its phytochemical analysis revealed a rich source of nutrients (Sugars, Minerals, Proteins, Vitamins, Fat and fibers) and Phytochemicals such as phytohormones, nitrogenous compounds, organic acids and enzymes (Tulecke *et al.*, 1991; Oettlé, 1993; Ma *et al.*, 2009; Wu & Hu, 2009; Yong *et al.*, 2009; Pragya, 2010). More importantly, the water from an immature coconut has been shown to have properties that can support the regulation of the endocrine system. It was confirmed to have oestrogen-like characteristics when administered to a group of menopausal rats and their oestrogen levels at the end of study were comparable to rats that were still ovulating (Nisaudah *et al.*, 2009). This present study therefore examines the effects of GCW on hyperprolactin-induced infertility following decreases in oestrogen, follicle stimulating and luteinizing hormones. The

effect on estrous cyclicity, follicular growth, ovulation, granulosa cell proliferation, oestrogen activities in the ovarian and pituitary tissues and ovarian oxidative stress were also experimented.

## **1.2 STATEMENT OF THE PROBLEM**

Among women in all the continents of the world, African women bear the largest burden of infertility as motherhood is considered a mandatory status (Hollos and Ulla 2008). Available evidence suggests that the social consequences of infertility are particularly profound for women as they suffer more of physical and mental abuse, abandonment, emotional imbalance and complication of marital dynamics which may sometimes lead to marital instability, divorce, polygamy or remarriage (Onah, 1992). There is a growing prevalence in endocrine-induced infertility where hyperprolactinaemia is a frequent occurrence (Evanthia *et al.*, 2009). Consistently high prolactin level is a common endocrine cause of infertility in women. This female reproductive dysfunction affects about one-third of infertile women worldwide (Corenblum *et al.*, 1993). In northern Nigeria, 33.4% of infertile women were reported to have high prolactin levels (Emokpae *et al.*, 2011). Hyperprolactinaemia has also been reported to be incident in other causes of female infertility; 80% incidence in galactorrhoea, anovulation 13.3%, polycystic ovarian women 20 – 30% and luteal phase deficiency 21.9% (Akande *et al.*, 2009; Geetu and Samar, 2013). The primary clinical treatments of hyperprolactinaemia is with dopamine agonist drugs such as; bromocriptine and cabergoline. The DA have been reported to present side effects in 20-78% of patients (Iffy *et al.*, 1996; Vilar *et al.*, 2008). Moreover, dopamine agonist drugs are relatively expensive and a long term therapy is needed to achieve result. There have been reports of the recurrence of hyperprolactinaemia in 63% of patients after 18 months of drug discontinuation (Kharlip *et al.*, 2009; Nachtigall and Valassi, 2010). For these reasons, GCW was experimented on hyperprolactin-induced infertility in rats to observe if it

would be effective in decreasing serum prolactin level and alterations caused by hyperprolactinaemia being a naturally occurring, cheaper and readily available substance with identified micronutrients.

### **1.3 RATIONALE FOR THE STUDY**

Studies have revealed that hyperprolactinaemia disrupts estrous cycling, decreases follicular and endometrial growth, impairs reproductive hormonal balance and conception (Rossi *et al* 2002; Panzan *et al.*, 2006; Verna *et al.*, 2006; Gomes *et al.* 2009; Rossi *et al.*, 2010). The major hormonal implication seen during hyperprolactinaemia is low production of oestrogen. It has been reported that excess prolactin inhibits follicular estradiol production in the ovary. Prolactin has been shown to have a direct inhibitory action on oestrogen biosynthesis of granulosa cells in the ovary (Uilenbroek and Linden 1998; Colao *et al.*, 2006). The water from an immature coconut fruit has been reported to have oestrogen-like properties which can further be explored especially as it relates to hormone regulation and its consequent actions on reproductive organs and reproductive processes (Nisaudah *et al.*, 2009). Additionally, high plasma concentration of prolactin has been associated with the induction of oxidative stress on reproductive organs (Ashok *et al.*, 2005). Oxidative stress has been identified to play a key role in the pathogenesis of sub-fertility. Reactive oxygen species have been implicated in recurrent pregnancy loss and inhibition of reproductive hormone signalling, oocyte maturation, ovarian steroidogenesis and ovulation (Iborra *et al.*, 2005). The reports of GCW oestrogenic characteristic in addition to its antioxidant property make it a substance of choice in this regard (Alleyne, *et al.*, 2005a).

#### **1.4 AIM OF THE STUDY**

The aim of this study is to assess the effect of oral administration of GCW on estrous acyclic pattern, anovulation, insensitivity of oestrogen receptors in the ovary and pituitary gland, disrupted reproductive hormonal profile and oxidative stress in metoclopramide-induced hyperprolactinaemia in female Sprague-Dawley rats.

#### **1.5 SPECIFIC OBJECTIVES**

The specific objectives are to:

1. Determine the effects of the administration of GCW on metoclopramide-induced estrous acyclic pattern in female Sprague-Dawley (S-D) rats
2. Investigate the histomorphological effects of GCW on the ovaries of metoclopramide-treated female S-D rats
3. Investigate the Immunohistochemical effects of GCW on oestrogen receptor activities in the ovaries and pituitary gland of metoclopramide-treated female S-D rats
4. Evaluate the effects of GCW on reproductive hormonal milieu and oxidative stress markers in metoclopramide-treated female S-D rats

#### **1.6 SIGNIFICANCE OF STUDY**

Treatment modality has been shifting towards natural products as they are readily available and affordable (Gupta *et al.*, 2005). Natural substances have also been reported to cause little or no side effects and act via multiple sites in reversing illnesses which make them more desirable than the conventional treatment option (Yildiz and Fatih, 2005). This study further investigated the possible site of action of GCW which is important in establishing the potential characteristics of GCW as it relates to hyperprolactinaemia. The outcome from this study will add to the body of



knowledge as it has not been documented and will also provide a safe alternative in the management of hyperprolactinaemia

## 1.7 DEFINITION OF TERMS

**Antioxidant:** These are substances that scavenge free radicals in the body to prevent damage produced by oxidative stress in tissues (Halliwell, 1995).

**Dopamine:** An organic chemical substance that inhibits the secretion of prolactin from the anterior pituitary gland (Ben-Jonathan and Hnasko, 2001).

**Free radicals:** An atom or group of atoms that have an unpaired electron that takes part in chemical reactions and is therefore able to produce derangement in cellular biochemical processes (Pallavi *et al.*, 2012).

**Green coconut water:** Liquid found within the cavity of an immature coconut fruit.

**Hyperprolactinaemia:** A consistently elevated serum level of prolactin in a non-lactating and non-pregnant subject (Victor and Vieweg, 2003).

**Immunohistochemistry:** A process of detecting antigens in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens (Ramos-Vara and Miller, 2014).

**Infertility:** It refers to the inability to achieve and carry a live pregnancy to full term (Makar and Toth, 2002; Cooper *et al.*, 2010).

**Lethal dose:** It is a standard measure of the toxicity of a material that will kill half of the sample population of a specific test animal in a specified period (Krishna, 2014).

**Metoclopramide:** Is a chemical substance that is used to induce lactation (Gabay, 2001).

**Oestrogen:** Reproductive hormone that stimulates the growth of reproductive tissue.

**Oestrogen receptor:** Is an intracellular protein that is activated by oestrogen (Dahlman-Wright *et al.*, 2006).

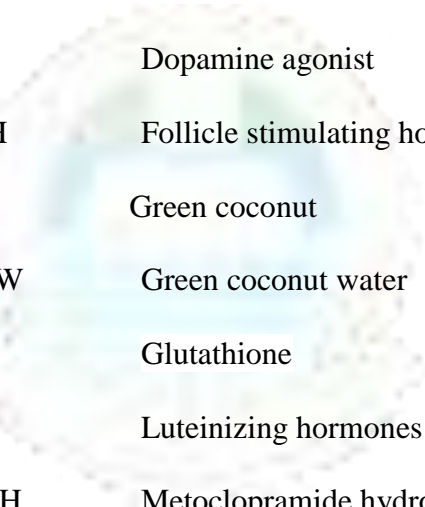
**Oxidative stress:** A disturbance caused by imbalance between the production of reactive oxygen species (pro-oxidants) and antioxidants in favour of the pro-oxidants in the body (Bayani, 2009).

**Prolactin:** A peptide hormone responsible for lactation (Bole-Feysot *et al.*, 1998).

**Stereology:** The study of estimating geometrical quantities of three-dimensional properties of microscopic objects seen in two dimensions (Kubinova *et al.*, 2004).

**Volume density:** The mass per unit volume of a substance (Hough *et al.*, 1991).

## 1.8 LIST OF ABBREVIATIONS



CAT	Catalase
DA	Dopamine agonist
FSH	Follicle stimulating hormone
GC	Green coconut
GCW	Green coconut water
GH	Glutathione
LH	Luteinizing hormones
MCH	Metoclopramide hydrochloride
MDA	Malondialdehyde
OR-Ag <sub>b</sub> <sub>c</sub>	Oestrogen antigen-antibody complex
PIF	Prolactin inhibitory factor
PRF	Prolactin releasing factor
PRL	Prolactin
SD	Sprague-Dawleys
SOD	Superoxide dismutase
VD	Volume density

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 PROLACTIN

##### 2.1.1 Brief Description of Prolactin

Prolactin (PRL) is a polypeptide hormone that stimulates milk production. PRL was originally discovered by Stricker and Grueter in 1928 as a hormone with 'lacto- genic' activity in non-human animals such as; birds, reptiles, amphibians and fishes (Riddle *et al.*, 1932). In 1970, a distinct human PRL was identified as a separate entity even though it has a remarkable homology with molecules of growth hormones and placental lactogen (Friesen, 1995). The secretion of prolactin is pulsatile as it increases during sleep, stress, food ingestion, chest wall stimulation, sexual intercourse and trauma. However, its sustainably high level in a non-lactating and non-pregnant individual calls for clinical attention (Melmed and Jameson, 2008). The normal serum level of PRL in human varies with sex. The normal PRL levels ranges between 10 – 20 and 10 – 25 ng/ml in men and women respectively (Citrome, 2008); although, the level may rise substantially after child birth to 150-200 ng/ml for the initiation and maintenance of lactation (Freeman *et al.*, 2000).

##### 2.1.2. Chemical Structure of Prolactin

In human, mature prolactin has a molecular mass of 22KDa and it is composed of 199 amino acids polypeptide residues arranged in a single chain with three intramolecular disulfide bonds between six cysteine residues (Cys4-Cys11, Cys58-Cys174, Cys191-Cys199). It has a half-life of approximately 50 minutes in circulation and metabolizes in the liver and kidney (Freeman

*et al.*, 200). Prolactin is synthesized as a pro-hormone following cleavage of a signal peptide bond, indeed chemically; prolactin appears in a multiplicity of post-translational forms ranging from size variants of isoforms to chemical modification. The size variants of prolactin include the monomeric 22 kDa and bigger size variants of 50 kDa and 150 kDa forms. Although, 85% of circulating prolactin is monomeric, however, big variants of 50 kDa and prolactin-IgG complexes of 150 kDa also circulate but in lower percentage. The bigger size variants are degraded into monomeric form by reducing its disulfide bonds (Gibney *et al.*, 2005). Furthermore, chemical modifications such as dimerization, polymerization, phosphorylation or glycosylation may also occur (Sabharwal *et al.*, 1992).

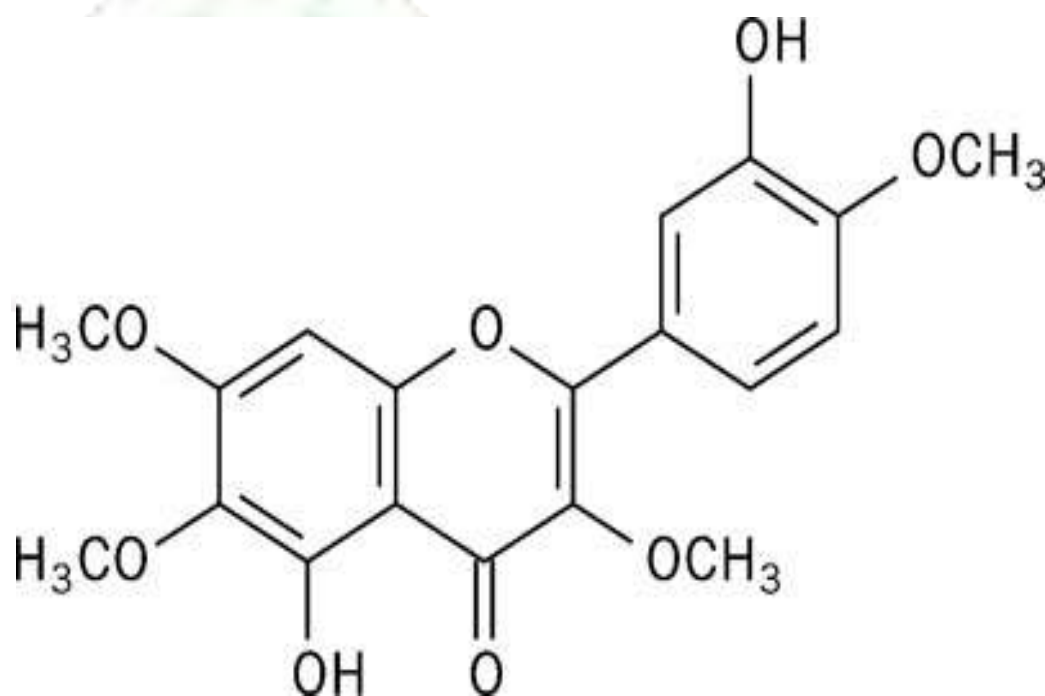


Figure1: Chemical structure of prolactin (Pixshark.com, accessed May, 2015).

### **2.1.3 The Roles of Prolactin**

The conventional role of PRL is the initiation and maintenance of lactation. During pregnancy, high circulating concentrations of oestrogen and progesterone inhibit the action of prolactin on milk production. Following delivery, the resultant decrease in oestrogen and progesterone levels stimulate the increase in prolactin secretion which leads to the induction of lobulo-alveolar growth of the mammary gland. Suckling act by the infant stimulates mechanoreceptors in the nipple, thereby stimulating milk let-down mechanism and milk production in the breast (American Academy of Pediatrics, 2005). PRL is one of the most versatile hormones of the pituitary gland in terms of biological actions. Prolactin appears to be important in several non-lactation aspects of reproduction. In some mammalian species such as rodents, birds and dogs, prolactin was shown to have stimulatory effects on reproductive or maternal behaviours such as; nest building and retrieval of scattered young ones. PRL also provides the body with sexual gratification after intercourse hence it is use as an indicator for the estimation of sexual satisfaction. Other roles include contribution to surfactant synthesis of fetal lungs at the end of pregnancy and immune tolerance of the fetus by the maternal organism during pregnancy (Nelson, 2005). Many studies have documented significant roles for prolactin activity beyond the reproductive system. It effects on water and salt balance, development and metabolism have been reported. It has also been shown to have profound influence on hematopoiesis and the regulation of blood clotting. It stimulates the proliferation of oligodendrocyte precursor cells which differentiate into oligodendrocytes. The mechanism by which a single hormone can modulate so many unrelated functions is traced to its structural polymorphism, multiple sites of synthesis and processing, divergent intracellular signaling pathways, target genes and ubiquitous receptor

distribution throughout the body (Benker *et al.*, 1990 ; Bole-Feysot *et al.*, 1998; Halbreich *et al.*, 2003; Marieb and Hoehn, 2006; Gregg *et al.*, 2007).

## **2.2 PROLACTIN SECRETION**

PRL is primarily produced by the lactotrophic cells in the anterior lobe of the pituitary gland. The extra-pituitary production was first detected in decidualized endometrial cells and subsequently in the immune cells, lacrimal gland, thymus, spleen, skin fibroblasts, sweat glands and mammary gland itself (Freeman *et. al.*, 2000). PRL can be found primarily in the serum. It can also be found in several fluid compartments in the body which include; cerebrospinal fluid, amniotic fluid, tears, breast milk, follicular fluid and sweat. The secretion of PRL is under the control of the activities of the prolactin inhibiting factors and prolactin releasing factors (Bartholomew *et al.*, 2007).

### **2.2.1 Inhibition of Prolactin Secretion**

Prolactin secretion is under tonic inhibitory control by prolactin-inhibiting factor (PIF) which is primarily dopamine agonist (DA). DA is a catecholamine neurotransmitter that is primarily secreted by specialized tuberoinfundibular neurons in the hypothalamus and released into the pituitary hypophyseal portal system where it inhibits the synthesis of prolactin by the lactotrophs (Velasco and Luchsinger, 1998). DA biosynthesis begins with amino acid tyrosine. The majority of circulating tyrosine originates from dietary sources such as egg, cheese, fish, avocado and almond. Tyrosine enters the tuberoinfundibular neurons by sodium and energy dependent uptake mechanism. It is converted into dopamine following the action of two enzymes; tyrosine hydroxylase and l-aromatic amino acid decarboxylase which act in sequential pattern. The product formed is translocated into secretory vesicles for storage. The fusion of DA secretory

vesicles with the plasma membrane of the lactotrophic cells results in the release of dopamine into the synaptic cleft or extracellular space which in turns suppresses the high intrinsic secretory activities of the lactotrophs. (Nagatsu and Stjarne, 1998). Additionally, gamma-aminobutyric acid, angiotensin II, neuropeptide and atrial natriuretic peptide have been reported to stimulate tyrosine-hydroxylase (TH) activity to increase DA secretion. Apomorphine and various ergot alkaloidal drugs such as bromocriptine and cabergoline have also been shown to mimic DA agonist action (Silveira and Franci, 1999; Ben-Jonathan and Hnasko, 2001). PRL may also regulate its own release by acting on the hypothalamic dopaminergic system in a short loop feedback mechanism (Gillam *et al.*, 2004; Selvarajah *et al.*, 2005; Atmaca *et al.*, 2006). It is well established that prolactin inhibits its own secretion in an autocrine-paracrine manner by activating neuroendocrine dopaminergic neurons in the hypothalamus (Horwitz *et al.*, 1994).

### **2.2.2 Stimulation of Prolactin Secretion**

Factors that interfere with the release of dopamine from the hypothalamus or stimulate intracellular activity of the lactotrophic cells to routinely raise serum prolactin levels are prolactin-releasing factors (PRF). (Lin *et al.*, 2004). Thyrotropin-releasing hormone, epidermal growth factor, vasoactive intestinal peptide and galanin act as PRF. Epidermal growth factor stimulates prolactin synthesis and secretion in pituitary cells by increasing the intracellular concentration of free calcium ion (Aanestad *et al.*, 1993; Marie *et al.*, 1993). Calcium plays a pivotal role in stimulus– secretion coupling in endocrine cells. Indeed, it has been demonstrated that an increase in the intracellular calcium concentration considerably stimulates secretory-granule fusion and thus hormone secretion ( Zorec *et al.*, 1991). Neurotoxins such as; lead, glutamate, nitric oxide, botulinum and tetrodotoxin are known to pose poisonous or destructive on

nerve tissues; hence disrupt its activities. Exposure to monosodium glutamate resulted in 40% reduction in the number of tuberoinfundibular neurons which are responsible for the secretion of dopamine (Bodnar *et al.*, 2001). Some psychostimulants (amphetamine, methylphenidate, modafinil), pathologic (Risperidone, Amisulpride, serotonin and tricyclics) and contraceptive drugs have also been reported to obstruct the action of prolactin inhibiting factor (PIF), hence its usage stimulates prolactin secretion (Kulick *et al.*, 2005). In addition, PRL release has been reported to be stimulated by some physiological stimulations such as: food intake, sexual intimacy, stress and breast mechanical stimulation. More so, sleep onset, alcohol intake and minor surgical procedures have also been shown to cause increase in prolactin secretion (Freeman *et al.*, 2000; Melmed *et al.*, 2005).

## **2.3 OVERVIEW OF HYPERPROLACTINAEMIA**

Hyperprolactinaemia is a consistently high serum level of PRL in a non-pregnant and non-lactating individual. It is a common disorder that is identified in endocrine- induced infertility in female (Blackwell, 1992). The causes of hyperprolactinaemia have been associated to several metabolic, pathological and pharmacological conditions.

### **2.3.1 Pituitary Disorders**

The pituitary gland is often referred to as the 'master gland' because it controls the functions of other endocrine glands. The lactotrophic cells known to be responsible for the secretion of prolactin are located in the anterior lobe of the pituitary gland. Pituitary adenomas are non-cancerous tumors of the pituitary gland. It is a common disorder of the pituitary gland. Lactotrophic adenomas (prolactinomas) are prolactin-secreting pituitary tumors and are the most common pituitary adenomas. It accounts for approximately 45% of all pituitary tumors (Shlomo *et al.*, 2011). The



pituitary gland produces excess prolactin in prolactinomas due to the increase in lactotrophic cell proliferation. This leads to an increase in the number of cells-secreting prolactin and consequently hyperprolactinaemia (Biller, 1999; Alikasifoglu *et al.*, 2001). The size of adenomas varies from microprolactinomas (less than 10 mm) which are the most common with approximately 90% of prolactinomas, macroprolactinomas (more than 10 mm) to giant prolactinomas, which are more than 4 cm (Gillam *et al.*, 2006). The symptoms of lactotrophic adenomas fall into two categories; the symptoms that result from the elevated serum prolactin which include hypogonadism, amenorrhea and galactorrhea. Secondly, the pressure of the mass effect from compression of surrounding tissues. Pressure on the optic nerve may impair vision and on the pituitary gland itself may affect its function (Webster *et al.*, 1994; Sanfilippo, 1999).

Pituitary stalk is the connection between the hypothalamus and the pituitary gland through which DA are transported. Additionally, pituitary stalk lesion may obstruct the delivery of dopamine from the hypothalamus to the prolactin secreting cells of the pituitary gland. Therefore, the obstruction of the pituitary stalk may also result in increased PRL secretion due to the interruption of dopamine flow from the hypothalamus through the hypophyseal-portal circulation to the lactotrophs (Burger *et al.*, 2001).

### **2.3.2 Hypothalamic Disorders**

The effects of the hypothalamus on pituitary gland are mediated in the gonads through the hypothalamic–pituitary–gonadal axis. Damage to the hypothalamus will impact the responsiveness of the pituitary to secrete its hormones. Hypothalamic disease may cause insufficient or inhibited signaling to the pituitary gland leading to deficiencies of one or more of its hormones. Dopamine is secreted by tuberoinfundibular neurons in the hypothalamus and

released into the pituitary hypophyseal portal system where it inhibits the synthesis of prolactin by the lactotrophs (Velasco and Luchsinger, 1998). Any disorder that affects the activity of the tuberoinfundibular neurons in the hypothalamus may lead to hyperprolactinaemia. This is due to the impairment of the activity of dopamine to suppress the release of prolactin (Sylvia, 2004). Disorder in the hypothalamus may be caused by tumor, malnutrition, genetic disorders, radiation, surgery and physical injury.

### **2.3.3 Deficiencies in the Dopaminergic system**

Dopamine is the key regulator of PRL and any disruption in the dopaminergic system will result in increased PRL secretion. The depletion in the central dopamine and catecholamine stores has been attributed to stress, poor nutrition and certain drugs. The effect of Stress was investigated on dopamine nerve activity in female rats. The researchers found that acute restraint stress decreases intracellular activities of dopamine-secreting neurons and consequently decreasing the synthesis of dopamine (Lucas *et al.*, 2004). Dopamine depletion has also been attributed to poor nutrition. It has been shown that certain food possess neurotoxic effect on the dopaminergic neurons. Moreover, alcohol and caffeine have been shown to reduce the central dopamine level in the brain while the consumption of fruits and vegetables provide antioxidants which help protect dopaminergic neurons from oxidative damage (Sathyanarayana *et al.*, 2008). Additionally, certain drugs; such as amphetamine, alpha-methyldopa, phenothiazines, butyrophenones and reserpine have been reported to deplete dopamine level (Fidelis *et al.*, 2008).

The dopamine-release mechanism is an important factor in determining the quantity of dopamine that is released. Therefore its obstruction should not be compromised in the brain. There is evidence of defects in process of dopamine exocytosis. In electrophysiological study, electrically

evoked dopamine release showed widespread dysfunction in the mechanisms of releasing dopamine in an obese prone rat. The defect thus leads to decrease in DA level (Brenda *et al.*, 2008). The action of dopamine is mediated at its receptors. More so, the sensitivity of dopamine receptors may be diminished or lost, which explains the lack of response on lactotrophic cell to increased endogenous dopamine stimulation (Luciano, 1999; Carmichael and Braunstein, 2009). Furthermore, the decrease in dopamine receptors has been shown to reduce the impact of DA in suppressing PRL secretion due to decrease action sites (Gene-Jack *et al.*, 2001)

#### **2.3.4 Neurological diseases**

Parkinson's disease has been reported to cause increase in the release of PRL. Parkinson's disease is a degenerative condition that causes tremor and motor impairment. A decrease in dopamine (DA) release has been implicated in Parkinson's disease due to depletion of dopamine-producing cells in the substantia nigra (Raúl *et al.*, 2004). In addition, hypothyroidism presents typical elevation of thyroid-stimulating hormone (TSH) by negative feedback on hypothalamus. Elevated TSH stimulates prolactin release from the pituitary, either by a direct effect on prolactin-secreting cells or by reducing dopamine (Molitch, 1999). Furthermore, chronic renal failure or impairment may lead to excessive accumulation of prolactin in the blood. This is probably secondary to decrease in the clearance rate by the kidney as about 80% of patients on haemodialysis have been reported with consistent high prolactin levels (Serri *et al.*, 2003).

#### **2.3.5 Medications**

Several drugs have been shown to cause significant increase in serum prolactin concentrations. Antidepressant drugs (selective serotonin reuptake inhibitors, monoamine oxidase inhibitors and

tricyclics), anti-hypertensive drugs (methyldopa and verapamil), gastroprokinetic drugs (metoclopramide) and anti-psychotic medications (risperidone and haloperidol) can cause elevated PRL level. Functional studies in humans have revealed that drugs stimulate PRL release by disrupting DA synthesis, blockage of DA receptors, reduction in central dopamine store and stimulation of the lactotrophic cells (Mark, 2005).

## **2.4 HYPERPROLACTIN-INDUCED INFERTILITY IN FEMALE**

Most symptoms of hyperprolactinaemia in the reproductive system are due to both direct action of prolactin on target tissues and indirect effects mediated by the decrease in gonadotropin pulsatile secretion which leads to gonadal dysfunction. High serum prolactin level has been associated with the inhibition of the hypothalamic gonadotropin releasing hormone (GnRH), suppression of preovulatory gonadotropin surge and consequent inhibition of gonadal function (Yazigi *et al.*, 1997). Amenorrhea, anovulation, reduced libido and orgasmic dysfunction are major clinical manifestation in hyperprolactin women (Ginsburg, 1992; Sanfilippo, 1999).

### **2.4.1 Reproductive Hormone Disorders**

Hypothalamic control of prolactin secretion on anterior pituitary hormones is primarily inhibitory, whereas the hypothalamic control on the secretion of other anterior pituitary hormones is stimulatory. Hence the secretion of prolactin increases whereas that of the other anterior pituitary hormones decreases and vice versa. PRL interferes with the release of gonadotropin-releasing hormone. This leads to decrease in the release of gonadotropins which are basically follicle stimulating hormones and luteinizing hormone (Matsuzaki *et al.*, 1994). FSH is known to stimulate follicular maturation. The decrease in FSH level decreases follicular development hence the prevention of the production of mature egg (Nawroth, 2005). The decrease in FSH and

consequent inhibition in follicular development have been implications in low oestrogen production which is attributed to decrease in the number of granulosa cells in the ovarian follicle (Ashkenazi, 2005). Estrogen has been shown to amplify the amount of luteinizing hormone (LH) release which is in response to increasing gonadotropin-releasing hormone. The disrupted oestrogen release consequently prevent LH-surge which is important for the initiation of ovulation and the conversion of residual follicle into corpus luteum that in turn produces progesterone to prepare the endometrium for possible implantation (Mahesh, 2011). Receptors are sites where the actions of the hormones are mediated. It has been reported that the blockage of gonadotropins at receptor sites can cause FSH and LH insensitivity. The insensitivity of gonadotropins at its receptor sites lead to poor initiation and hence gonadotropins become inactivated to stimulate follicular growth and initiate ovulation (Craven *et al.*, 2006).

#### **2.4.2 Ovarian Dysfunction**

Follicle-stimulating hormone (FSH) is a hormone that stimulates the growth of primary ovarian follicles in the ovary. It is synthesized by the gonadotrophic cells of the anterior pituitary gland. The decrease in the secretion of FSH demonstrated during hyperprolactinaemia inhibits follicular development which prevents the production of mature eggs (Nawroth, 2005). Hyperprolactinaemia affects ovarian steroidogenesis as high serum prolactin has been shown to reduce granulosa cell proliferation which leads to a decrease in follicular estradiol production (Colao *et al.*, 2006). The action of Luteinizing hormone (LH) in the initiation of ovulation and development of the corpus luteum is under the control of oestrogen. Therefore, low oestrogen secretion causes loss of feed-back mechanism on LH. The loss of positive oestrogen feedback on LH secretion inhibits normal LH pulsatility (Craven *et al.*, 2006). Hence, the disruption in luteinizing activities leads to luteal-phase defect and the prevention of preovulatory LH- surge.

This prevent the release of egg from the ovarian follicle and prevent the conversion of the residual follicle into a corpus luteum (Wuttke *et al.*, 2001). Anovulation, amenorrhea and luteal phase deficiency-induced infertility have been indicated in ovarian dysfunction, which is a major implication in hyperprolactinaemia (Emokpae *et al.*, 2011).

### **2.4.3 Estrous Acyclicity**

The maintenance of estrous cyclicity is under the control of reproductive hormones. Therefore, disruptions in hormonal profiles have been attributed to estrous acyclicity. During experimental induction of hyperprolactinaemia in rodent, prolonged transmission from estrus to proestrus followed by long permanent diestrus was observed. The vaginal smear cytology remained leukocytic, independent of the day of estrous cycle. Available data indicated that elevated PRL concentrations have profound effects on reproductive cyclicity by disrupting ovarian steroid secretion and preovulatory neurochemical events (Borovaya and Volkova, 1994).

### **2.4.4 Oxidative stress**

Reproductive cells and tissues remain stable when free radical production and the scavenging antioxidant remain balanced. The role of reactive oxygen species (ROS) in various diseases of the female reproductive system has been established to affect a variety of physiological functions in the reproductive tract. It has been reported that free oxygen radicals may react with cellular macromolecules to produce destructive effects. There are cumulative evidence which suggest that oxidative stress is involved in conditions such as abortions, preeclampsia, hydatidiform mole, fetal embryopathies, preterm labour and intrauterine growth retardation (Van, 2009). Prolactin is a major stress-induced hormone and its secretion follows psychological, environmental or physical stress. High prolactin evokes oxidative stress in the arcuate nucleus of the hypothalamus limiting the synthesis of dopamine agonist and consequently hyperprolactinaemia (Franci *et al.*, 1992;

Mohan *et al.*, 2011). A positive correlation among serum prolactin and nitrite suggested that hyperprolactinaemia could contribute to infertility by inducing oxidative damage which is implicated by high lipid peroxidation. Oxidative stress markers such as; superoxide dismutase (SOD), glutathione peroxidase (GSH) and catalase (CAT) have been identified to be low while malondialdehyde (MDA) concentration was high within the ovary, endometrium, fallopian tubes, embryo, placenta and the peritoneal fluid of oxidative stressed women inflicting significant damage to reproductive structures (Veena *et al.*, 2008). In addition, decreased semen parameter mediated by the alteration of the hormone feedback mechanism has been reported in male (Oremosu and Akang 2014).

## **2.5 MECHANISM OF METOCLOPRAMIDE INDUCED-HYPERPROLACTINAEMIA**

Metoclopramide hydrochloride (MCH) is a white crystalline, odourless substance that is soluble in water. It is an antiemetic and gastroprokinetic agent commonly used to prevent vomiting and also used to enhance gastrointestinal motility by increasing the frequency of gastric contraction to facilitate gastric emptying (Patterson *et al.*, 1999). The data available indicate that metoclopramide stimulates pituitary PRL secretion by dopamine antagonistic properties in rat. It was reported that MCH blocks dopamine receptors on lactotrophic cells in the anterior pituitary gland. Some data have also reported that MCH stimulates the metabolic activity of lactotrophic cells and also depletes the central DA store (Regina *et al.*, 2011).

## **2.6 PREVALENCE OF HYPERPROLACTINAEMIA IN NIGERIA**

In Nigeria, hormonal disorders and anovulation are the common causes of female infertility and these are common implications in hyperprolactinaemia (Olooto *et al.*, 2012). A clinical study reported 33.4% of hyperprolactinaemia in infertile women in northern Nigeria (Emokpae *et al.*,



2011). It is also incident in other causes of female infertility such as; galactorrhoea (80%), polycystic ovarian syndrome (20–30%), luteal phase deficiency (13.3%) and hypergonadotropic hypogonadism (1.7%) (Akande *et al.*, 2009).

## **2.7 COCONUT PALM**

The word 'Coconut' was first mentioned in 1555 in Spain. The exact origin of coconut is subject to controversy as some historians believe that the coconut palm originated from Southeast Asia while others believe that it originated from the southern American region. This long and varied history is due to the rapid distribution of the coconut palm over most of the tropical islands and coasts. Presently, the coconut tree flourishes in all tropical territories around the world (Werth, 1993). The coconut palm family is one of the oldest, most diverse and beautiful plant in existence. The slender, leaning and ringed trunk of the palm tree rises to a height of 30 meters from a swollen base to a crown of graceful giant of feather-like pinnate leaves. The pinnate leaves are of 4–6 m long and pinnae are 60–90 cm long. The leaf stalks are thornless and are about 3 to 5 feet in length. The flowers are small, light yellow and usually in clusters. The coconut palm thrives on sandy soil and highly tolerant of salinity. It prefers areas with abundant sunlight, regular rain fall and high humidity for optimum growth (Chan and Craig 2006; Bourke and Tracy 2009).

### **2.7.1 Coconut Fruit**

The botanical name of coconut is *Cocos nucifera*. It belongs to the family of arecaceae (Palm family). It is referred to as agbôn in Yoruba, akuoyibo in Igbo and kwakwa in Hausa. The coconut fruit is made up of the exocarp, mesocarp and endocarp layer. The exocarp is the outermost layer which is usually seen in brown, yellow or green colour depending on the stage of maturation, the middle mesocarp is composed of fibres called coir and the endocarp is the harden shell which



encloses the seed, embryo or endosperm (Bourke and Tracy 2009). The endosperm is located within the shell. The solid endosperm is the coconut meat while the liquid endosperm is the coconut water (Fife, 2005).

### **2.7.2 Brown Coconut Fruit**

The coconut fruit reaches maturity at twelve months. The outer shell at full maturity has a coarse brown hair-like texture and the endosperm layer is firm, dense, rich and chewy. Towards the end of maturation, the volume of water in the cavity decreases considerably which may be due to absorption by the endosperm tissue or evaporation (Chou, 1998; Jarimopas and Ruttanadat, 2007).

### **2.7.3 Green Coconut Fruit**

The green coconut (GC) is an immature coconut fruit with green exocarp. It is also referred to as a young or tender coconut because it is harvested before maturity at about 6 months of age. The physical characteristics, mechanical strength, acoustic and physiological properties have been reported to change as the coconut matures (Jarimopas and Ruttanadat, 2007). There are linear changes with maturity in the thickness of the flesh. The GC has little flesh or meat lining, which is very soft, moist, creamy and gel-like in texture. The meat overtime, slowly grows solid due to fluid absorption. The GC produces sounds which progresses from solid to hollow as the fruit ages. A possible explanation for this, is that the immature fruit is full of water, which makes the fruit exhibits no sound. However, when the fruit is mature, the small amount of water causes gap development in the cavity of the fruit and the sound becomes hollow (Gatchalian *et al.*, 1994). The husk of GC is moist and hard at its tender age. The husk gradually loses moisture and become softer as the fruit matures. The shell rupture force and firmness also increases with maturity

(Mohsenin, 1996). The shell is thinner and gradually becomes woody and thicker with increasing age (Chou, 1998).

#### **2.7.4 Green Coconut Water**

Green coconut water (GCW) or juice is the clear liquid occupying the inner cavity of an immature coconut fruit. GCW is technically referred to as the liquid endosperm due to its cytoplasmic origin which serves as a suspension for nourishing the embryo during fruit development. In the early developmental stage of the fruit, the entire endosperm is in a liquid form containing free nuclei generated by a process of several cycles of division during their nuclear phase of development. The Cytokinetic process begins, progressing from the periphery towards the centre, forming the solid endosperm layer deposited onto the rind as meat (Patrick and Offler, 2001). The cellularization process continues with development but does not fill up the entire embryonic cavity; hence the multinucleate liquid endosperm fails to fully develop into solid mass, leaving the cavity solution-filled. The GCW is gradually but not completely replaced with the solid endosperm as development progresses and this leaves the immature coconut with more volume of water. There are more phytonutrients in the GCW as nutrients are transferred to other part of the fruit as maturity proceeds (Patrick and Offler, 2001). Hence, the GCW is at its purest and contains more inorganic ions which contribute to its therapeutic properties (Anurag and Rajamohan, 2003). The coconut is harvested at an immature green stage mainly because of the high volume of water and phytochemical contents (Janick and Paull, 2008).

### 2.7.5 Phytochemical Analysis of GCW

Over decades, researchers have reported that GCW contains protein (arginine, alanine, cysteine and serine), vitamins (thiamin B1, riboflavin B2, niacin B3, pantothenic B5, pyridoxine B6, folate, and folic & ascorbic acid), carbohydrates (cellulose, Fructose, sucrose and glucose), fat and fiber (Vigliar *et al.*, 2006). It is also a rich source of minerals (  $K^+$  being the most abundant,  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Fe^{2+}$ ,  $P^+$ ,  $Na^+$ ,  $Cl^-$  ) and trace elements (zinc, selenium, iodine, sulphur, manganese, boron and molybdenum) (Tulecke *et al.*, 1991), Phytohormones that have been reported include, cytokinin, auxin and diphenylurea (Ma *et al.*, 2008). It has also been reported to contain nitrogenous compounds (ethanolamine and ammonia), organic acids (lauric, tartaric, malic, citric, acetic, pipecolic, succinic, shikimic and quinic acids) and enzymes (acid phosphatase, catalase, dehydrogenase diastase, peroxidase, RNA polymerases) (Yong *et al.*, 2009).

### 2.8 GCW USAGE IN TRADITIONAL MEDICINE

The green coconut water is a valuable source of medicine that literally comes in its own container. For thousands of years, coconut products have held a respected and valuable place in folk medicine. It is commonly referred to as the fluid of life, dew from heaven, miracle water and nature's sport. Coconut water has a host of yet scientifically unproven but traditional uses in cultures all over the world. From ancient times in Africa, reports support the position that about 85% of the population rely on coconut fruit in traditional medicine. Coconut is used to treat a variety of health problems such as; asthma, colds, cough, dropsy, dysentery, earache, fever, flu, gingivitis, gonorrhea, jaundice, lice, scabies, scurvy, skin infections, sore throat, typhoid and ulcers. In the Indian and Caribbean folk medicine, it is believed to be highly beneficial in eliminating kidney stones and treating urinary tract infections (Corner, 1966). It has been reported

to be used in the treatment of some reproductive problems such as; irregular and painful menstruation and also taken during pregnancy to give the unborn babies strength and vitality. It is also used to boost semen quality and induce libido (Sofowora, 1993).

## **2.9 RESEARCH STATUS OF GCW**

There are increasing scientific evidence that support the role of coconut water in medicinal application.

### **2.9.1 Green Coconut Water and Reproduction**

It was reported that GCW demonstrated oestrogen-like properties when administered in several groups of menopausal rats. The rats demonstrated oestrogen levels comparable to rats that still had their ovaries (Nisaudah *et al.*, 2009). It has been reported that GCW contains sterol which is known to be involved in the synthesis of steroid hormones *in vivo* (Punghmatharith, 1998). In addition, GCW has been shown to aid the maintenance of pregnancy as the number of fetuses delivered at the end of the gestation period corresponded to the number of implantation sites counted on the 10th day of pregnancy in mice. Furthermore, GCW was shown to promote diuresis with minimal loss of electrolytes. It can therefore be used in women with threatened or recurrent abortion (Pragya, 2010; Kennedy *et al.*, 2013). Increased libido has been reported in women and men in their mid-60s after drinking GCW. It has also been shown to improve sexual vitality, boost sperm count and enhance motility (Oettlé, 1993).

### **2.9.2 Antioxidant and Antibiotic Properties**

Coconut water contains numerous antioxidant compounds that have the ability to scavenge free radicals in the body. It was suggested that the naturally occurring vitamin C in coconut water,

along with other vitamins and amino acids, may be responsible for its antioxidant effects (Fonseca *et al.*, 2009; Yong *et al.*, 2009). Furthermore, micronutrients play vital role in the antioxidant system. Kinetin was shown to act as a strong antioxidant both under *in vitro* and *in vivo* conditions. A study done by Olsen *et al.* demonstrated that kinetin protected DNA from oxidative damage mediated by Fenton reaction by inhibiting the formation of 8-oxo-2'-deoxyguanosine, which is a common marker of oxidative damage in DNA. (Olsen, *et al.*, 1999; Verbeke *et al.*, 2000). Coconut water was reported to reduce oxidative stress induced in the liver in rat (Mantena *et al.*, 2003; Loki and Rajamohan, 2003). Lauric acid has unfavourable effects on many microorganisms. GCW contains lauric acid which is converted into monolaurin. Monolaurin exhibits great antiviral, antiprozoal and antibacterial activities which fight against worms, parasites and lipid-coated viruses (Alleyne *et al.*, 2005a).

### **2.9.3 Hydration**

The most promising claim of GCW is its usage as a premium mode of re-hydration (Georg *et al.*, 2004). Its electrolytes content is similar to plasma hence its international acclaim for use as oral rehydration to replace fluids and electrolytes loss. It has been used to treat dehydration caused by dysentery, cholera and diarrhoea (Adams & Bratt 1992). It has been reported to contain five key minerals at fifteen times higher than sport drinks (Douglas *et al.*, 2012). It is documented that during the Pacific Second World War of 1941-45 and Vietnam War, both sides in the conflict regularly used coconut water siphoned directly from the nut to give emergency plasma transfusions to wounded soldiers. This is due to its sterility, pyrogen-free and non-haemolytic properties. It has also been reported that patient can safely receive as much as one quarter to one third of their body weight of coconut water intravenously to gain body fluid (Campbell-falck *et al.*, 2000).

#### **2.9.4 Anti-aging**

Coconut water is the richest natural dietary source of cytokinins. Researchers have suggested the possibility that consuming a rich source of cytokinins will produce anti-aging effect on the body cells by slowing down considerably the normal sequence of aging and reducing the risk of developing degenerative and age related diseases. Cytokinins has been tested for the possible treatment for aging spots, wrinkles, stretch marks, sagging and dry skin. Its application stimulates cell division of connective tissue which replaces older and damaged tissues with functionally new ones. In the regulation of cell growth, cytokinins also prevent disorders that may lead to the development of cancer. Normal cells are kept healthy while cancerous cells are programmed to die, preventing them from growing and spreading (Rattan and Clark 1994).

#### **2.9.5 Enhances Weight Loss**

Coconut water is a natural electrolyte and isotonic beverage which enhances weight loss in a completely natural form by increasing the metabolic rate and fat burning process in the body (Jones, 1997). It has been reported that the administration of immature coconut water for 28 days demonstrated a significant decrease in total body weight in mice (Eze and Chukwuemeka, 2011).

#### **2.9.6 Improves Cardiovascular Health**

A very important aspect of cardiovascular health is the proper management of blood pressure (Alleyne *et al.*, 2005b). According to researchers, individuals with high blood pressure usually have low potassium levels. Therefore, drinking coconut water on a regular basis can be quite effective in regulating blood pressure due to its high concentration of potassium (Anurag and Rajamohan, 2003). A study found that 71% of volunteers with high blood pressure saw

improvements after drinking coconut water twice-daily for 2 weeks. Similarly, some recent studies have found that coconut oil or water can help increase HDL (good) cholesterol, which makes it a wonderful natural treatment for maintaining good cardiovascular health (Nevin and Rajamohan 2004). Antithrombotic activities of the tender coconut water in experimental myocardial infarction were evaluated. It was found that tender coconut water can reduce thrombosis which is the main factor involved in the pathogenesis of myocardial infarction. Moreover, treatment with coconut water seems to be more natural, less expensive and without side effects. Thus, it provides an accessible source for the treatment of coronary vascular diseases in developing countries. This result may provide some useful information for further application of tender coconut water as a cardiotonic medicinal food (Prathapan and Rajamohan, 2010).

#### **2.9.7 Dissolve Kidney Stone**

Medical researchers have shown that the consumption of coconut water can be very effective in dissolving kidney stones. Eugenio Macalalag demonstrated its effectiveness in patients suffering from kidney and urethral stones after consuming coconut water for 2 to 3 times a week. It resulted in significant decrease in the size of kidney stones. He also reported that when coconut water was injected through urethral catheters up to where the stones were lodged (bukolysis), there were disintegration of the stones and finally expelled without the need for surgery (Macalalag and Macalalag, 1997).

#### **2.9.8 Anti-ulceric property**

A warm crude extract of coconut milk and coconut water were investigated for their anti-ulcerogenic effects in male Wistar albino rats. The rate of reduction in the mean ulceric area in the treated rats was similar to that of sucralfate, a conventional cytoprotective agent in ulcer



management. The results showed that coconut water and milk via macroscopic observation had protective effects on the ulcerated gastric mucosa (Nneli and Woyike, 2008).

## **2.10 ANATOMY OF THE FEMALE REPRODUCTIVE SYSTEM**

The female reproductive system is composed of several distinct organs which perform the following functions:

- a) Production of eggs
- b) Reception site for sperm during copulation
- c) Providing a conducive environment for fertilization
- d) Providing shelter and nourishment to the growing embryo (Baird *et al.*, 2012).

### **2.10.1 The Gross Structure of Mammalian Ovary and Uterus**

The ovary is almond shaped paired organ which produces the egg. The ovaries are grayish-pink in colour and present an uneven surface. Each ovary is about 4 cm in length, 2 cm in width and about 8 mm in thickness and weighs about 3 gram. In human, each ovary usually takes turns every month in releasing the egg. It presents a lateral and a medial surface, an upper or tubal and a lower or uterine extremity and an anterior or mesovarian and a posterior free border. The lateral surface is in contact with the parietal peritoneum, which lines the ovarian fossa while the medial surface is to a large extent covered by the fimbriated extremity of the uterine tube. The tubal extremity is near the external iliac vein and attached the fimbria of the uterine tube and a fold of peritoneum called the suspensory ligament of the ovary, which is directed upward over the iliac vessels. The uterine end is directed downward toward the pelvic floor, it is usually narrower than the tubal. The mesovarian border is straight attached to the broad ligament by a short fold called



the mesovarium. Between the two layers of the mesovarium are blood vessels and nerves passing through to the hilum of the ovary (Blackburn and Flemming, 2011)

The uterus or womb is pear-shaped hollow muscular organ. The uterus is located within the pelvic cavity immediately dorsal to the urinary bladder and ventral to the rectum. The uterus measures about 7.5 cm in length, 5 cm in breadth, and 2.5 cm in thickness. It weighs about 35 gram. The uterus can be divided anatomically into four portions. The isthmus is a slight constriction, midway between the apex and the base of the uterus and corresponding to this in the interior, is a narrowing of the uterine cavity called the internal orifice. The portion above the isthmus is termed the body and that below is the cervix. The fundus is a part of the body which lies above a plane passing through the points of entrance of the uterine tubes. The uterus is primarily supported by the pelvic diaphragm, perineal body and the urogenital diaphragm and secondarily supported by broad ligament of uterus. The function of the uterus is to carry the fertilized ovum until delivery. The fertilized ovum becomes an embryo, attaches to the endometrium, creates a placenta and develops into a fetus (gestates) until childbirth. The uterine cavity opens into uterine tubes one on either side, while below its cavity communicates with that of the vagina. The uterus undergoes series of transformation as it responds to changes in hormone secretion during different stages in reproductive cycle. After ovulation, the mucous membrane and the endometrial glands of the uterus have reached its maximum size for implantation. In the absence of pregnancy, the corpus luteum degenerates due to decrease in progesterone and the endometrial lining is shed and discharged as menstruation (Isachenko *et al.*, 2009; Oktay and Oktem 2008). The uterine tubes (Fallopian tubes or oviducts) help transport the ovum from the ovary to the site of fertilization, transport spermatozoa from the site of deposition to the site of fertilization, provide appropriate environment for fertilization and transport the fertilized ovum (embryo) to the uterus where

implantation and further development occur . The uterine tubes can be divided into three major parts; the infundibulum, ampulla and isthmus. The infundibulum is a funnel-shaped lateral end that is closely related to the ovary. Its margins have 20 to 30 fimbriae (finger-like processes that spread over the surface of the ovary) which help to trap the oocyte into the ampulla. The ampulla is the widest and longest part of the uterine tube; it is the region where fertilization occurs. The isthmus of the uterine tube is the medial short, narrow and thick-walled end (Blackburn and Flemming, 2011).

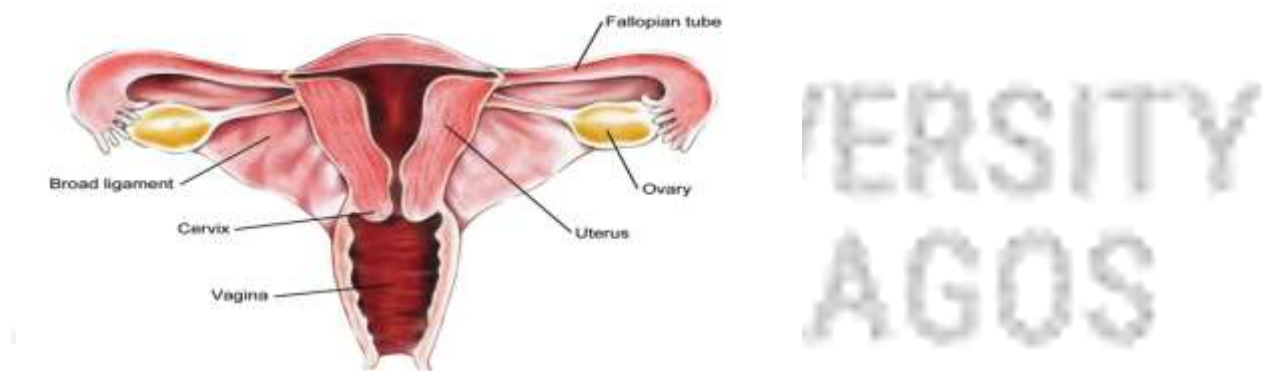


Figure 2: Structure showing the ovary and uterus ( [www.imgkid.com](http://www.imgkid.com), accessed May, 2015).

### **2.10.2 General Histology of the Ovary and Uterus**

The ovary consists of the cortex and medulla. The cortex is the outer region which lies immediately internal to the tunica albuginea. It contains the ovarian follicles which is the functional unit of the ovary. The cortex undergoes fluctuations in size and microscopic appearance that correlate with stages of the reproductive cycle. Internal to the cortex is the

medulla, consisting of blood and lymph vessels, nerves and connective tissue. The medulla, which contains no germinal elements, exhibits no significant cyclical activity. The hilus is the depression along the mesovarian margin, through which the ovarian ligament is attached and through which the blood vessels enter the ovary (Young, 2006).

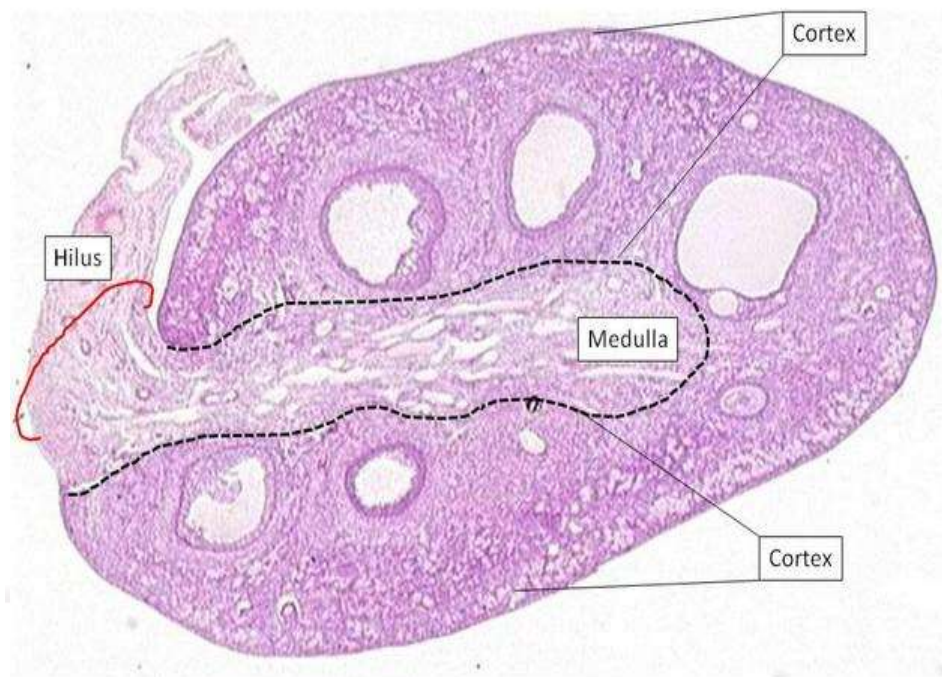


Figure 3: Microscopic structure of the ovary (Histology Lab Final, Michigan University, College of Veterinary Medicine).

The uterus is made up of an endometrium (innermost lining), myometrium (middle tunica muscularis) and the perimetrium (outer tunica serosa). The endometrium is the innermost lining of the uterus which comprises the tunica mucosa and the tunica submucosa. The tunica mucosa is subdivided into the lamina epithelialis usually simple columnar epithelium and lamina propria which consists of loose connective tissue. The myometrium is the middle tunica muscularis layer

of the uterus. It is composed of a thick inner circular layer and a thinner outer longitudinal layer of smooth muscle. The region in between the two layers of smooth muscle contains large blood vessels called the stratum vasculare. The Perimetrium is the outer tunica serosa of the uterus. It has the typical composition of loose connective tissue with a large number of lymphatic vessels (Young, 2006).

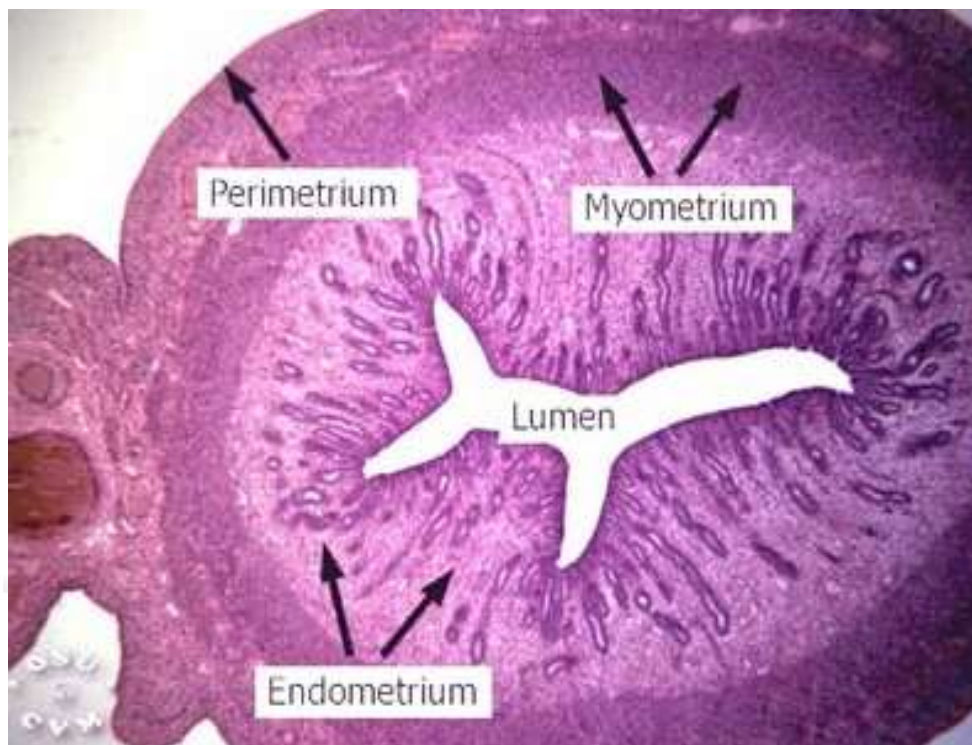


Figure 4: Microscopic structure of the uterus (onlinehealthcaredegrees.com, accessed May, 2015).

### **2.10.3 Ovulation**

Ovulation is the process in which a mature ovarian follicle ruptures and discharges an ovum or egg. The process is initiated by the mid-cycle surge of the pituitary luteinizing hormone (LH). This results from the peak of oestrogen levels during the end of follicular phase that induces substantial biochemical, molecular and cellular changes culminating in the release of a mature

ovum (Russell and Robker, 2007). During the follicular (pre-ovulatory) phase of the menstrual cycle, the ovarian follicle undergoes series of developmental stages which is stimulated by follicle stimulating hormone (FSH). By the 9<sup>th</sup> day of menstrual cycle, only one healthy secondary follicle remains, with the rest having undergone programmed cell death in a mechanism called apoptosis. The dominant follicle enlarges to release an ovum and produces large amount of estradiol. The oestrogen released in late follicular phase has a stimulatory effect on the hypothalamic gonadotropin-releasing hormone (GnRH) and hence causes a spike in LH secretion which triggers ovulation with blister formation (Chen *et al.*, 1993; Ashkenazi, 2005; Richards, 2007). Through a signal transduction cascade initiated by LH, proteolytic enzymes are secreted by the follicles which degrade follicular tissues at the site of the blister, forming a hole called the stigma. The follicle gradually approaches the surface of the ovary where the stigma is formed and the ovum is released. The ovum is released with the corona radiata and cumulus oophorus granulosa cells which undergo a period of proliferation and mucification with the secretion of hyaluronic acid cocktail that disperses and gathers the cumulus cell network in a sticky matrix around the ovum. This network stays with the ovum after ovulation and has been shown to be necessary for fertilization. During the luteal (post-ovulatory) phase, the ovum travels toward the fallopian tube for fertilization. After the discharge of the ovum the lining of the follicle is thrown into folds and vascular processes grow inward from the surrounding tissue. In this way the space is filled up and the corpus luteum is formed (Navarrete-Palacios *et al.*, 2003; Susan *et al.*, 2004; Watson & Stacy 2010).

#### **2.10.4 Oogenesis**

Oogenesis is the process of producing the female gamete. Oocytogenesis marks the initiation of Oogenesis which is the transformation of primordial follicles into primary oocyte or oogonia.



Primary oocyte reaches their maximum development at 20 weeks of gestational age, when approximately seven million primary oocytes have been created. It is believed that, when oocytogenesis is completed no additional primary oocyte is created, in contrast to the male process of spermatogenesis where gametocytes are continuously created (Johnson *et al.*, 2004). The succeeding phase of ootidogenesis occurs when the primary oocyte develops into an ootid. This is achieved by the process of meiosis I which begins during embryonic development, but halts in the diplotene stage of prophase I until puberty. At the time of birth all the surviving primary oocytes are surrounded by thin, single layers of follicular epithelial cells called the primordial follicle. Further developments take place as soon as a regular reproductive cycle is established during puberty. The primary oocyte is hormonally stimulated to enlarge and to complete the first meiotic division to become the secondary oocyte. The haploid secondary oocyte immediately initiates meiosis II. However, this process is halted at metaphase II until fertilization.

Folliculogenesis begins with the recruitment of primordial follicles into the pool of growing follicles that ends with either ovulation or death by atresia. Folliculogenesis can be divided into preantral or gonadotropin-independent phase and antral or gonadotropin-dependent phase. The preantral or gonadotropin-independent phase is characterized by the growth and differentiation of the oocyte. The preantral is further divided into three major stages: the primordial, primary and secondary follicle stages. The primordial follicles are considered the fundamental reproductive units of the ovary because they give rise to all dominant follicles. The primordial follicle contains a small primary oocyte (25  $\mu\text{m}$  in diameter) with a single layer of flattened or squamous granulosa cells closely apposed to the oocyte and a basal lamina. The primordial follicles do not have independent blood supply and thus have limited access to the endocrine system. A primary follicle is defined by the presence of cuboidal granulosa cells that are arranged in a single layer

surrounding the oocyte. The major developmental events that occur in the primary follicle include FSH receptor expression and oocyte growth and differentiation. In human, high level of plasma FSH accelerate primary follicular development. During the preantral period, the oocyte increases in diameter (120  $\mu\text{m}$ ) and develops its surrounding extracellular matrix, the zona pellucida. This step is termed the primary-to-secondary follicle transition. It involves a change in the arrangement of the granulosa cells from simple cuboidal epithelium to stratified or pseudo-stratified columnar epithelium. The major function of the granulosa cell is the production of oestrogen as well as growth factors which aid the development of the oocyte. The formation of secondary follicle also occurs with complete formation of the glycoprotein layer, the pellucid zone between the oocyte and follicular epithelium. The zona-pellucida or egg coat is a glycoprotein membrane that surrounds the plasma membrane of an oocyte that is usually released with the egg. It binds with the spermatozoa to initiate the acrosomal reaction before the male gamete can penetrate and zona reaction which leads to its modification to block polyspermy. The stroma ovarii outside the basal lamina organizes itself to become theca cells. The antral or gonadotropin-dependent phase is characterized by the tremendous increase of the size of the follicle (up to approximately 25-30 mm). During folliculogenesis, division of the granulosa cells increases and fluid-filled spaces develop within the cells. The spaces coalesce to form the antrum. Under the influence of pituitary gonadotropic hormones, many antral follicles thereafter continue to grow, forming large graafian follicles or tertiary follicle. At this stage, the ovum (secondary oocyte) is suspended within the fluid of the antrum (liquor folliculi) by a slender stalk of granulosa cells surrounded by cluster of granulosa cells, the cumulus oophorus. As secondary follicle development proceeds, two primary layers of theca cells appear; an inner theca interna that differentiates in the theca interstitial cells and an outer theca externa that differentiates into smooth muscle cells. Theca cells are thought to

be recruited from surrounding stroma tissue. The theca cell development is also accompanied by numerous blood vessels, presumably through angiogenesis. Consequently, blood circulate around the ovarian follicle, thereby bringing nutrients and gonadotropins to the developing follicle and removing waste and secretory products from it. Their function is enabled through the establishment of a vascular system providing communication with the pituitary axis throughout the reproductive cycle and delivering essential nutrients to these highly active cells. The theca cells are often the final follicular cell type to die during atresia. For those follicles that do ovulate, the theca cells then undergo hormone-dependent differentiation into luteinized theca cells of the corpus luteum (Chen *et al.*, 1993).

A fully grown follicle contains five distinct structural units; a fully grown oocyte surrounded by a zona pellucida, approximately nine layers of granulosa cells, a basal lamina, a theca interna, a theca externa and a capillary network in the theca tissue. A mature follicle is also characterized by a cavity called antrum which contains the follicular fluid (Chen *et al.*, 1993; Ashkenazi, 2005).

### **2.10.5 Endocrine Regulation of Oogenesis**

FSH is a glycoprotein synthesized by the gonadotrophic cells of the anterior pituitary gland. FSH release is controlled by the gonadotropin-releasing hormone (GnRH) produced in the hypothalamus via the hypophyseal portal veins. The FSH primarily controls the maturation of germ cells by stimulating the growth and recruitment of immature ovarian follicles in the ovary. The FSH also acts on the follicular cells to secrete progesterone which activate maturation promoting factor (MPF) in the primary oocyte. MPF causes germinal vesicle breakdown, chromatin condensation and progression to Metaphase II at the secondary oocyte stage. In early follicular phase, FSH is the major factor that rescues the follicles from apoptosis and hence



initiates follicular growth. In late follicular phase, FSH level declines, thus critical in selecting only the most advanced follicle to proceed for ovulation. The release of LH is controlled by pulses of gonadotropin-releasing hormone (GnRH) from the hypothalamus which is subjected to oestrogen feedback from the gonads. The granulosa cells of the developing ovarian follicle synthesizes oestrogen (Nelson and Bulun, 2001; Lin *et al.*, 2004) and via the hypothalamic interface of positive feed-back effect, with a raise in LH release over 24- to 48-hour leads to LH surge which triggers ovulation. Thereby not only causing the release of egg but also initiating the conversion of the residual follicle into a corpus luteum that in turn produces progesterone which prepares the endometrium for implantation. At the end of menstruation, there is a slight rise in FSH which is necessary to start the next ovulatory cycle (Fowler *et al.*, 2003; Boulpaep and Boron 2005; Dickerson *et al.*, 2008; Radu *et al.*, 2010).

## **2.11 ESTROUS CYCLE**

Estrous cycle is a physiological activity which is controlled by reproductive hormones in female mammal. The estrous cycle is synonymous with menstrual cycle of human. The estrous animals reabsorb their endometrium in the absence of pregnancy and are generally only sexually active on the estrus phase of their estrous cycle. This is in contrast with the menstrual cycle in which the endometrium is shed through menstruation and animals are sexually active at any time of their cycle ( Susan *et al.*, 2004; Geoffrey *et al.*, 2007). The length of estrous cycle varies among species, but typically is more frequent in smaller animals. Monoestrous species, such as; bears, foxes, wolves and white-tailed deer undergo estrus once in a year while diestrous animal (dog) goes into estrus twice per year. Polyestrous species such as; rodents, cats, cows and pigs go through a succession of estrous cycles during the year (Heape, 1900). Puberty occurs with the establishment of estrous cycle in sexually mature female rats which onset results from the

establishment of pulsatile luteinizing hormone (LH) release by the eighth postnatal week (50-60 days of age) which leads to ovarian maturation. Before this time, the reproductive tract is inactive; the first proestrus, estrus, metaestrus and diestrus periods then follow and are interrupted by anestrus phases. (Hubscher *et al.* 2005). Histological changes in vaginal smears, morphological appearances of the reproductive organs and general behavioural scheme have been used to characterize the different phases of estrous cycle in rodents (Marcondes *et al.*, 2002; Hubscher *et al.* 2005).

### **2.11.1. Metestrus**

Metestrus occurs in the absence of conception prior to ovulation. The vagina smear histology during this phase shows leukocytes, a few cornified and basophilic cells. This phase is typically brief and may last for six to eight hours in rats. During this phase, the signs of oestrogen stimulation subside and corpus luteum starts to form. The uterine lining begins to secrete small amount of progesterone. Early metestrus is marked by the mid region of the vagina showing a complete detachment of the cornified epithelium, generally with residual squames present in the lumen. There is a continued desquamation of epithelial cells throughout the phase, with a progressive loss of the stratum granulosum and upper germinativum (stratum spinosum). The uterine endometrial epithelium shows continued vacuolar degeneration with variable leukocyte infiltration. The ovarian corpora lutea may still contain a fluid-filled central cavity generally devoid of fibrous tissue and the cytoplasm of the new corpora lutea are somewhat less basophilic than at estrus, a previous phase (Mccracken *et al.*, 1999; Spornitz, *et al.*, 1999 ; Marcondes *et al.*, 2002).

### 2.11.2. Diestrus

Diestrus is characterized by the activity of the corpus luteum that produces progesterone. In the absence of pregnancy, the diestrus phase terminates with the regression of the corpus luteum. The vaginal smear histological appearance is characterized by little mucus with some leukocytes, nucleated basophilic cells and occasionally vacuolated cells. The diestrus occurs with the longest period of time which is usually about fifty-five to fifty-seven hours. The uterine lining is not shed but rather reorganized for the next phase. At early diestrus, the vaginal epithelium is at its lowest level of approximately three to seven cells layers of stratum germinativum. The stratum germinativum consists of stratum basal as a single layer of columnar epithelial cells and an outer stratum spinosum as multiple layers of polyhedral cells with variable infiltration by leukocytes. A notable epithelial cell proliferation occurs toward the end of the phase with thickening of the epithelium at the end of diestrus defining the characteristic for the practical staging for the end of diestrus and the beginning of proestrus. The uterus is small and inactive and the horns lack a prominent vasculature and generally show a slit-like lumen. They are lined by low cuboidal or columnar epithelium. There are few mitotic actions of endothelium in early diestrus which increase in activity during the progression of the phase. In the ovary, the newly formed corpora lutea from the previous ovulation have attained their maximal size and this is the best ovarian marker for diestrus, although degeneration of the corpora luteum is seen in late diestrus (McCracken *et al.*, 1999; Spornitz *et al.*, 1999; Marcondes *et al.*, 2002).

### 2.11.3 Proestrus

This phase is regulated by gonadotropic hormones which stimulate maturation of ovarian follicles and oestrogen secretion exert its biggest influence to induce ovulation. The vaginal smear histological appearance shows the presence of sheets or isolated nucleated epithelial cells and cornified cells. The duration of proestrus phase in rat is usually about twelve to fourteen hours. The vaginal epithelium shows high mitotic activities throughout the phase following the early formation of the stratum granulosum. There is a progressive development of the superficial mucoid layer (stratum mucification) or rete mucosum characterized by layers of cuboidal to ovoid cells with mucin-containing cytoplasmic vacuoles and the formation of a stratum corneum of dense cornified cells. At the end of the phase, the epithelium is fully cornified and generally shows a superficial mucoid layer exhibiting some desquamation of mucoid cells. The uterine lining starts to develop into progressive large cells forming a tall cuboidal to columnar epithelium. The endometrial vasculature becomes more prominent and the stroma shows some edema with the lumen generally becoming markedly dilated toward the end of the phase. The ovarian corpora lutea are degenerate with central fibrous tissue formation (McCracken et al., 1999; Spornitz *et al.*, 1999; Marcondes *et al.*, 2002).

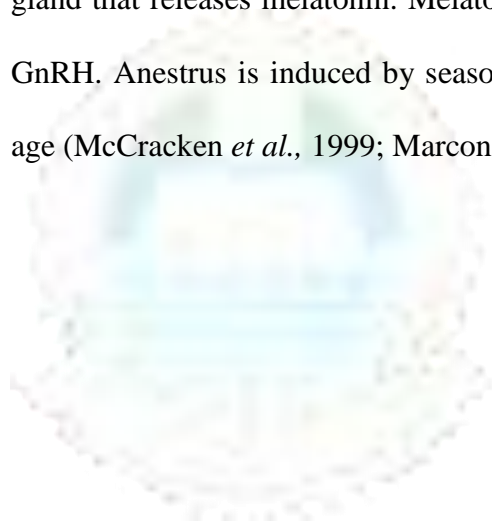
### 2.11.4 Estrus

Estrus refers to the phase when the female is sexually receptive "in heat" or "on heat" to the opposite sex. Some animal exhibits a sexually receptive behaviour such as; lordosis reflex, in which the animal spontaneously elevates her hindquarters and reddened colouration of the vulvae (Geoffrey *et al.*, 2007). The vaginal smear shows primarily non-nucleated cornified cells. Fertile mating on estrus phase of the cycle leads to pregnancy, but infertile mating leads to a state of

pseudo-pregnancy which usually lasts for about 12 to 14 days in rats .Estrus phase lasts for about twenty-five to twenty-seven hours. In the vagina, there is a loss of mitotic figures and a progressive shedding of the superficial mucoid and cornified layers. The endometrial epithelium defines the beginning of estrus with the appearance of cellular degeneration in the uterine glands (McCracken *et al.*, 1999; Spornitz *et al.*, 1999; Marcondes *et al.*, 2002).

#### **2.11.5 Anestrus**

Anestrus refers to the phase when the sexual cycle is at rest and this is controlled by the pineal gland that releases melatonin. Melatonin is thought to act by regulating hypothalamic activity of GnRH. Anestrus is induced by season, menopause, pregnancy, lactation, significant illness and age (McCracken *et al.*, 1999; Marcondes *et al.*, 2002).



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## **CHAPTER THREE**

### **METHODOLOGY**

#### **3.1 POPULATION, SOURCE AND MAINTENANCE OF EXPERIMENTAL ANIMALS**

A total of 375 (three hundred and seventy-five) female Sprague-Dawley rats weighing 145-170g were used for this study, and thirty male Sprague-Dawley rats of proven fertility were used for mating in the fertility test study. The rats were obtained from a breeding stock of the Nigerian Institute of Medical Research, Yaba, Lagos. The animals were authenticated by a taxonomist in the department of Zoology of the University of Lagos and were kept in standard ventilated plastic cages under standard laboratory conditions with a photoperiodicity of twelve hours light alternating with twelve hours of darkness. The animals were allowed to acclimatize for two weeks and were fed with commercially available rat chow from Livestock feeds Plc., Ikeja, Lagos and had free access to tap water. The experiment was carried out in two phases of the same experimental protocol, one being a repetition of the other to ascertain the reproducibility of findings and to minimize the effects of error on the results. The weights of the animals were measured at procurement, during the period of acclimatization and throughout the duration of the experiment. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles for the Care and Use of Animals (American Physiological Society, 2002) and were approved by the Departmental Committee on the Use and Care of Animals.

### **3.1.1 Determination of the phases of estrous cycle in the animals**

The phases of estrous cycle were established by daily cytological examination of fresh vaginal smear in the morning between 8:00 and 10:00 am. Approximately 0.2 ml of normal saline was drawn into the suction pipette. The tip of the pipette was pushed gently into the entrance of the vagina to a depth of 5 mm and the fluid was flushed into the vagina and back up into the pipette two or three times by gently squeezing and releasing the bulb of the pipette. The smear was gently dropped onto the glass slide and viewed under a light microscope with 40X objective lens. The first day of the estrous cycle was designated as metestrus, the vagina smear histology showed leukocytes amidst few squamous cells. The second day was the diestrus phase and this showed predominantly leukocytes. The third day showed large nucleated cells and this was designated as the proestrus phase. The fourth day was designated as the estrus phase and the vaginal cytology showed large flakes of squamous cells (Marcondes *et al.*, 2002).

## **3.2 PLANT MATERIAL**

### **3.2.1 Collection of green coconut fruits**

The immature coconut fruits were harvested at six months of age between November, 2011 and April, 2013 from Chief William Kudofoke coconut farm, Ajara-Topa, Badagry, Lagos. The average weight of the fruit was 1.55 kg. The fruit was authenticated at the Federal Institute of Forestry Research in Ibadan by Dr (Mrs.) A. O. Ugbogu with plant's accession No. FHI 109665 and sample was deposited in the Department of Botany of the University of Lagos.





FIGURE 5: Photograph of immature coconut fruits used in the study.

### **3.2.2 Extraction of the green coconut water**

The immature coconut fruits were washed and dehusked. A sterile iron rod was used to open the germinal pore. The extraction of the coconut water was done through the germinal pore, poured into a clean airtight bottle and kept in the refrigerator at 4°C. The GCW was replaced every three weeks (Bustamante, 2002).

### **3.2.3 The Lethal dose of GCW**

The animals were weighed and administered increasing geometric series of GCW in mg/kg with oropharyngeal canula and 1ml syringe through the oral and intraperitoneal route respectively.

### **3.2.4 Green coconut water oral dose**

A preliminary study was carried out with 10 ml/100 g b.w. of GCW daily in two divided doses (Nisaudah *et al.*, 2009) and a lower geometric volume of 5 ml/100 g b.w. of GCW daily was also effective.

### **3.2.5 Phytochemical analysis of GCW**

Qualitative and quantitative phytochemical analysis was conducted on green coconut water using standard procedures as described by Edeoga *et al.*, 2005.

#### **3.2.5.1 Cyanogenic glycoside**

Five millilitre of sample was weighed into a 250 ml conical flask and incubated for 16 hours at 38°C before being mixed with 95% methanol. The sample was filtered by double layer of filter paper and then distilled. The sample was transferred into a low-necked 500 ml flask connected to a steam generator. It was then steam-distilled for 1 hour with sodium bicarbonate solution in a 50 ml conical flask. 1 ml of starch indicator was added to 20 ml of the distillate and iodine-titrated solution after which percentage hydrocyanide content was calculated.

#### **3.2.5.2 Alkaloid**

Five grams of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 hours. This was filtered and the extract was

concentrated to one-quarter of its original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

#### **3.2.5.3 Tannin**

Five hundred milligrams of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and left for 1 hour. This was filtered into a 50 ml volumetric flask. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M iron chloride in 0.1 M hydrochloric acid and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes.

#### **3.2.5.4 Flavonoid**

Ten grams of the sample was mixed with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper of No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

#### **3.2.5.5 Phenol**

The sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 minutes. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The sample was left to react for 30 min for colour development. This was measured at 505 nm.

#### **3.2.5.6 Sugar**

Two millilitre of the solution sample in a test tube was added 1 ml each of Fehling's solution A and B. The mixture was heated in a water bath for 10 minutes. A brick-red precipitate indicated a reducing sugar. The sample was dried in the oven to a constant weight.

#### **3.2.5.7 Saponin**

Twenty grams of sample was put into a conical flask and 20% aqueous ethanol was added. The mixture was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue extracted with another 200 ml 20% ethanol. The extract was reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

#### **3.2.5.8 Sterol**

Two millilitre of acetic anhydride was added to 0.5 g ethanolic mixture of the sample with 2 ml tetraoxosulphate(VI) acid. The colour changed from violet to blue or green in the samples indicated the presence of steroids. After evaporation the sample was dried in the oven to a constant weight; the content was calculated as percentage.

### 3.3 HYPERPROLACTIN-INDUCING AGENT

Metoclopramide hydrochloride (MCH) was used as the hyperprolactin-inducing agent. The drug was purchased from Pfizer pharmaceutical, Ikeja Lagos. MCH was administered at a dose of 0.2 mg/100 g. b. w. daily for 28 days to induce hyperprolactinaemia (Raffaele *et al.*, 2009). The dose was calculated by simple proportion based on the animal's weekly weights and administered via oral route with the use of a metal oropharyngeal canula.

### 3.4 ANIMAL DISTRIBUTION, EVENTS AND DURATIONS.

The animals that demonstrated regular estrous cycling for three consecutive cycles were selected and randomly divided into six experimental groups A to F

#### 3.4.1 Experimental study groups A - F

(i) EXPERIMENTAL STUDY GROUP A (Induction and withdrawal groups)

In this group, 0.2 mg/100 g body weight of metoclopramide hydrochloride (MCH) was administered daily through the oral route for 28 days (A1). MCH administration was withdrawn and replaced with distilled water (DSTL) for 'short' term of 8 days (A2), for 'medium' term of 16 days (A3) and for 'long' term of 28 days (A4). There were 4 sub-groups of an induced group and 3 withdrawal groups;

(a) Induced group: A<sub>1</sub>:MCH<sub>28 days</sub>

(b) Withdrawal short term group: A<sub>2</sub>:MCH<sub>28 days</sub>; DSTL<sub>8 days</sub>

(c) Withdrawal moderate term group: A<sub>3</sub>: MCH<sub>28 days</sub>; DSTL<sub>16 days</sub>

(d) Withdrawal Long term group: A<sub>4</sub>: MCH<sub>28</sub> days; DSTL<sub>28</sub> days

In total A<sub>1</sub> to A<sub>4</sub> were made up of a population of 60 female S-D rats as shown below;

Sub-groups	Population	Events and distribution
A <sub>1</sub> :MCH <sub>28</sub> days	15	Estrous cycle was studied for 28 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 28 <sup>th</sup> day.
A <sub>2</sub> :MCH <sub>28</sub> days, DSTL <sub>8</sub> days	15	Estrous cycle was studied for 36 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 36 <sup>th</sup> day.
A <sub>3</sub> : MCH <sub>28</sub> days; DSTL <sub>16</sub> days	15	Estrous cycle was studied for 44 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 44 <sup>th</sup> day.

A<sub>4</sub>: MCH<sub>28 days</sub>; DSTL<sub>28 days</sub> 15

Estrous cycle was studied for 56 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 56<sup>th</sup> day.

Total duration was 56 days

(ii) EXPERIMENTAL STUDY GROUP B (Post- treated groups)

This group consists of animals that were initially fed with metoclopramide followed by the administration of 5 ml/100 g. b.w. and 10 ml/100 g. b.w. of GCW for short, medium and long term respectively. There were 6 sub-groups of low and high dose phases of GCW for 8, 16 and 28 days.

- (a) Low dose phase at 5 ml/100 g. b. w. of GCW: (3 sub-groups) B<sub>1</sub>: MCH<sub>28 days</sub> – GCW<sub>L8 days</sub>; B<sub>2</sub>: MCH<sub>28 days</sub> – GCW<sub>L16 days</sub>; B<sub>3</sub>: MCH<sub>28 days</sub> – GCW<sub>L28 days</sub>
- (b) High dose phase at 10 ml/100 g. b. w. of GCW: (3 sub-groups) B<sub>4</sub>: MCH<sub>28 days</sub> – GCW<sub>H8 days</sub>; B<sub>5</sub>: MCH<sub>28 days</sub> – GCW<sub>H16 days</sub>; B<sub>6</sub>: MCH<sub>28 days</sub> – GCW<sub>H28 days</sub>

In total B<sub>1</sub> to B<sub>6</sub> were made up of a population of 90 female S-D rats as shown below;

Sub-groups	Population	Events and distribution
B <sub>1</sub> : MCH <sub>28 days</sub> – GCW <sub>L8 days</sub>	15	Estrous cycle was studied for 36 days, 5 rats were sacrificed on proestrus phase for



histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 36<sup>th</sup> day.

B2: MCH<sub>28 days</sub> – GCW<sub>L16 days</sub> 15

Estrous cycle was studied for 44 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 44<sup>th</sup> day.

B3: MCH<sub>28 days</sub> – GCW<sub>L28 days</sub> 15

Estrous cycle was studied for 56 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 56<sup>th</sup> day.

B4: MCH<sub>28 days</sub> – GCW<sub>H8 days</sub> 15

Estrous cycle was studied for 36 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 36<sup>th</sup> day.

B5: MCH<sub>28 days</sub> – GCW<sub>H16 days</sub>

15      Estrous cycle was studied for 44 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 44<sup>th</sup> day.

B6: MCH<sub>28 days</sub> – GCW<sub>H28 days</sub>

15      Estrous cycle was studied for 56 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 56<sup>th</sup> day.

Total duration was 56 days

(iii) EXPERIMENTAL STUDY GROUP C (Co-administered groups)

This group consists of animals that were concurrently treated with metoclopramide hydrochloride and green coconut water. There were 2 sub-groups of low and high dose phases of GCW.

(a) Low dose phase at 5 ml/100 g. b. w. of GCW = C1: (MCH+ GCW<sub>L</sub>)<sub>28 days</sub>

(b) High dose phase at 10 ml/100 g. b. w. of GCW = C2: (MCH+ GCW<sub>H</sub>)<sub>28 days</sub>

A total of 30 female SD-rats were used as shown below;

Sub-groups	Population	Events and distribution
C1: (MCH+ GCW <sub>L</sub> ) <sub>28 days</sub>	15	Estrous cycle was studied for 28 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 28 <sup>th</sup> day.
C2: (MCH+ GCW <sub>H</sub> ) <sub>28 days</sub>	15	Estrous cycle was studied for 28 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 28 <sup>th</sup> day.

Total duration was 28 days

#### (iv) EXPERIMENTAL STUDY GROUP D (Pre-treated groups)

This group consists of animals that were initially fed with green coconut water followed by the administration of metoclopramide hydrochloride. There were 6 sub-groups of low and high doses of GCW for short, medium and long term.

(a) Low dose at 5 ml/100 g. b. w. : (3sub-groups) D1: GCW<sub>L8 days</sub> – MCH<sub>28 days</sub>; D2: GCW<sub>L16 days</sub> – MCH<sub>28 days</sub>; D3: GCW<sub>L28 days</sub> – MCH<sub>28 days</sub>

(b) High dose at 10 ml/100 g. b. w. : (3sub-groups) D4: GCW<sub>H8 days</sub> – MCH<sub>28 days</sub>; D5: GCW<sub>H16 days</sub> – MCH<sub>28 days</sub>; D6: GCW<sub>H28 days</sub> – MCH<sub>28 days</sub>;

A total of 90 female SD-rats were used as shown below;

Sub-groups	Population	Events and distribution
D1: GCW <sub>L8 days</sub> – MCH <sub>28 days</sub> ,	15	Estrous cycle was studied for 36 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 36 <sup>th</sup> day.
D2: GCW <sub>L16 days</sub> – MCH <sub>28 days</sub>	15	Estrous cycle was studied for 44 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 44 <sup>th</sup> day.
D3: GCW <sub>L28 days</sub> – MCH <sub>28 days</sub>	15	Estrous cycle was studied for 56 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were

sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 56<sup>th</sup> day.

D4: GCW<sub>H8 days</sub> – MCH<sub>28 days</sub> 15

Estrous cycle was studied for 36 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 36<sup>th</sup> day.

D5: GCW<sub>H16 days</sub> – MCH<sub>28 days</sub> ,

15 Estrous cycle was studied for 44 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 44<sup>th</sup> day.

D6: GCW<sub>H28 days</sub> – MCH<sub>28 days</sub> ,

15 Estrous cycle was studied for 56 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 56<sup>th</sup> day.

Total duration was 56 days

(v). EXPERIMENTAL STUDY GROUP E (GCW- treated groups)

This group consists of animals fed with green coconut water only. There were 6 sub-groups of low and high doses of GCW for short, medium and long term;

(a) Low dose phase of 5 ml/100 g. b. w. of GCW: (3sub-groups) E1: GCW<sub>L8days</sub>; E2: GCW<sub>L16days</sub>; E3: GCW<sub>L28days</sub>.

(b) High dose phase of 10 ml/100 g. b. w. of GCW: (3sub-groups) E4: GCW<sub>H8days</sub>; E5: GCW<sub>H16days</sub>; E6: GCW<sub>H28days</sub>

A total of 90 female SD-rats were used as shown below;

Sub-groups	Population	Events and distribution
E1: GCW <sub>L8 days</sub>	15	Estrous cycle was studied for 8 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 8 <sup>th</sup> day.
E2: GCW <sub>L16 days</sub>	15	Estrous cycle was studied for 16 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and

5 rats were subjected to fertility testing at the end of 16<sup>th</sup> day.

E3: GCW<sub>L28 days</sub>

15

Estrous cycle was studied for 28 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 28<sup>th</sup> day.

E4: GCW<sub>H8 days</sub>

15

Estrous cycle was studied for 8 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 8<sup>th</sup> day.

E5: GCW<sub>H 16 days</sub>

15

Estrous cycle was studied for 16 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 16<sup>th</sup> day.

E6: GCW<sub>H28 days</sub>

15

Estrous cycle was studied for 28 days, 5 rats were sacrificed on proestrus phase for



histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 28<sup>th</sup> day.

Total duration was 28 days.

(vi) EXPERIMENTAL STUDY GROUP F (control group)

This group consists of animals fed with distilled water only. There were 3 sub-groups of 8, 16 and 28 days.

A total of 45 female S-D rats were used as shown below

Sub-groups	Population	Events and distribution
F <sub>1</sub>	15	Estrous cycle was studied for 8 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at the end of 28 <sup>th</sup> day.
F <sub>2</sub>	15	Estrous cycle was studied for 16 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and

5 rats were subjected to fertility testing at the end of 28<sup>th</sup> day.

F<sub>3</sub>

15

Estrous cycle was studied for 28 days, 5 rats were sacrificed on proestrus phase for histomorphological study, 5 rats were sacrificed on estrus phase for ova count and 5 rats were subjected to fertility testing at

Total duration was 28 days

### 3.4.2 The male group

This group comprises of male rats of proven fertility. The animals were kept in separate cages strictly for mating. In all, they were made up of 30 rats. The coupling was done in female to male ratio of 2:1.

### 3.4.3 The experimental design

**GROUP A:** In this group, a dose of MCH at 0.2 mg/100 g body weight daily for 28 days was used to induce hyperprolactinaemia (A1: MCH<sub>28 days</sub>). The administration metoclopramide hydrochloride was withdrawn and replaced with the administration of distilled water for 'short' term, which refers to the withdrawal of MCH for brief period of 8days (A2: MCH<sub>28 days</sub>; DSTL<sub>8 days</sub>), the withdrawal for 'medium' term refers to the duration of 16 days (A3: MCH<sub>28 days</sub>; DSTL<sub>16 days</sub>) and 'long' term refers to the duration of 28 days (A4: MCH<sub>28 days</sub>; DSTL<sub>28 days</sub>). In sub-groups A1, A2, A3 and A4, estrous cycle of the rats were studied daily, 5 rats were sacrificed in the morning of proestrus for biochemical, hormonal and histomorphological investigations, 5

rats were sacrificed in the morning of estrus for ova count study and the remaining 5 rats were used for coupling at the end of 28, 36, 44 and 56 days in A1, A2, A3 and A4 respectively.

**GROUP B:** A dose of metoclopramide hydrochloride at 0.2 mg/100 g body weight/day for 28 days was administered and then post-treated with low dose of 5 ml/100 g. b. w. of GCW for short term (B1: MCH<sub>28 days</sub>; GCW<sub>L8 days</sub>), medium term (B2: MCH<sub>28 days</sub>; GCW<sub>L16 days</sub>) and long term (B3: MCH<sub>28 days</sub>; GCW<sub>L28 days</sub>) for 8, 16 and 28 days respectively. High oral dose of 10 ml/100 g. b. w. of GCW in two divided doses was also used for post-treatment for short term (B4: MCH<sub>28 days</sub>; GCW<sub>H8 days</sub>), medium term (B5: MCH<sub>28 days</sub>; GCW<sub>H16 days</sub>) and long term (B6: MCH<sub>28 days</sub>; GCW<sub>H28 days</sub>) for 8, 16 and 28 days respectively. In sub-groups (B1 and B4), (B2 and B5) and (B3 and B6), estrous cycle of the rats were studied daily, 5 rats were sacrificed on the morning of proestrus for biochemical, hormonal and histomorphological investigations, 5 rats were sacrificed on the morning of estrus for ova count study and the remaining 5 rats were used for coupling at the end of 36, 44 and 56 days respectively.

**GROUP C:** This group is made up of subgroups C1 and C2. The animals were concurrently treated with metoclopramide hydrochloride and green coconut water daily for 28 days. In C1, the animals received, MCH (0.2 mg/100 g body weight/day) and low dose of 5 ml/100 g. b. w. of GCW; C1: (MCH + GCW<sub>L</sub>)<sub>28 days</sub> while C2 animals received MCH (0.2 mg/100g body weight/day) and high dose of 10 ml/100 g. b. w. of GCW; C2: (MCH + GCW<sub>H</sub>)<sub>28days</sub>. In sub-groups C1 and C2, estrous cycle of the rats were studied daily, 5 rats were sacrificed on the morning of proestrus for biochemical, hormonal and histomorphological investigations, 5 rats were sacrificed on the morning of estrus for ova count study and the remaining 5 rats were used for coupling at the end of 28days

**GROUP D:** In this group, the rats were initially pre-treated with green coconut water before the induction of hyperprolactinaemia. The rats received low dose of GCW at 5 ml/100 g. b. w. for short term (D1: GCW<sub>L8 days</sub>; MCH<sub>28 days</sub>), medium term (D2: GCW<sub>L16 days</sub>; MCH<sub>28 days</sub>) and long term (D3: GCW<sub>L28 days</sub>; MCH<sub>28 days</sub>) for 8, 16 and 28 days respectively, followed by the administration of metoclopramide hydrochloride. The animals also received high dose of GCW at 10 ml/100 g. b. w. for short term (D4: GCW<sub>H8 days</sub>; MCH<sub>28 days</sub>), medium term (D5: GCW<sub>H16 days</sub>; MCH<sub>28 days</sub>) and long term (D6: GCW<sub>H28 days</sub>; MCH<sub>28 days</sub>) for 8, 16 and 28 days respectively, followed by the administration of metoclopramide hydrochloride. In sub-groups (D1 and D4), (D2 and D5) and (D3 and D6), estrous cycle of the rats were studied daily, 5 rats were sacrificed on the morning of proestrus for biochemical, hormonal and histomorphological investigations, 5 rats were sacrificed on the morning of estrus for ova count study and the remaining 5 rats were used for coupling at the end of 36, 44 and 56 days respectively.

**GROUP E:** The animals in this group received GCW only. The rats were treated with low dose of GCW at 5 ml/100 g. b. w. for short term (E1: GCW<sub>L8 days</sub>), medium term (E2: GCW<sub>L16 days</sub>) and long term (E3: GCW<sub>L28 days</sub>) for 8, 16 and 28 days respectively. The animals also received high dose of GCW at 10 ml/100 g. b. w. for short term (E4: GCW<sub>H8 days</sub>), medium term (E5: GCW<sub>H16 days</sub>) and long term (E6: GCW<sub>H28 days</sub>) for 8, 16 and 28 days respectively. In sub-groups (E1 and E4), (E2 and E5) and (E3 and E6), estrous cycle of the rats were studied daily, 5 rats were sacrificed on the morning of proestrus for biochemical, hormonal and histomorphological investigations, 5 rats were sacrificed on the morning of estrus for ova count study and the remaining 5 rats were used for coupling at the end of 36, 44 and 56 days respectively.

**GROUP F:** The animals in this group received distilled water only. The estrous cycle of the rats were studied daily, 5 rats were sacrificed on the morning of proestrus for biochemical, hormonal

and histomorphological investigations, 5 rats were sacrificed on the morning of estrus for ova count study and the remaining 5 rats were used for coupling. The results from this group were compared with that of the experimental groups.

### **3.5 PROTOCOL SCHEDULE**

Blood samples were obtained while the animals were alive from the orbital venous sinus. All sacrifices were done by cervical dislocation. Incisions were made on the ventral surface of the pelvic region and the ovaries and oviducts were removed for histomorphological, biochemical and ova count studies.

### **3.6 TISSUE PROCESSING FOR HISTOLOGICAL STUDY**

The ovaries were carefully dissected out and trimmed of fat. The ovaries were weighed and then fixed in 10% formal saline. The fixed tissues were transferred into ascending grades of alcohol for dehydration. On day 1, the tissues were placed in 70% alcohol for 7 hours, then transferred into 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for one hour each and finally cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax at 58°C. Three changes of molten paraffin wax at one hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections were orientated perpendicular to the long axis of the ovaries. Serial sections of 5 µm thick cut by the rotary microtome were obtained from a solid block of tissue and floated in water bath. The sections were picked with clean slides onto which egg albumin had been coated and dried on the hot plate at 52°C. The mounted sections were dewaxed in xylene and then hydrated in descending grades of alcohol (90%, 70% and 50%). The sections were then stained with Haematoxylin for 10 minutes after which rinsed and differentiated in 1% acid alcohol for 10 seconds before rinsing in water. The sections

were then counter stained in Eosin for one minute, rinsed in water and dehydrated in 50%, 70%, 90% and absolute alcohol. The sections were finally cleared in xylene and a drop of mountant was placed on the section and covered with cover slip (Henriques, 1981).

### **3.7 IMMUNOHISTOCHEMISTRY**

The animals were perfused to extract the pituitary gland. The tissue was immediately fixed in Bouin's fluid. The ovarian and pituitary tissues were processed and antigen retrieval procedure was performed by heating section in citric acid using microwave as a source of heat. This was followed with equilibration which was done by bringing tissue to water (Shi *et al.*, 1999). The sections were blocked with peroxidase and protein block reagents to prevent interactions from receptors that have the same properties with the oestrogen receptors. The sections were then incubated with the primary oestrogen receptor antibody with catalogue number 790-4324 manufactured by Ventana, United Kingdom, to form antigen-antibody complex. A secondary antibody was added to enlarge the antigen-antibody complex and a polymer to alight the brown colouration of the complex.

### **3.8 OVA COUNT**

The fimbriated part of the oviduct was dissected out and trimmed of fat. The cavity of the oviduct was opened to expose the ova, suspended on a glass slide with one or two drops of normal saline and covered with a cover slip for stable positioning. The cavity of the oviduct was viewed on a light microscope at X100 magnification and the ova seen were counted and recorded (Murphy and Mahesh 1985).

### **3.9 HORMONAL ASSAYS**

Blood samples were obtained from the orbital venous sinus with microhematocrit tube inserted into the medial canthus between 7- 8 a.m. in the morning and immediately spurned at 3,000 rpm for 10 minutes. The serum was separated and frozen at  $-80^{\circ}\text{C}$  until the hormonal assays were carried out.

#### **3.9.1 Prolactin assay**

This was carried out using the enzyme immunoassay (ELISA) kit of catalogue number: 4226Z. The double-antibody immunoassay assay utilized anti-PRL antibody for solid phase (microtiter wells) immobilization and mouse monoclonal anti-PRL antibody enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the PRL molecules being sandwiched between the solid phases. After incubation for 60 minutes at room temperature, the wells were washed with water to remove unbound labelled antibodies. A solution of tetramethylbenzidine (TMB) was added and incubated for 20 minutes which resulted in the development of blue colouration. The colour development was stopped with hydrochloric acid and the colour was changed to yellow and measured spectrometrically at 450 nm. The concentration of PRL was directly proportional to the colour intensity of the test sample. The value for each set of reference standards, specimens, controls and samples were calculated by constructing a standard curve. The mean absorbance values obtained from each reference standard was plotted against its concentration in mIU/ml on graph paper with absorbance values on vertical or Y-axis and concentration on horizontal or X-axis. The mean values for each specimen was determined with the corresponding concentration of PRL in



mIU/ml from the standard curve. The hormonal assay for an individual sample was conducted in duplicate.

### **3.9.2 Follicle stimulating hormone assay**

This was carried out using the enzyme immunoassay (ELISA) kit of catalogue number: 4224Z. The assay utilized a polyclonal anti-FSH antibody for solid phase (microtiter wells) immobilization and mouse monoclonal anti-FSLH antibody enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies which resulted in the FSH molecules being sandwiched between the solid phases. After incubation for 60 minutes at room temperature, the wells were washed with water to remove unbound labelled antibodies. A solution of TMB was added and incubated for 20 minutes for the initiation of blue colouration. The colour development was stopped with hydrochloric acid and the colour was changed to yellow and measured spectrometrically at 450 nm. The concentration of FSH was directly proportional to the colour intensity of the test sample. The value for each set of reference standards, specimens, controls and samples were calculated by constructing a standard curve. The mean absorbance values obtained from each reference standard was plotted against its concentration in mIU/ml on graph paper with absorbance values on vertical axis and concentration on horizontal axis. The mean values for each specimen was determined with the corresponding concentration of FSH in mIU/ml from the standard curve.

### **3.9.3 Luteinising hormone assay**

This was carried out using the enzyme immunoassay (ELISA) kit of catalogue number: 4225Z. The assay utilized anti-LH antibody for solid phase (microtiter wells) immobilization and mouse monoclonal anti-LH antibody enzyme (horseradish peroxidase) conjugate solution. The test

sample was allowed to react simultaneously with the antibodies, resulting in the LH molecules being sandwiched between the solid phase and enzyme linked antibodies. After 60 minutes of incubations at room temperature, the wells were washed with water to remove unbounded antibodies. A solution of TMB was added and incubated for 20 minutes which resulted in the development of a blue colouration. The colour development was stopped with hydrochloric acid and the colour was changed to yellow and measured spectrometrically at 450 nm. The concentration of LH was directly proportional to the colour intensity of the test sample. The value for each set of reference standards, specimens, controls and samples were calculated by constructing a standard curve. The mean absorbance values obtained from each reference standard was plotted against its concentration in mIU/ml on graph paper with absorbance values on vertical axis and concentration on horizontal axis. The mean values for each specimen was determined with the corresponding concentration of LH in mIU/ml from the standard curve.

#### **3.9.4 Oestrogen assay**

This assay employs the competitive inhibition enzyme immunoassay technique using enzyme immunoassay (ELISA) kit of catalogue number: ab108667. Absorbance was measured with an automatic spectrophotometer at 450 nm. A standard curve was obtained by plotting the concentration of the standard versus the absorbance and the concentration of oestrogen was read from the standard curve.

#### **3.9.5 Dopamine assay**

The serum level of dopamine was assayed using commercial kits of catalogue number: KA1887, from Abnova (Walnut, USA). Dopamine was extracted by cis-diol-specific affinity gel, acylated and derivatized enzyme using microtiter plate. The derivatized standards, controls and samples

analytes compete for a fixed number of antiserum binding sites. The free antigen and antigen-antiserum complexes were removed by washing. The antibody bound to the solid phase was detected by anti-rabbit IgG - peroxidase conjugate as a substrate. The absorbance of the solution in the well was read using microplate reader set at 450nm.

### **3.10 ORGAN BIOCHEMICAL ASSAY**

The tissues after extraction were removed of fat and immediately immersed in ice. The organs were then homogenized with 0.1 M phosphate buffer and laboratory sand (acid washed sand). The resulting homogenate was centrifuge for 15 minutes and the supernatant was decanted and stored at -20°C until biochemical analysis was carried out.

#### **3.10.1 Determination of Catalase (CAT)**

The assay utilized enzyme activity on hydrogen peroxide. Hydrogen peroxide was prepared with phosphate buffer; 0.2 ml of sample was added to 1.8 ml of 30 mM of hydrogen peroxide substrate in a 2 ml curvette. The phosphate buffer was used as a blank. The absorbance for the test sample, blank and standard was read with a spectrophotometer against a blank at 240nm at 30seconds interval for 1minute. The enzyme activity was calculated using the molar extinction coefficient of  $40\text{M}^{-1}\text{cm}^{-1}$  expressed as unit/mg protein (Aksenes and Njaa, 1981).

#### **3.10.2 Determination of Superoxide dismutase (SOD)**

SOD activity was determined by its ability to inhibit the auto-oxidation of epinephrine adrenochrome. The reaction mixture (3 ml) which contained 2.95 ml 0.05 M sodium carbonate buffer of pH 10.2, 0.02 ml of homogenate and 0.03 ml of epinephrine was used to initiate the

reaction. The enzyme activity was calculated by measuring the change in absorbance at 480 nm for every 30 seconds for 150 seconds (Sun and Zigma, 1978).

### **3.10.3 Determination of Glutathione (GSH)**

The reduced GSH content of the tissue was estimated by its reducing activity on Ellman's reagent. Trichloroacetic acid at 10% was centrifuged with the homogenate. 1.0 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5, 5-dithiobisnitro benzoic acid in 100 ml of 1% sodium nitrate and 3 ml of phosphate buffer). The absorbance was read at 412 nm using the spectrophotometer and a plot of absorbance versus concentration of reduced GSH was obtained (Sedlak and Lindsay, 1968).

### **3.10.4 Determination of Malondialdehyde (MDA)**

The MDA concentration was determined as described by Buege and Aust (1978). 2 ml of 0.375% trichloroacetic acid, thiobarbituric acid and hydrochloric acid were added to 1.0 ml of the homogenate. This was mixed vigorously and heated for 15 minutes in a water bath at 80-90<sup>0</sup>C. The sample was cooled in cold water for 15 minutes. The clear pink supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm in a spectrophotometer and the concentration was calculated using the molar absorptivity of malondialdehyde.

## **3.11 FERTILITY STUDIES**

At the end of the experiments, animals were mated with sexually matured male S-D rats of proven fertility on the estrus day of the cycle. The fertility of the male rats were established with 100% pregnancies recorded prior to the coupling with the female rats. The female rats were smeared the

next morning and presence of sperm plug was used to ascertain mating. This day was taken as the first day of pregnancy and the animals were sacrificed on 20<sup>th</sup> day of gestation. The fetuses were removed by ventral laparotomy, counted, weighed and observed for congenital malformations.

### **3.12 MEASUREMENT OF WEIGHT**

#### **3.12.1 Total body weight gain**

The body weight was measured weekly by a weighing balance. The differences in the weights were estimated as the weight gain and expressed as g/100 g body weight of the corresponding weight.

#### **3.12.2 Ovarian weight**

The weight of the ovary was measured by a sensitive electronic balance. The weight values were expressed as g/100 g body weight of the animals.

### **3.13 STEREOLOGICAL MEASUREMENTS**

Stereology is often defined as the science of estimating three dimensional information from two dimensional sections. Stereological evaluation of ovarian histology has been used on its own to measure follicular development and thus provide information on the physiological status of the ovary. Accurate stereological estimation of ovarian follicles at various stages of development are important indicator of the process of folliculogenesis in relation to the endocrine signals and mechanisms that control the growth and maturation of the oocytes (West, 1990). A Graafian follicle is a three dimensional structure with a central antrum surrounded by a variety of different cell types such as; the theca cells and granulosa cells and the antrum . The stereological evaluation of follicular component was carried out by counting grid method. A counting grid with set of regularly spaced points

was placed on a bounded follicular section. All points hitting profiles were counted. The volume density ( $V_D$ ) was calculated as proportion of volume by simple percentile fraction;

Volume density ( $V_D$ ) = Profile counted / Total specimen profile X100 (Gundersen *et al.*, 1988).

### 3.14 STATISTICAL ANALYSIS

Data was analysed using SPSS 16.0 computer software package (SPSS Inc; Chicago U.S.A). The results were expressed as mean  $\pm$  standard error. Statistical comparisons were made using analysis of variance (ANOVA) with Scheffe's *post hoc* test for within group and between groups comparison. The level of significance was considered at  $p < 0.05$



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## CHAPTER FOUR

### RESULTS

#### 4.1 QUANTITATIVE DETERMINATION OF PHYTOCHEMICAL CONSTITUENTS

The phytochemical analysis of GCW revealed the following constituents

Table1: Qualitative and Quantitative Analysis of GCW (mg/100 g GCW)

Cyanogenic glycoside	$10.49 \pm 0.31$
Alkaloid	$32.09 \pm 0.38$
Tannin	$18.17 \pm 0.64$
Flavonoid	$21.03 \pm 0.32$
Phenol	$32.85 \pm 1.19$
Sugar	$71.07 \pm 0.37$
Sterol	$22.58 \pm 0.10$

#### 4.2 THE LETHAL DOSE (LD<sub>50</sub>) OF GCW

The LD<sub>50</sub> of GCW was greater than 5000 mg/ kg body weight.



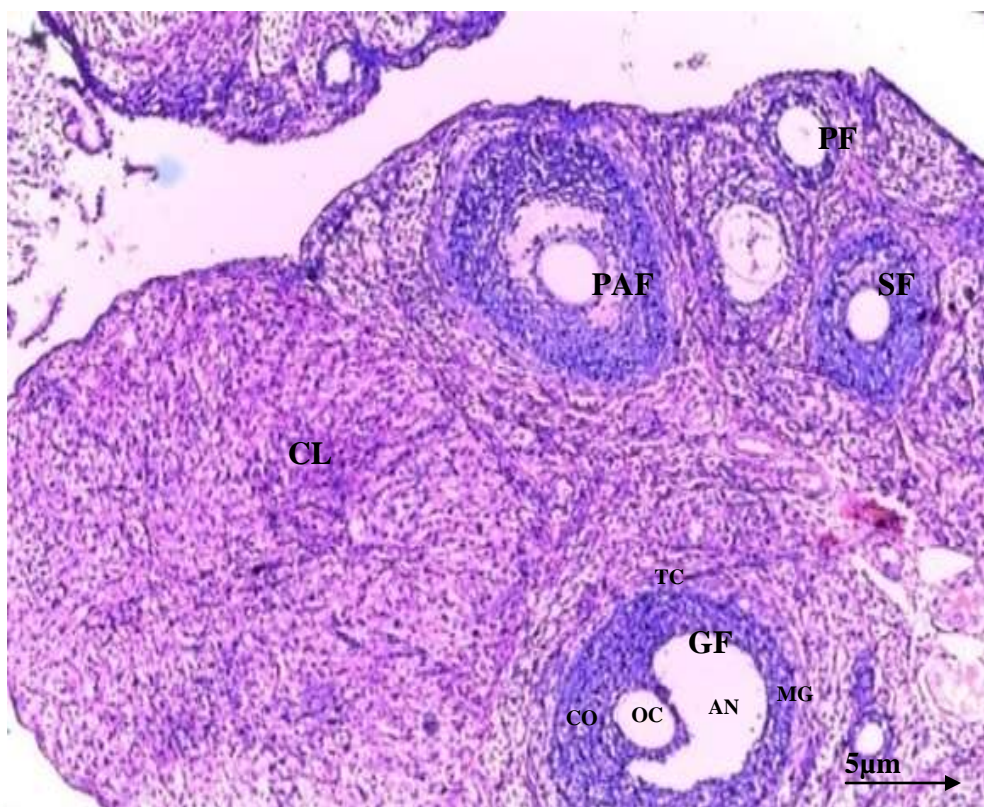
#### **4.3. HISTOLOGICAL STUDIES OF THE OVARIES OF FEMALE SPRAGUE-DAWLEY RATS**

The control section showed normal follicular growth. According to Pedersen and Peter's follicular classification in rodent, follicles were seen at different stages of development ranging from primary, secondary to the tertiary or mature stage (Pedersen and Peters 1968). The histological section of the control group showed small primary or primordial follicles with a single layer of granulosa cells, the medium or secondary follicle with multiple layers of granulosa cells, the preantral follicle seen with free fluid filled spaces within the granulose cells and large tertiary or Graafian mature follicles with a mature oocyte. At this level, the mature follicle presented granulosa cell differentiations into corona radiata, cumulus oophorous and membrana granulosa and a single large antrum. There was visibility of the theca cells alignment around the follicle. There was also presence of corpus luteum which reflects recent ovulation (Plate 1). The induced group showed poor follicular growth with only visible primary follicles. There were absence of secondary, preantral and mature follicles which implies that the primary follicles did not proceed in follicular development. There was absence of corpus luteum which reflects that ovulation has not occurred recently (Plate 2). The histological sections of the group withdrawn for 8 days were similar with the induced with follicles seen at the primary and secondary stages (Plate 3). The groups withdrawn for 16 and 28 days showed few follicles in the mature stage when compared with the control (Plate 4-5).

The histological sections of the post-treated group for 8 days were similar to the expression in the induced group. The section view showed poor follicular development of predominantly primary and secondary follicles. There was absence of mature follicles which implies that the primary follicles did not proceed in follicular development. There was no visible corpus luteum which reflects that ovulation has not occurred recently (Plate 6-7). The groups post-treated for 16 days

showed few follicles at the mature stage (Plate 8-9) while the expression in the sections of the groups post-treated for 28days were comparable with the control group with more number of follicle at the mature stage when compared with the withdrawal group of the same experimental duration (Plate 10-11) .

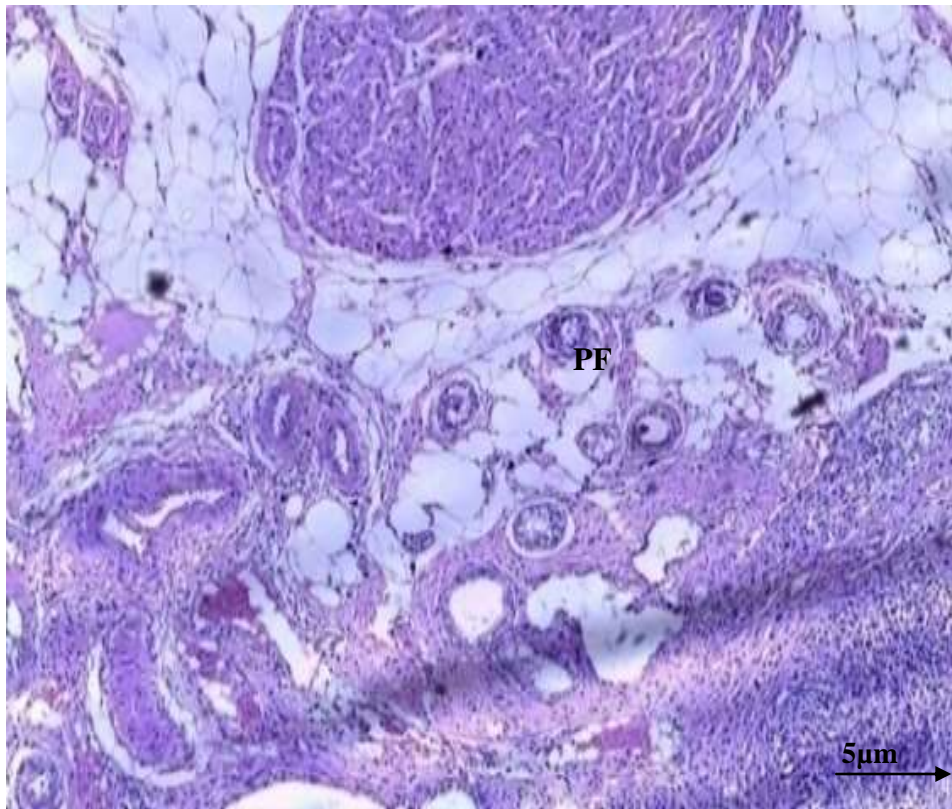
The histological findings of the co-administered group revealed poor follicular development with follicles seen at the primary and secondary follicular stages (Plate 12-13). The histological findings of the ovaries of the pre-treated groups showed poor follicular growth with follicles seen at the primary and secondary follicular stages. There was absence of mature follicles which implies that the primary follicles did not proceed in follicular development. (Plate 14-19). The histological sections of ovary of all green coconut water treated groups were similar to the expression shown in the control group. The section showed follicles at different stages of development with the number of mature follicle comparable with the control group (Plate 20-25).



**Plate 1:** Photomicrograph of the ovary of the control Sprague-Dawley rat; shows follicles at different stages of development which includes the primary, secondary and mature follicles at magnification of X100.

Abbreviations: AN (Antrum); CL (corpus luteum) ; CO(Cumulus oophorous ); CR( Corona radiata); CT (corpus luteum); GF(Graafian Follicle); MG (Membrane granulosa ); OC(Oocyte); PAF (Preantrum Follicle); PF (Primary Follicle); SC (Secondary Follicle); TC (Theca cells).

Stains: Haematoxylin and Eosin.

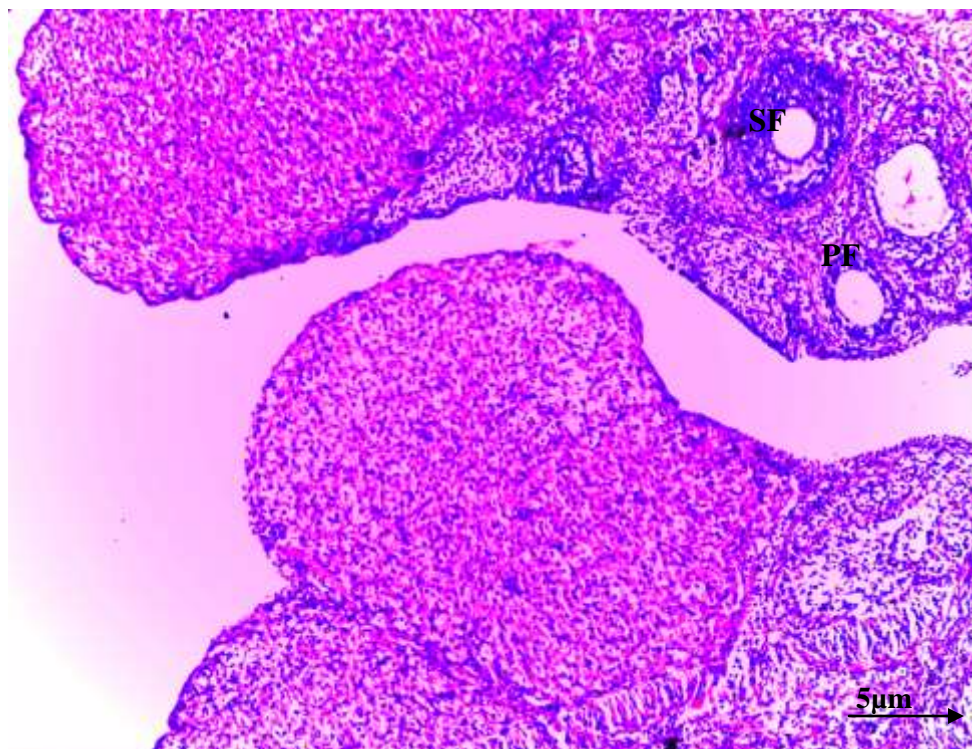


**Plate 2:** Photomicrograph of the ovary of Sprague-Dawley treated with 0.2 mg/100 g. b. w. 28 days metoclopramide hydrochloride; shows poor follicular growth with follicles seen only at the primary follicular stage at magnification of X100.

Abbreviations: PF (Primary Follicle)

Stains: Haematoxylin and Eosin.

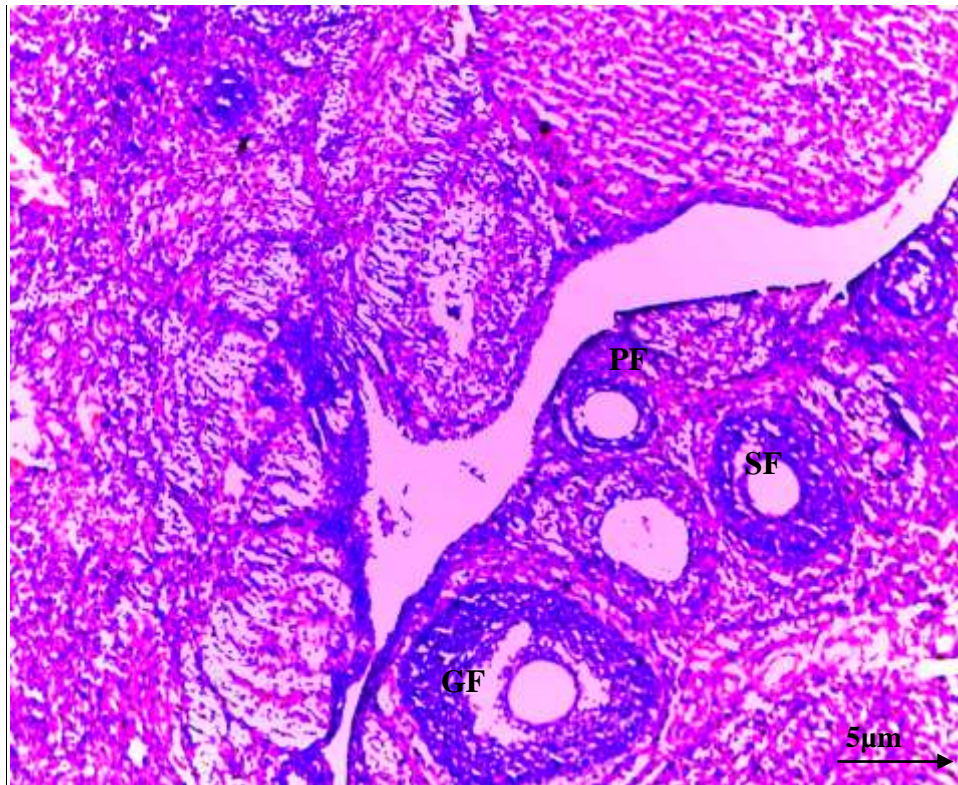




**Plate 3:** Photomicrograph of the ovary of Sprague-Dawley rats treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days and withdrawn for 8 days; shows follicles at primary and secondary follicular stages at magnification of X100.

Abbreviation: PF (Primary Follicle); SF (Secondary Follicle).

Stains: Haematoxylin and Eosin.

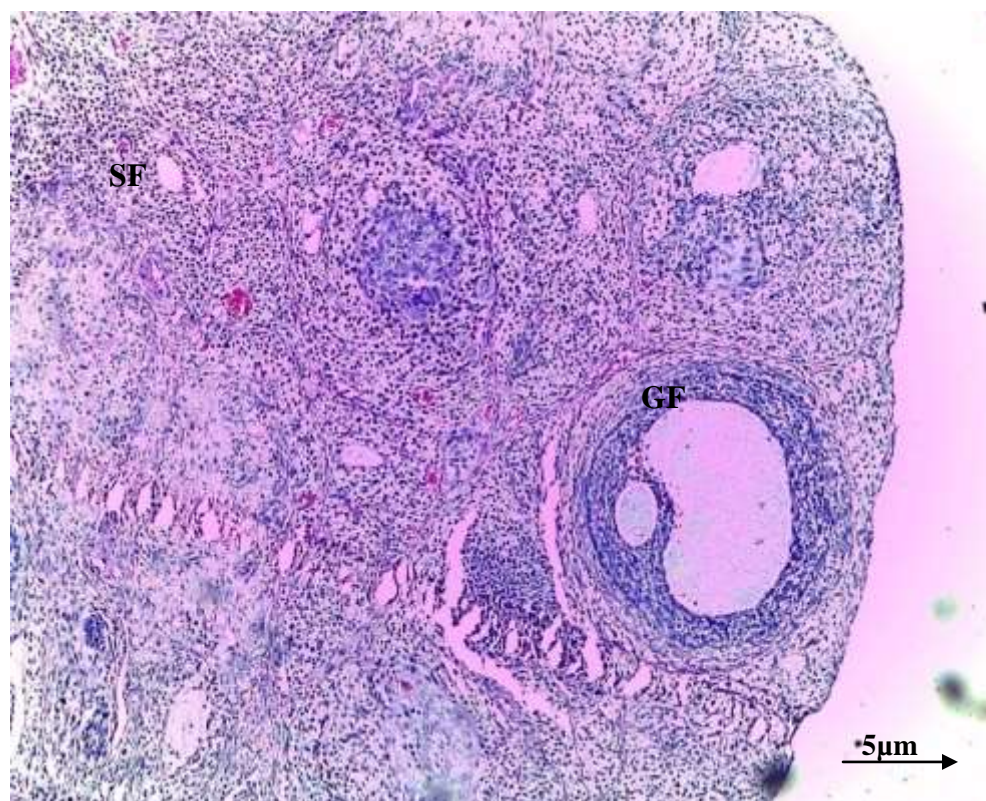


**Plate 4:** Photomicrograph of the ovary of Sprague-Dawley rats treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days and withdrawn for 16 days; shows few follicles at the mature stage at magnification of X100

Abbreviations: PF (Primary Follicle; SF (Secondary Follicle); GF (Graafian Follicle)

Stains: Haematoxylin and Eosin.



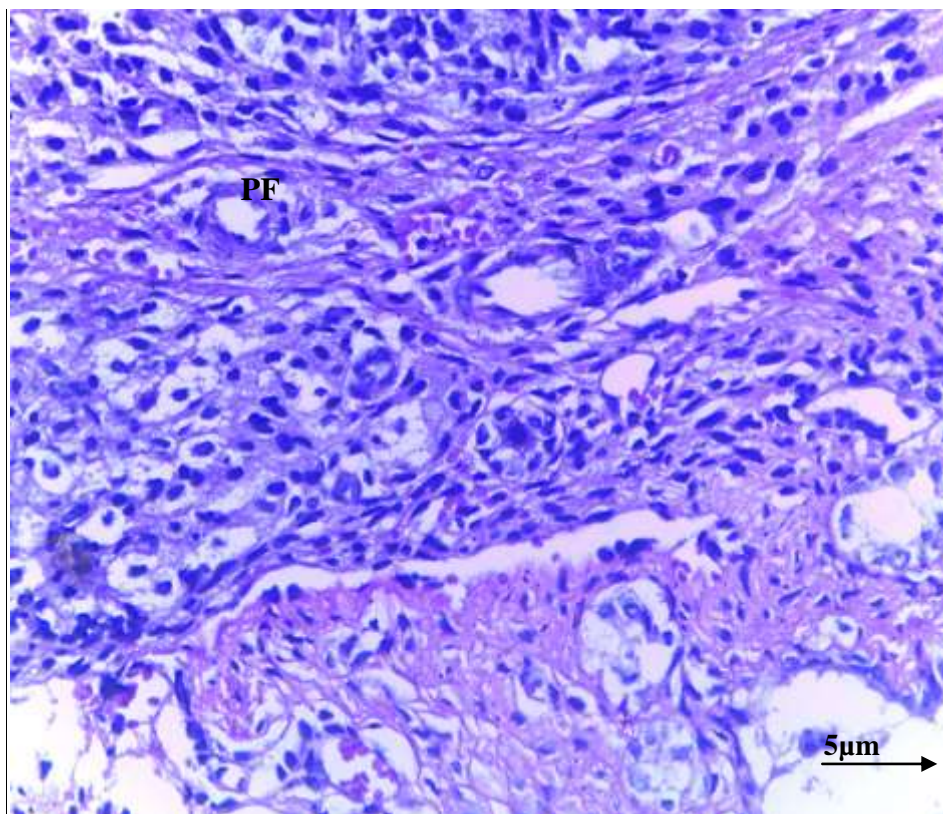


**Plate 5:** Photomicrograph of the ovary of Sprague-Dawley rats treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days and withdrawn for 28days; shows a follicle at the mature stage at magnification of X100.

Abbreviations: GF (Graafian Follicle); SF (Secondary Follicle).

Stains: Haematoxylin and Eosin.

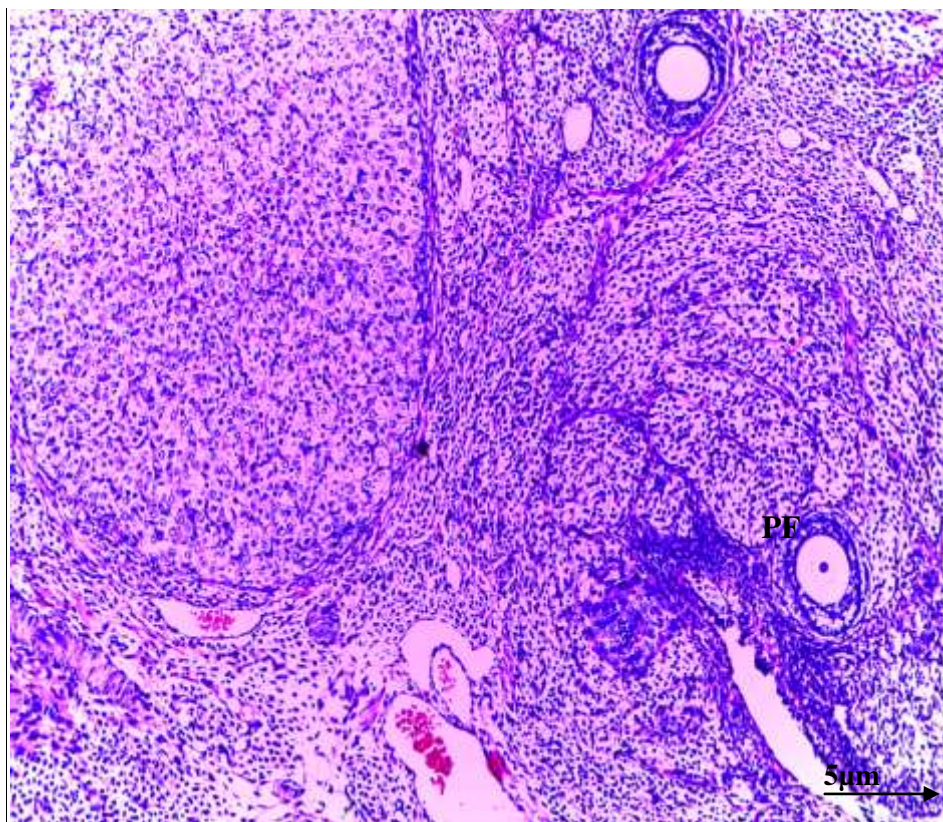




**Plate 6:** Photomicrograph of the ovary of Sprague-Dawley rats, treated with 0.2 mg/100 g. b. w. of metoclopramide hydrochloride for 28 days followed by 5 ml/100 g. b. w. of green coconut water for 8 days; shows poor follicular development with follicles seen at the primary and secondary stages at magnification of X100.

Abbreviations: PF (Primary Follicle).

Stains: Haematoxylin and Eosin.

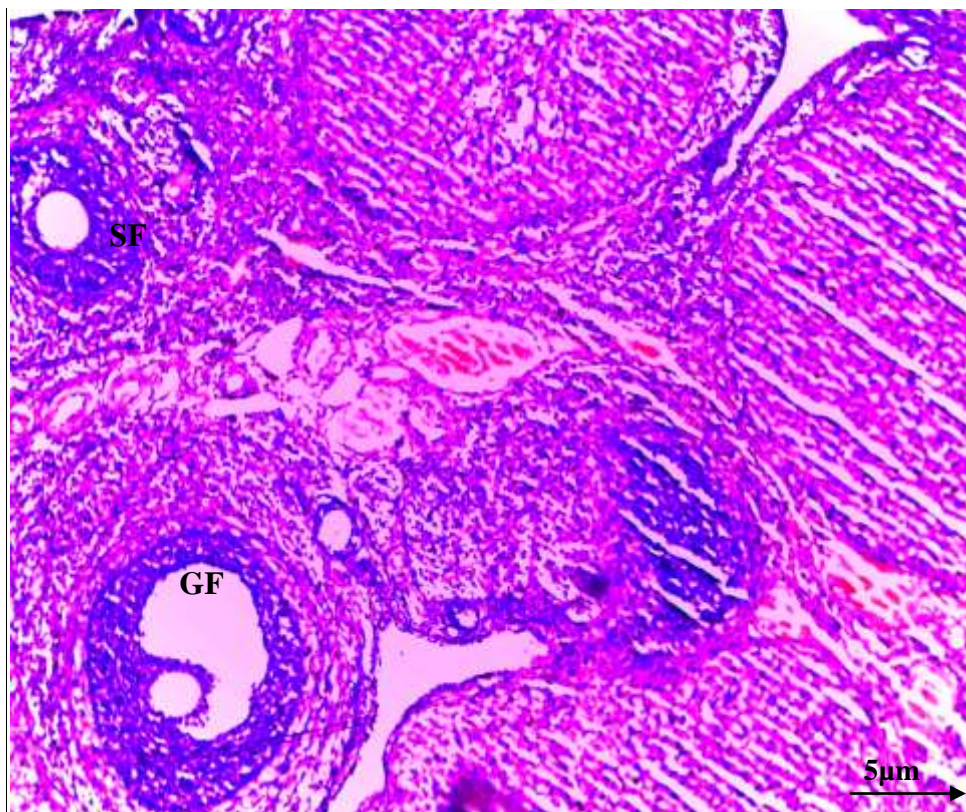


**Plate 7:** Photomicrograph of the ovary of Sprague-Dawley rats, treated with 0.2 mg/100 g. b. w. of metoclopramide hydrochloride for 28 days followed by 10 ml/100 g. b. w. of green coconut water for 8 days; shows poor follicular development with follicles seen at the primary stage of follicular development at magnification of X100.

Abbreviations: PF (Primary Follicle)

Stains: Haematoxylin and Eosin.

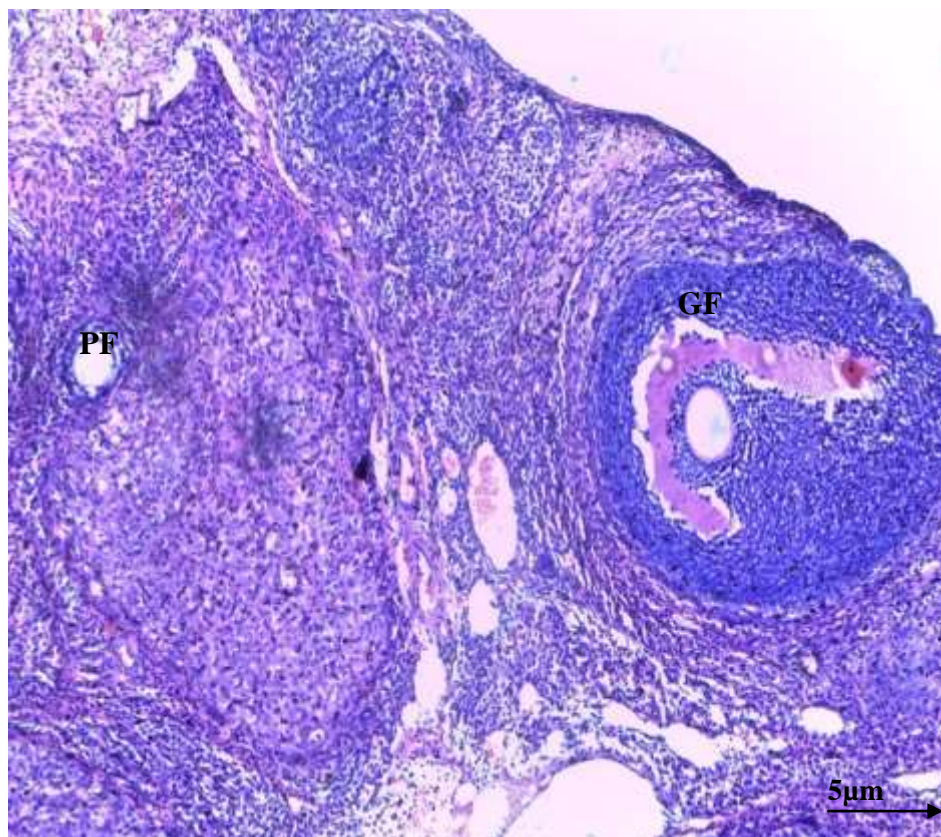




**Plate 8:** Photomicrograph of the ovary of Sprague-Dawley rats, treated with 0.2 mg/100 g. b. w. of metoclopramide hydrochloride for 28 days followed by 5 ml/100 g. b. w. of green coconut water for 16 days; shows a follicle at the mature stage at magnification of X100.

Abbreviations: GF (Graafian Follicle); SF (Secondary Follicle).

Stains: Haematoxylin and Eosin.

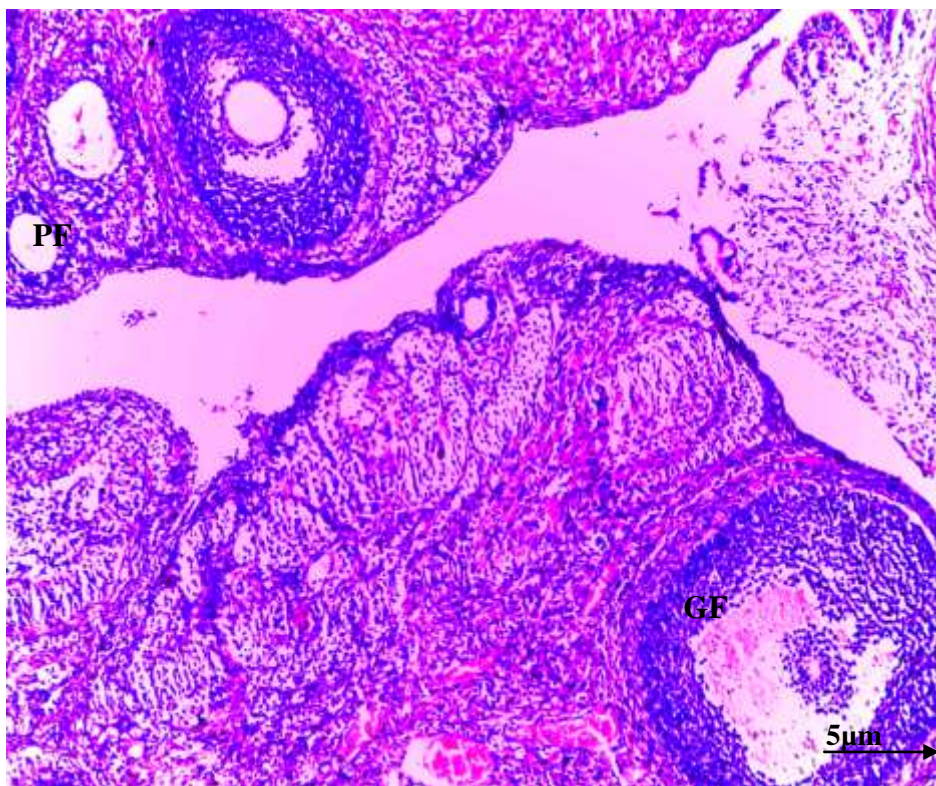


**Plate 9:** Photomicrograph of the ovary of Sprague-Dawley rats, treated with 0.2 mg/100 g. b. w. of metoclopramide hydrochloride for 28 days followed by 10ml/100 g. b. w. of green coconut water for 16 days; shows a follicle at the mature stage at magnification of X100.

Abbreviations: GF (Graafian Follicle); PF (Primary Follicle)

Stains: Haematoxylin and Eosin.

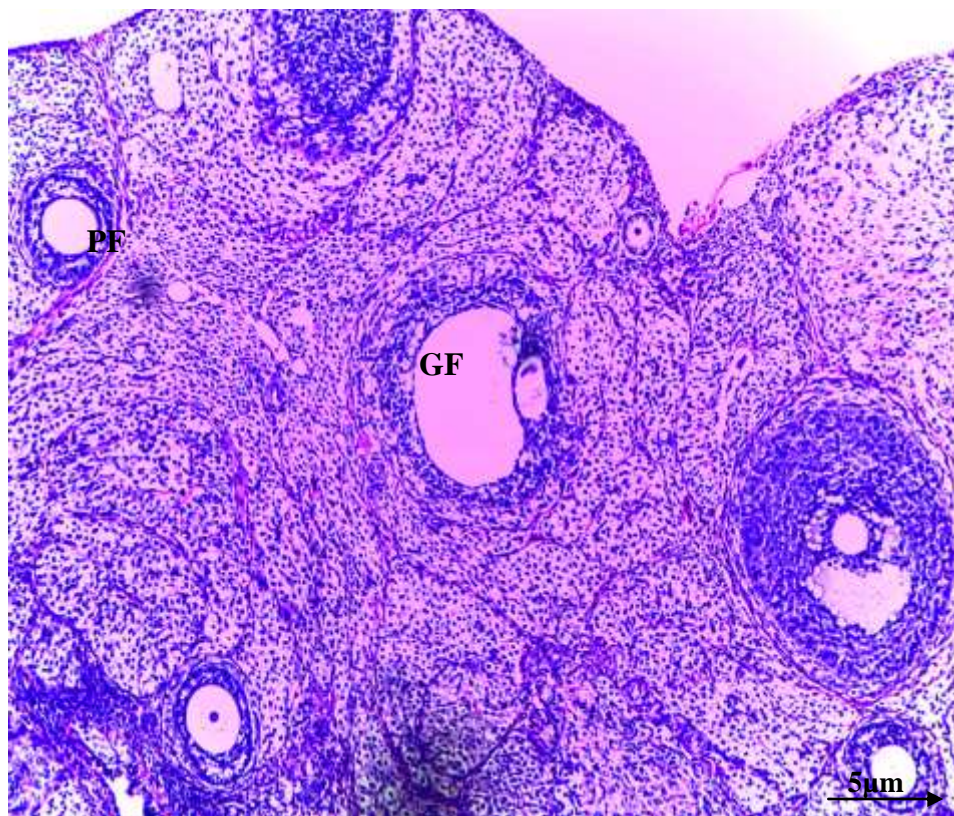




**Plate 10:** Photomicrograph of the ovary of Sprague-Dawley rats, treated with 0.2 mg/100 g. b. w. of metoclopramide hydrochloride for 28 days followed by 5 ml/100 g. b. w. of green coconut water for 28 days; shows more number of follicle at the mature stage at magnification of X100.

Abbreviations: GF (Graafian Follicle); PF (Primary Follicle)

Stains: Haematoxylin and Eosin.

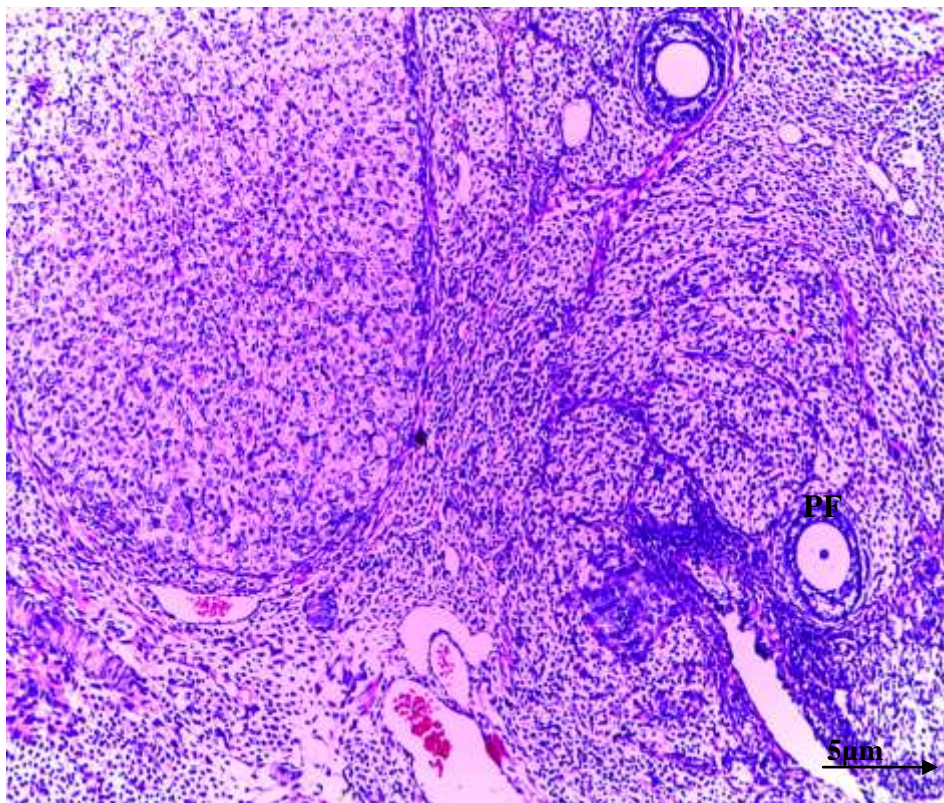


**Plate 11:** Photomicrograph of the ovary of Sprague-Dawley rats, treated with 0.2 mg/100 g. b. w. of metoclopramide hydrochloride for 28 days followed by 10 ml/100 g. b. w. of green coconut water for 28 days; shows more number of follicles at the mature stage at magnification of X100.

Abbreviations: GF (Graafian Follicle); PF (Primary Follicle)

Stains: Haematoxylin and Eosin.



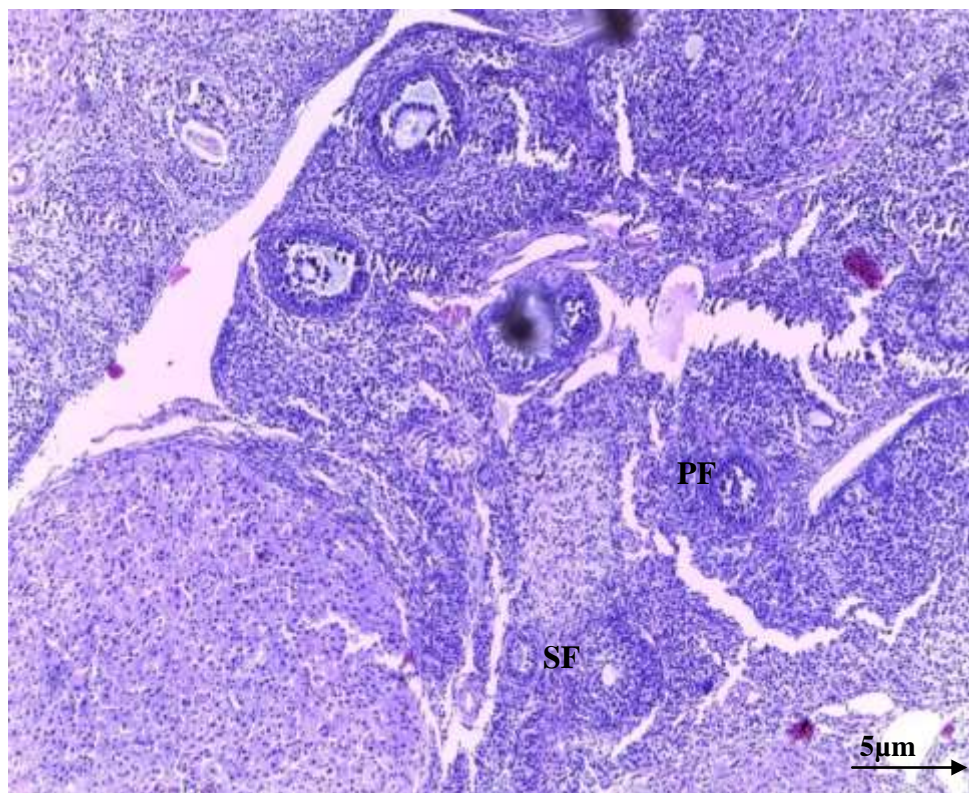


**Plate 12:** Photomicrograph of the ovary of Sprague-Dawley rats treated concurrently with 0.2 mg/100 g. b. w. metoclopramide hydrochloride and 5 ml/100 g. b. w. of green coconut water for 28 days; shows poor follicular development with follicles seen at the primary stage of follicular development at magnification of X100.

Abbreviation: PF (Primary Follicle).

Stains: Haematoxylin and Eosin.

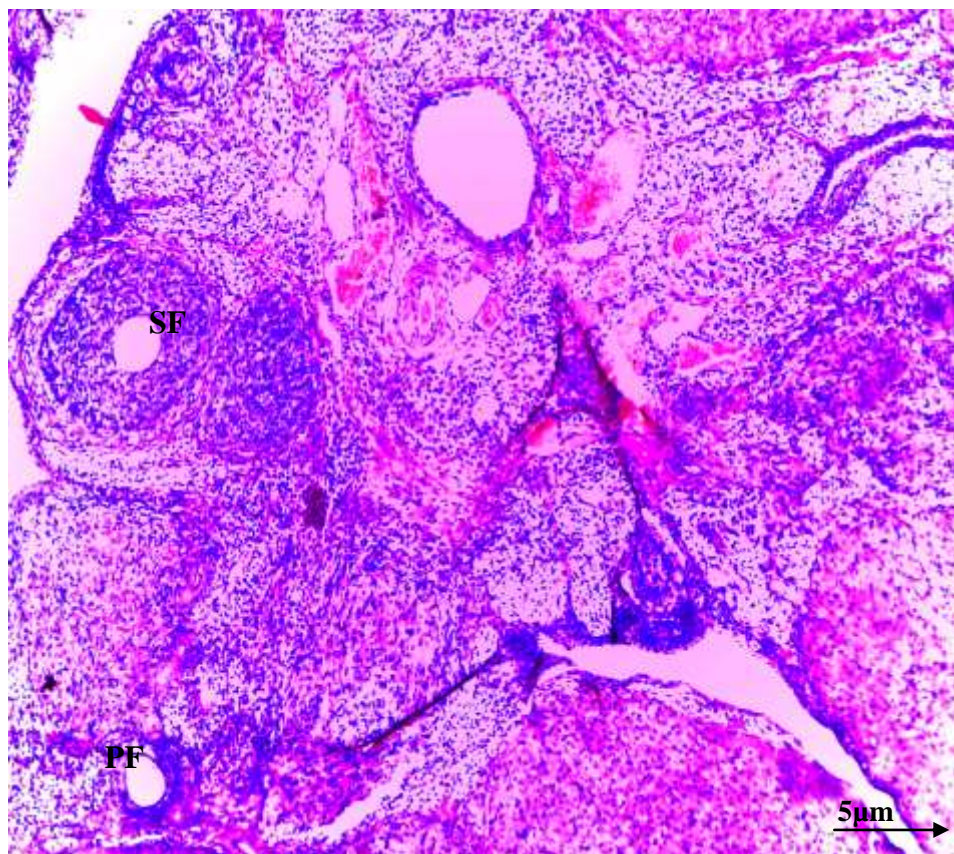




**Plate 13:** Photomicrograph of the ovary of Sprague-Dawley rats treated concurrently with 0.2 mg/100 g. b. w. metoclopramide hydrochloride and 10 ml/100 g. b. w. of green coconut water for 28 days; shows poor follicular development with follicles seen at the primary and secondary follicular stages at magnification of X100.

Abbreviation: PF (Primary Follicle); SF (Secondary follicle).

Stains: Haematoxylin and Eosin.

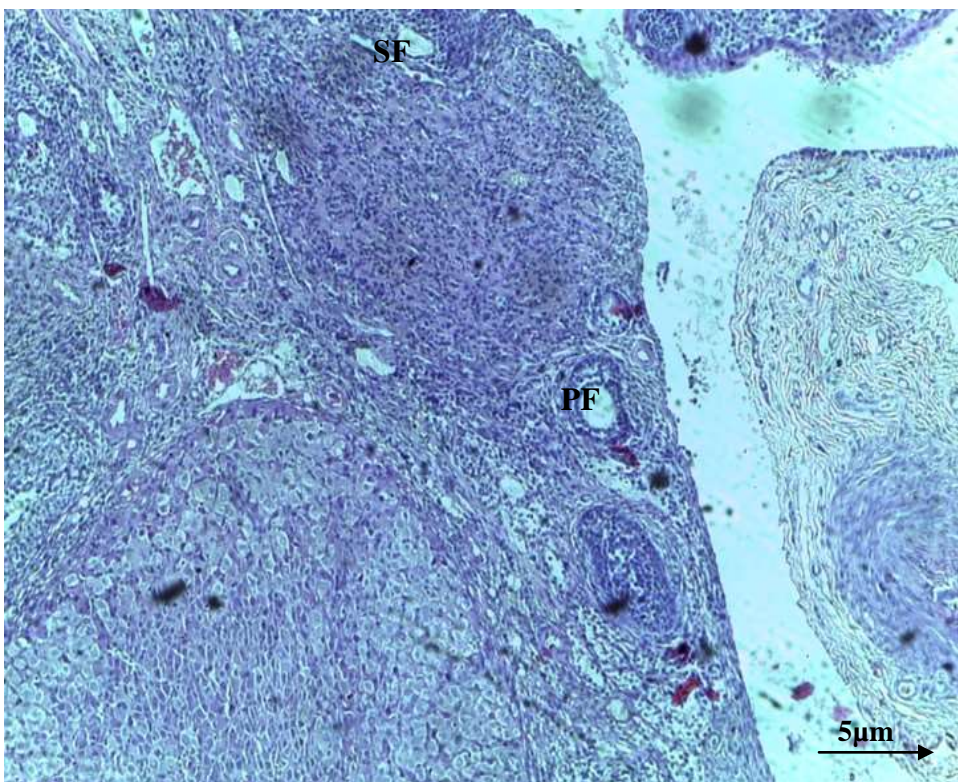


**Plate 14:** Photomicrograph of the ovary of Sprague-Dawley rat, pretreated with 5 ml/100 g. b. w. of green coconut water for 8 days followed by 0.2 mg/100 g. b. w. of metoclopramide hydrochloride for 28 days; shows poor follicular development with follicles seen at the primary and secondary follicular stages at magnification of X100.

Abbreviations: PF (Primary Follicle); SF (Secondary Follicle)

Stains: Haematoxylin and Eosin.

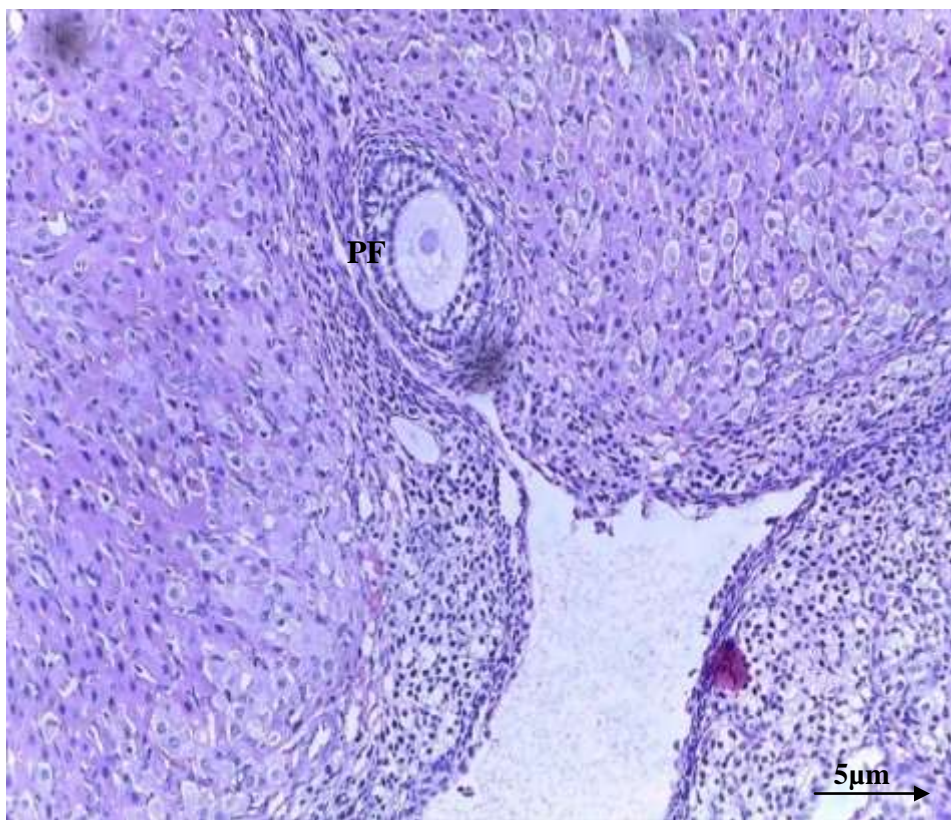




**Plate 15:** Photomicrograph of the ovary of Sprague-Dawley rat, pretreated with 10 ml/100 g. b. w. of green coconut water for 8 days followed by 0.2 mg/100 g. b. w. of metoclopramide hydrochloride for 28 days; shows poor follicular development with follicles seen at the primary and secondary follicular stages at magnification of X100.

Abbreviations: PF (Primary Follicle); SF (Secondary Follicle)

Stains: Haematoxylin and Eosin.

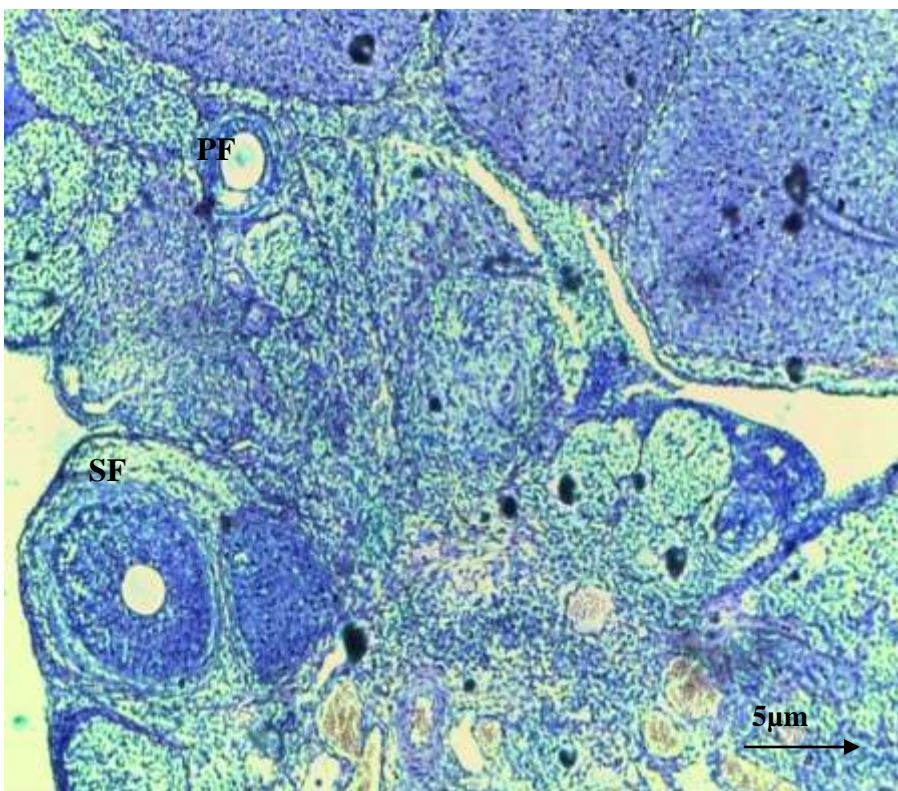


**Plate 16:** Photomicrograph of the ovary of the Sprague-Dawley rat, pretreated with 5 ml/100 g. b. w. of green coconut water for 16 days followed by 0.2 mg/100 g. b. w. of metoclopramide hydrochloride for 28 days; section shows poor follicular development with follicles seen at the primary stage of follicular development at magnification of X100.

Abbreviations: PF (Primary Follicle)

Stains: Haematoxylin and Eosin.

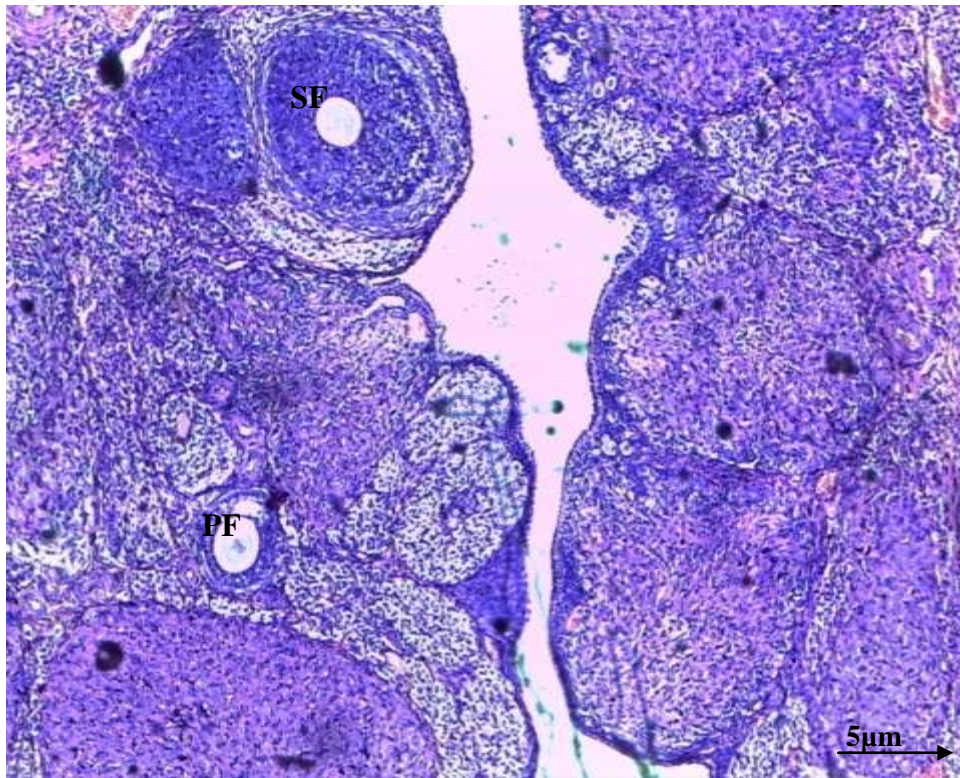




**Plate 17:** Photomicrograph of the ovary of the Sprague-Dawley rat, pretreated with 10 ml/100 g. b. w.. of green coconut water for 16 days followed by 0.2 mg/100 g. b. w. of metoclopramide hydrochloride for 28 days; shows poor follicular development with follicles seen at the primary and secondary follicular stages at magnification of X100.

Abbreviations: PF (Primary Follicle); SF (Secondary Follicle)

Stains: Haematoxylin and Eosin.

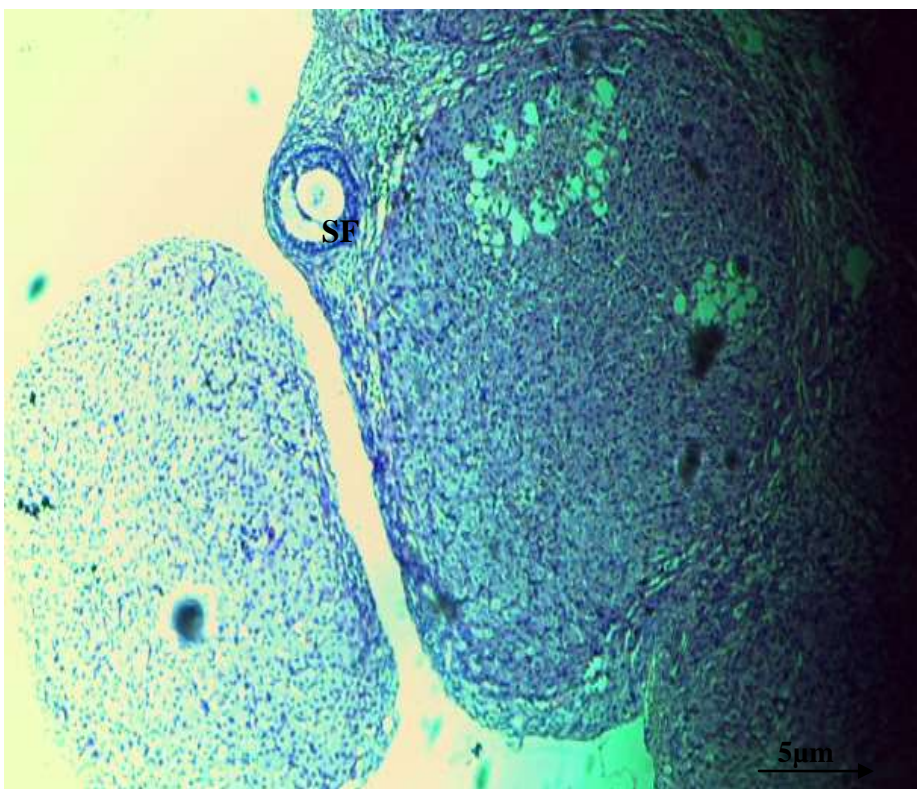


**Plate 18:** Photomicrograph of the ovary of Sprague-Dawley rat, pretreated with 5 ml/100 g. b. w. of green coconut water for 28 days followed by 0.2 mg/100 g. b. w. of metoclopramide hydrochloride for 28 days; shows poor follicular development with follicles seen at the primary and secondary follicular stages at magnification of X100.

Abbreviations: PF (Primary Follicle); SF (Secondary Follicle)

Stains: Haematoxylin and Eosin.



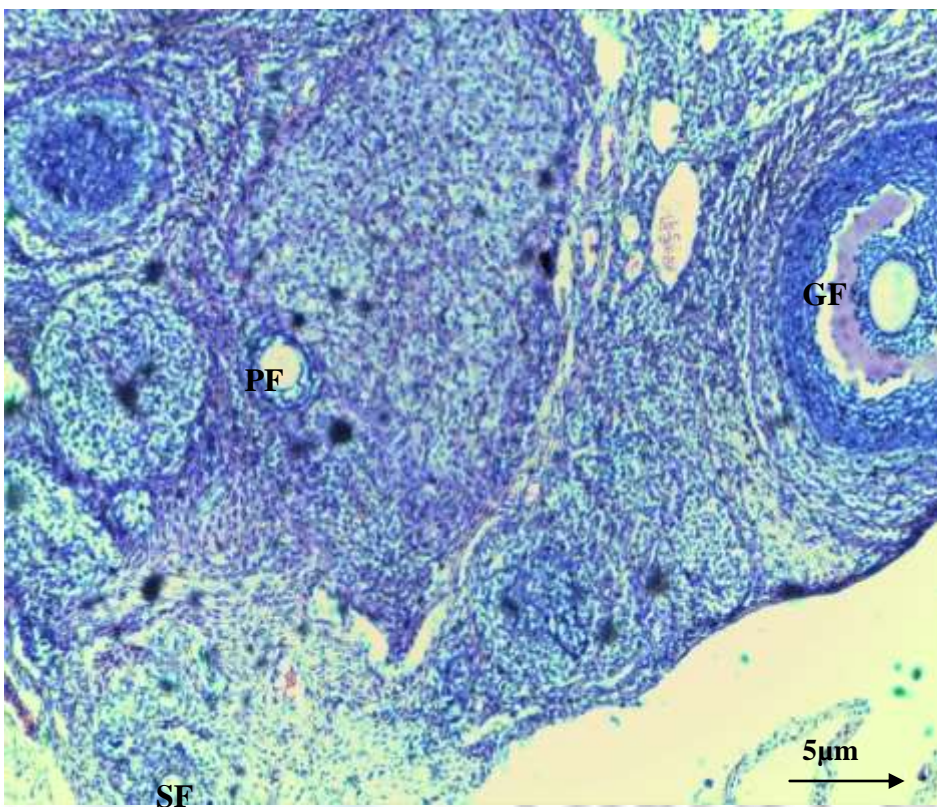


**Plate 19:** Photomicrograph of the ovary of Sprague-Dawley rat, pretreated with 10 ml/100 g. b. w. of green coconut water for 28 days followed by 0.2 mg/100 g. b. w. of metoclopramide hydrochloride for 28 days; shows poor follicular development with follicles seen at the secondary follicular stage at magnification of X100.

Abbreviations: SF (Secondary Follicle)

Stains: Haematoxylin and Eosin.

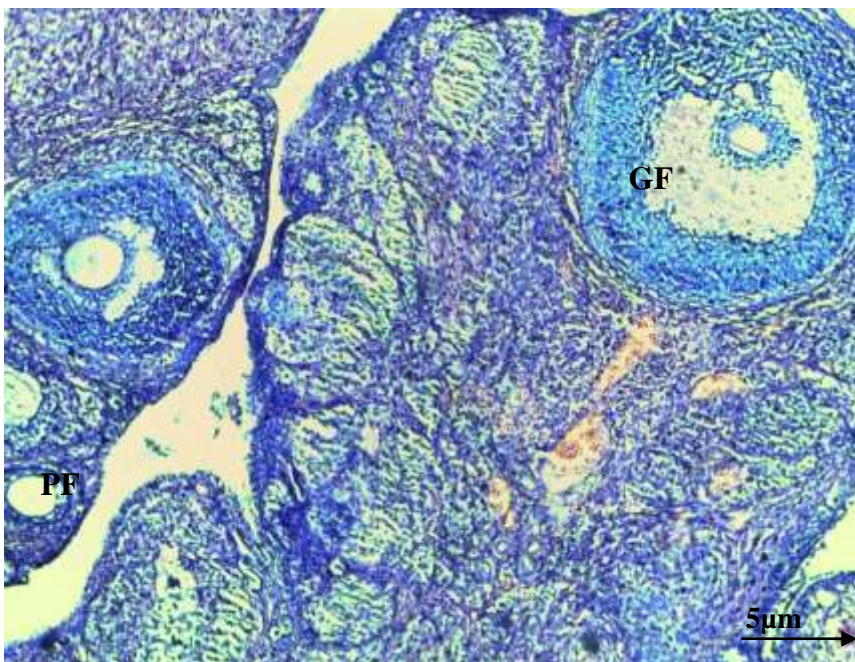




**Plate 20:** Photomicrograph of the ovary of Sprague-Dawley rats treated with 5 ml/100 g. b. w. of green coconut water for 8 days; shows follicles at different stages of development at magnification of X100.

Abbreviations: PF (Primary Follicle); SF (Secondary Follicle); GF (Graafian Follicle).

Stains: Haematoxylin and Eosin.

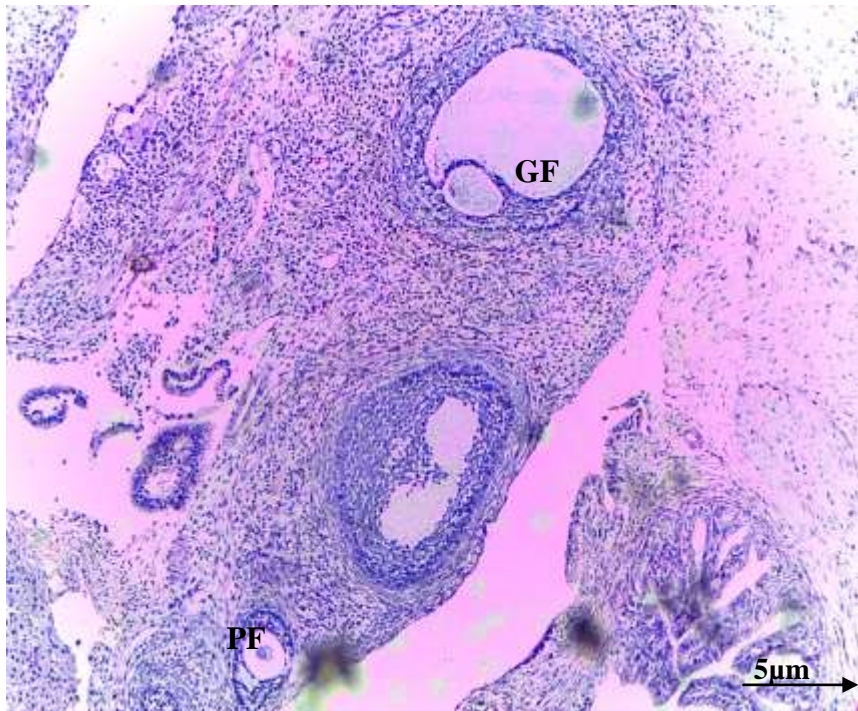


**Plate 21:** Photomicrograph of the ovary of Sprague-Dawley rats treated with 10 ml/100 g. b. w. of green coconut water for 8 days; shows follicles at different stages of development with more number of follicles at the mature stage at magnification of X100.

Abbreviations: PF (Primary Follicle); GF (Graafian Follicle).

Stains: Haematoxylin and Eosin.

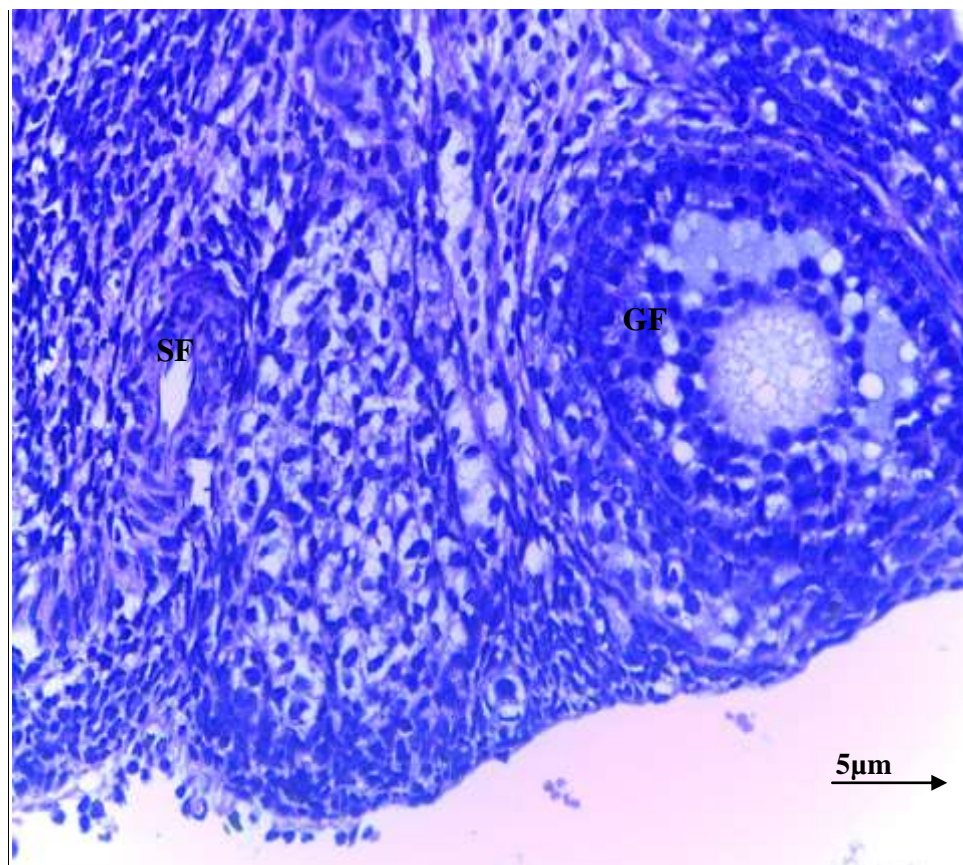




**Plate 22:** Photomicrograph of the ovary of Sprague-Dawley rats treated with 5 ml/100 g. b. w. of green coconut water for 16 days; shows follicles at different stages of development with more number of follicles at the mature stage at magnification of X100.

Abbreviations: PF (Primary Follicle); GF (Graafian Follicle).

Stains: Haematoxylin and Eosin.

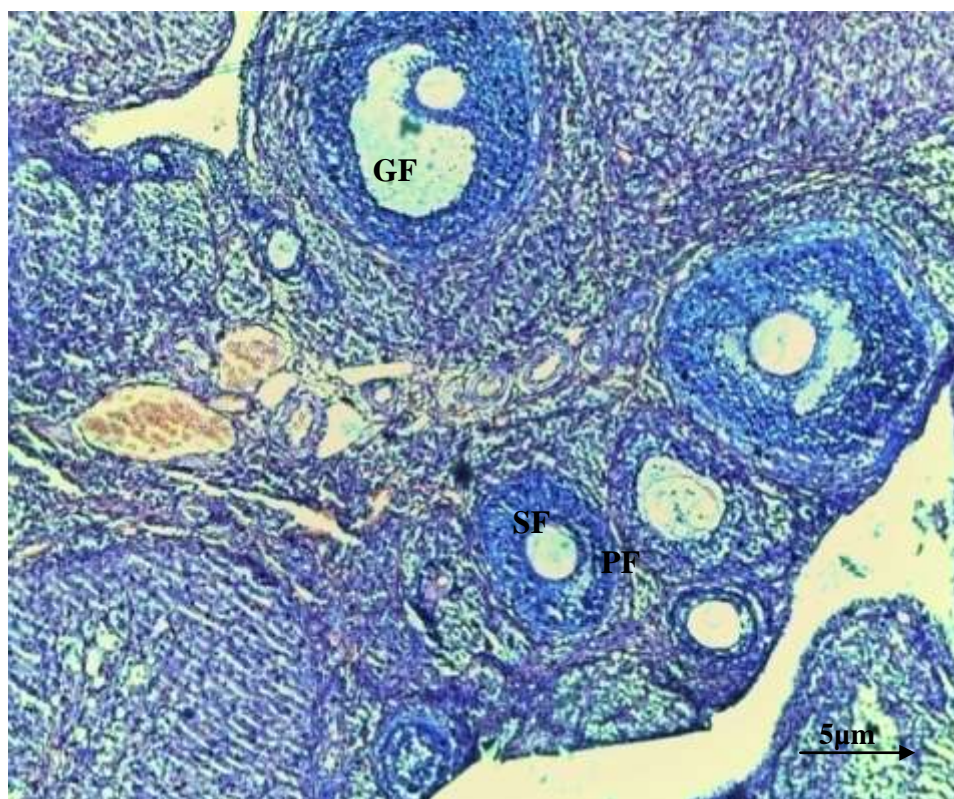


**Plate 23:** Photomicrograph of the ovary of Sprague-Dawley rats treated with 10 ml/100 g. b. w. of green coconut water for 16 days; shows follicles at different stages of development with more number of follicles at the mature stage at magnification of X100.

Abbreviations: SF (Secondary Follicle); GF (Graafian Follicle).

Stains: Haematoxylin and Eosin.

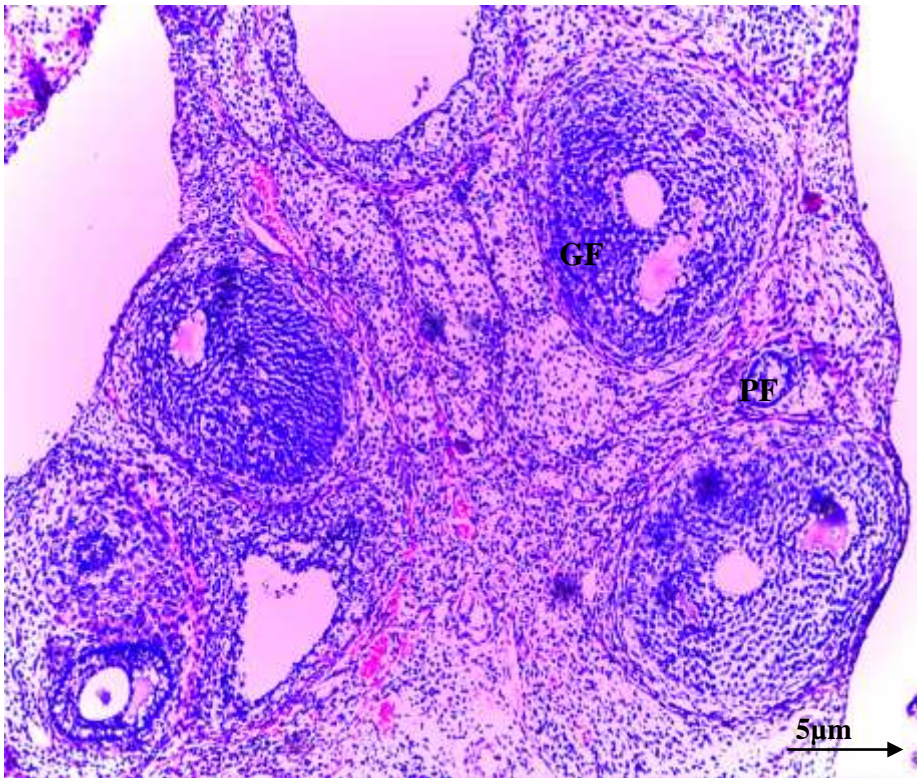




**Plate 24:** Photomicrograph of the ovary of Sprague-Dawley rats treated with 5 ml/100 g. b. w. of green coconut water for 28 days; shows follicles at different stages of development with more number of follicles at the matured stage at magnification of X100.

Abbreviations: PF (Primary Follicle); SF (Secondary Follicle); GF (Graafian Follicle).

Stains: Haematoxylin and Eosin.



**Plate 25:** Photomicrograph of the ovary of Sprague-Dawley rats treated with 10 ml/100 g. b. w. of green coconut water for 28 days; shows follicles at different stages of development with more number of follicles at the matured stage at magnification of X100.

Abbreviations: PF (Primary Follicle); GF (Graafian Follicle).

Stains: Haematoxylin and Eosin.

#### 4.4 STEREOLOGICAL EVALUATION OF THE VOLUME DENSITIES OF FOLLICULAR COMPONENTS

Stereological evaluation was carried out in the groups that revealed mature follicles in their histological sections. The control group demonstrated normal values in follicular components with the highest value in granulosa cell volume density ( $43.75 \pm 5.79$ ), followed by the antrum ( $18.75 \pm 6.05$ ) then the theca cells ( $15.10 \pm 3.61$ ). The withdrawal groups demonstrated significant differences in the volume densities of follicular components. The 16 and 28 days withdrawal groups showed mature follicles that demonstrated significant decrease in the volume densities of the granulosa cells ( $27.20 \pm 6.32$  and  $24.39 \pm 5.77$  versus  $43.75 \pm 5.79$ ;  $p < 0.05$ ) and increase in the volume density of the antrum ( $38.57 \pm 4.00$  and  $36.58 \pm 4.00$  versus  $18.75 \pm 6.05$ ;  $p < 0.05$ ) when compared with the values obtained from the control. The values of the theca cell volume densities in this group were comparable with the control. These expressions demonstrated the highest value of follicular components in antrum volume density, followed by the granulosa cell then the theca cells. The volume densities of antrum, theca cells and granulosa cells in the groups post-treated with GCW for 16 and 28 days were comparable with the values obtained from the control. The groups treated with GCW only for short, medium and long term also showed comparable values in the volume densities of their follicular components when compared with the control values.



**TABLE 2:** Stereological Evaluation of Volume Densities of the Antrum, Granulosa Cells and Theca Cells of Mature Ovarian Follicle of Female Sprague-Dawley rats

GROUP	SUB-GROUP DETAIL	ATR <sub>VD</sub> (%)	GC <sub>VD</sub> (%)	TC <sub>VD</sub> (%)
Control	DSTL	18.75 ± 6.05	43.75 ± 5.79	15.10 ± 3.61
Withdrawal	MCH <sub>28 days</sub> -WD <sub>16 days</sub>	38.57 ± 4.00*	27.20 ± 6.32*	13.66 ± 2.54
	MCH <sub>28 days</sub> -WD <sub>28 days</sub>	36.58 ± 4.00*	24.39 ± 5.77*	14.71 ± 2.49
Post-treated	MCH <sub>28 days</sub> - GCW <sub>16 days M/d</sub>	17.70 ± 3.56	42.75 ± 5.23	15.10 ± 3.60
	MCH <sub>28 days</sub> - GCW <sub>16 days H/d</sub>	18.55 ± 4.18	42.17 ± 5.70	16.01 ± 4.21
	MCH <sub>28 days</sub> - GCW <sub>28 days M/d</sub>	19.25 ± 7.46	43.22 ± 5.10	15.22 ± 3.40
GCW-treated	MCH <sub>28 days</sub> - GCW <sub>28 days H/d</sub>	18.20 ± 7.96	49.09 ± 5.10	15.21 ± 3.61
	GCW <sub>8 days H/d</sub>	18.19 ± 4.65	47.00 ± 4.21	16.22 ± 3.44
	GCW <sub>8 days H/d</sub>	18.19 ± 4.65	47.00 ± 4.21	16.13 ± 3.00
	GCW <sub>16 days M/d</sub>	19.09 ± 5.15	44.55 ± 7.14	16.22 ± 3.44
	GCW <sub>16 days H/d</sub>	18.19 ± 4.85	40.44 ± 6.21	17.23 ± 3.08
	GCW <sub>28 days M/d</sub>	18.07 ± 7.70	46.55 ± 5.10	16.13 ± 3.33
	GCW <sub>28 days H/d</sub>	18.00 ± 5.07	47.55 ± 5.19	15.13 ± 3.31

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5. \*Significant differences;  $p < 0.05$

**Key for table 2:** DSTL: Distilled water; MCH: 0.2 mg/100 g. b.w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>M/d</sub>: 5 ml/100 g. b.w. of green coconut water; GCW<sub>H/d</sub>: 10 ml/100 g. b.w. of green coconut water; -: treated followed by; VD: Volume density; ATR: Antrum; GC: Granulosa cell; TC: Theca cell.

## **4.5 IMMUNOHISTOCHEMISTRY OF OESTROGEN RECEPTOR ACTIVITIES**

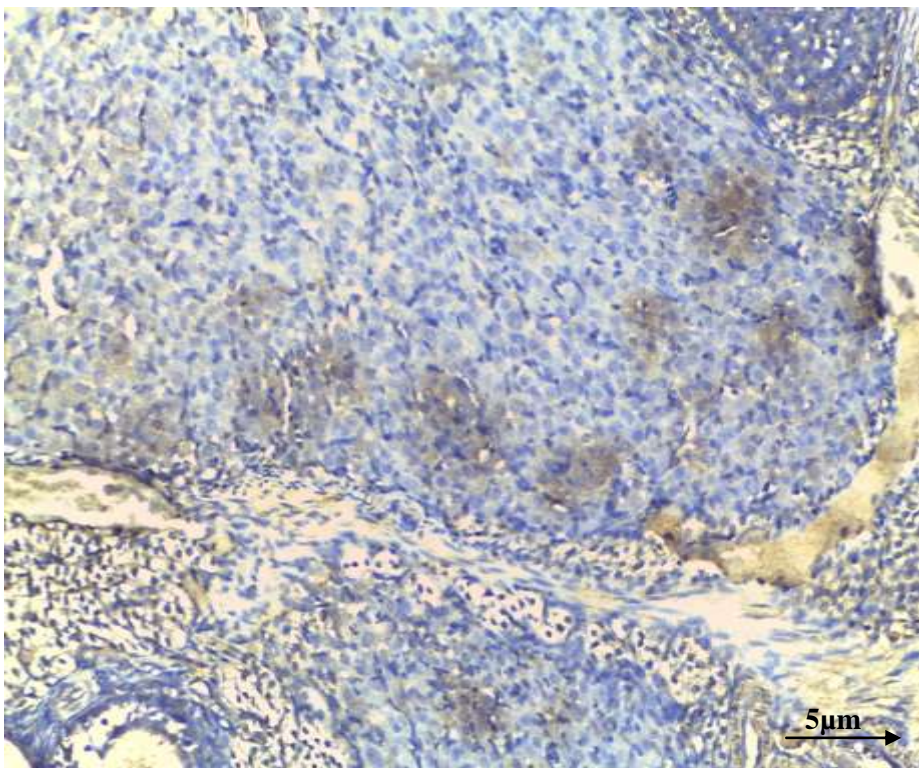
### **4.5.1. In the Ovaries**

The oestrogen antigen-antibody complexes in the ovaries of both the experimental and control groups were expressed in brown colouration. Immunohistochemical sections of the ovary of the control group showed high and extensive expression of oestrogen antigen-antibody complexes (OR-Agb<sub>c</sub>), this is demonstrated in the high intensity of brown colouration (Plate 26). The induced group showed mild expression of OR-Agb<sub>c</sub> in its ovarian section (Plate 27). The expression of OR-Agb<sub>c</sub> in the ovary of the group withdrawn for 8 days was mild (Plate e 28). The group withdrawn for 16 days showed moderate expression of OR-Agb<sub>c</sub> (Plate 29) while 28 days withdrawal group showed high but non-extensive expression of OR-Agb<sub>c</sub> (Plate 30). The expressions of OR-Agb<sub>c</sub> in the ovary of the groups post-treated with low and high doses of GCW for 8 days were mild (Plate 31 -32). In the groups post-treated for 16 and 28 days, the expressions of OR-Agb<sub>c</sub> were high and extensive (Plate 33 - 36). In the co-administered groups, expressions of OR-Agb<sub>c</sub> in the ovaries were mild (Plate 37 - 38). The immunohistochemical sections of the ovaries of the rats pre-treated with GCW at low and high doses for 8, 16 and 28 days showed mild expression of OR-Agb<sub>c</sub> (Plate 39 - 44). The immunohistological sections of the ovaries of GCW-treated rats at low and high doses for 8, 16 and 28 days showed high expression of OR-Agb<sub>c</sub> (Plate 45 - 50).



**Plate 26:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with distilled water; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

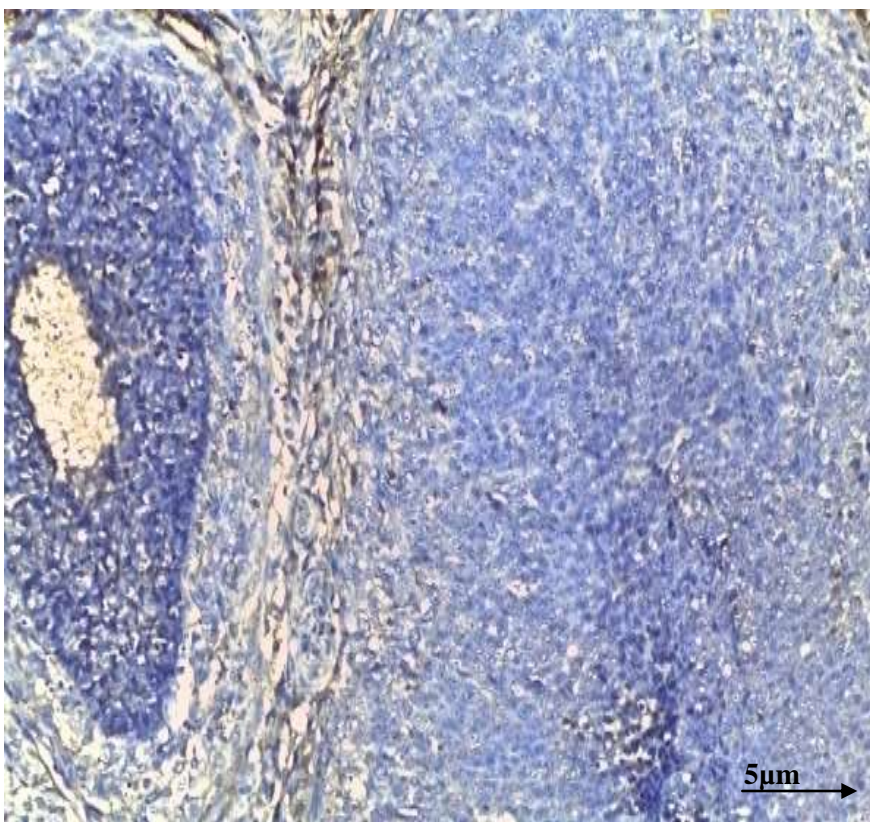
Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 27:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

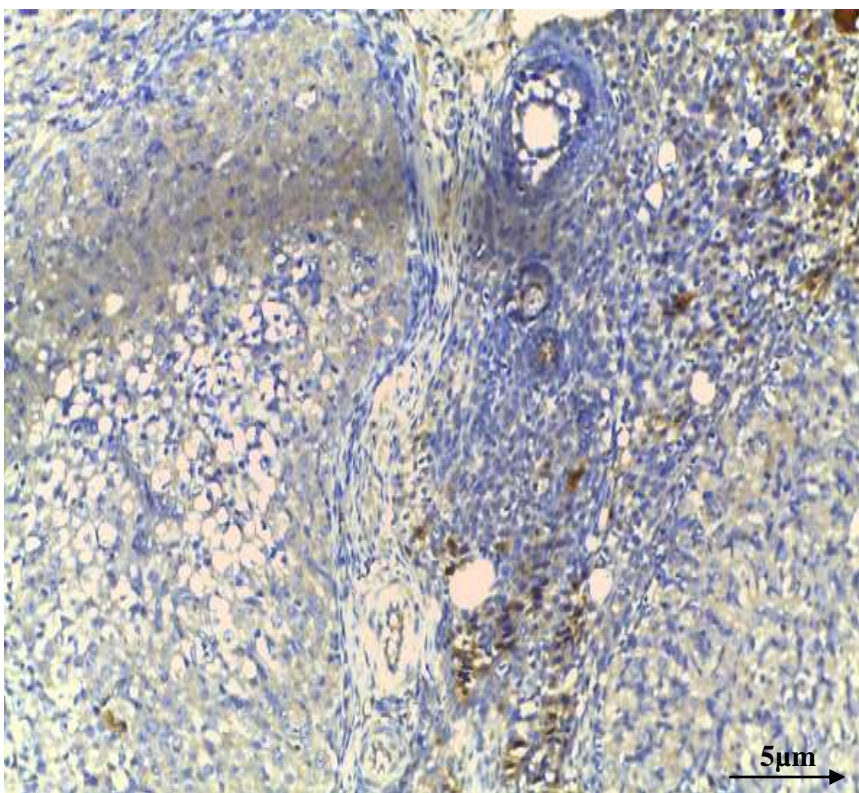
Antibody: Rabbit monoclonal oestrogen receptor antibody





**Plate 28:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days and withdrawn for 8 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

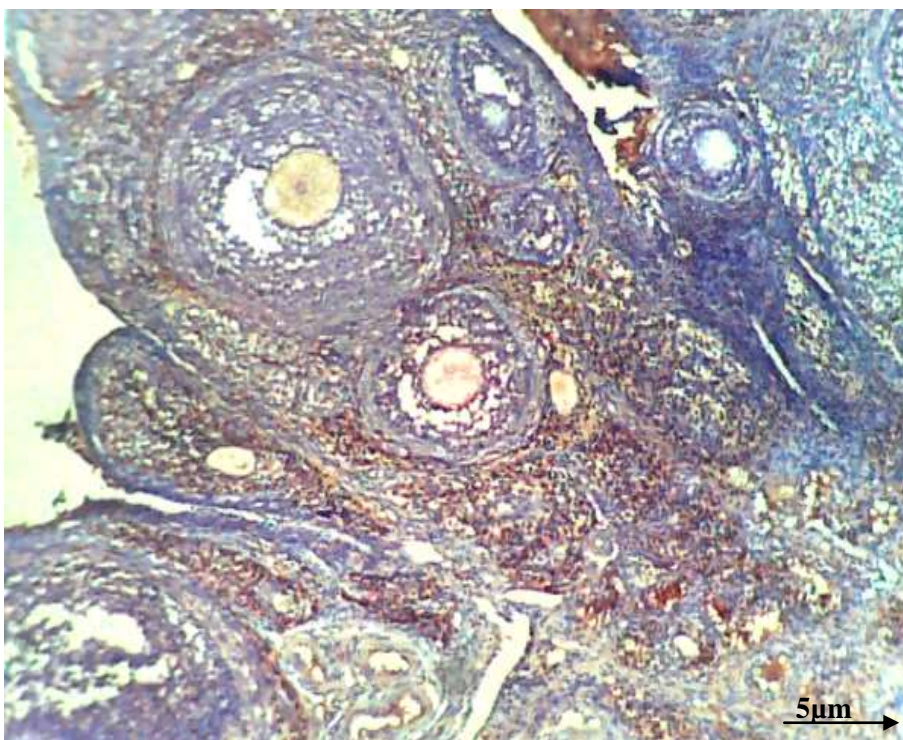
Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 29:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days and withdrawn for 16 days; shows moderate expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody

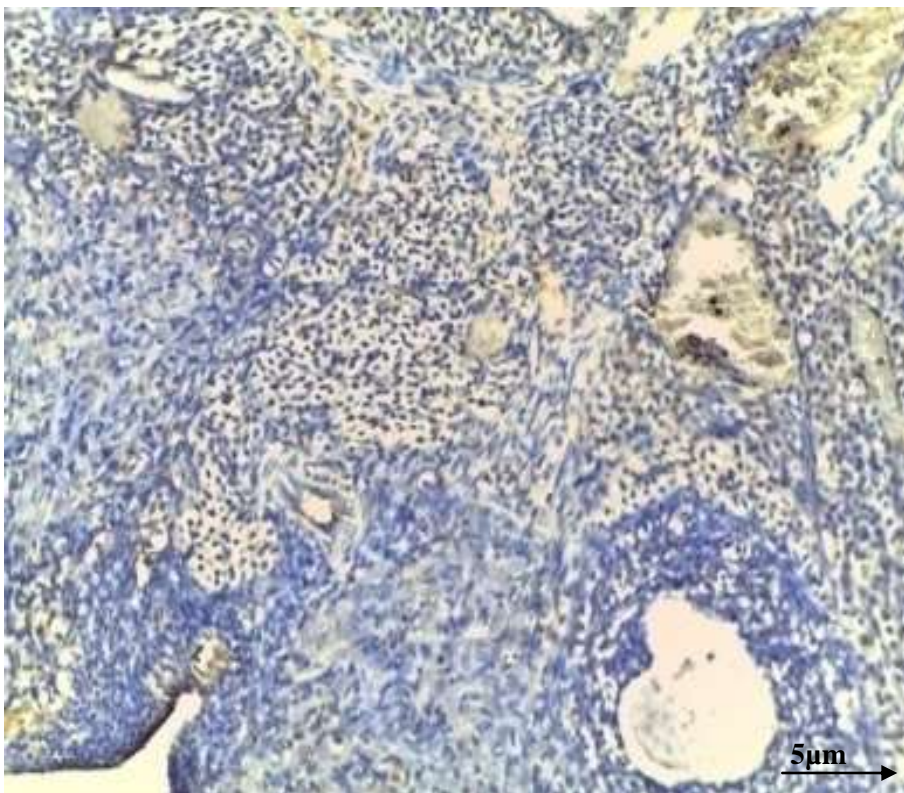




**Plate 30:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days and withdrawn for 28 days; shows high but none-extensive expression of oestrogen antigen-antibody complex at magnification of X100.

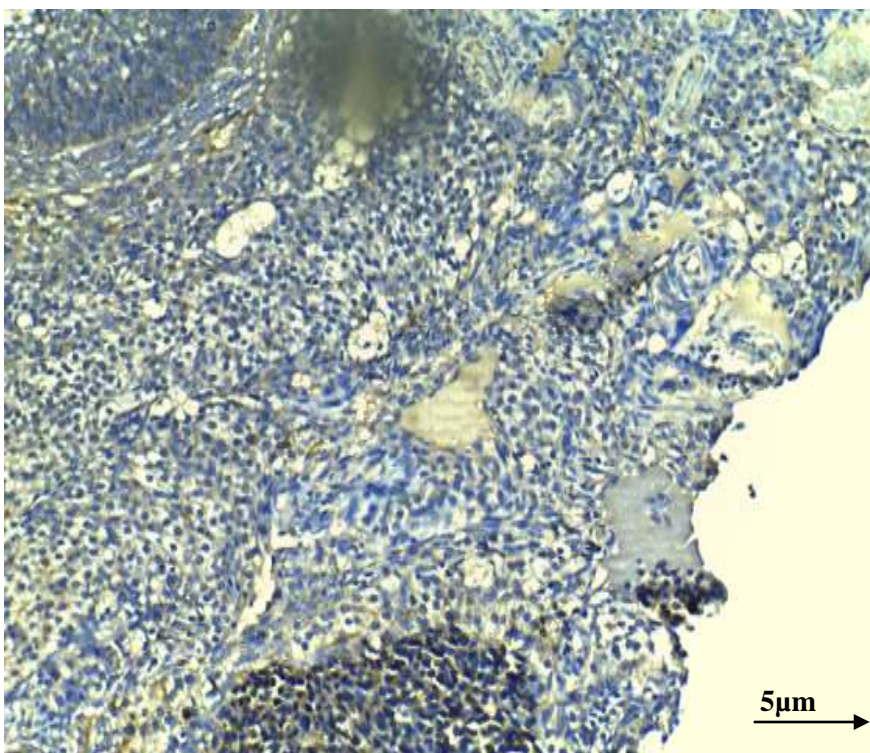
Antibody: Rabbit monoclonal oestrogen receptor antibody





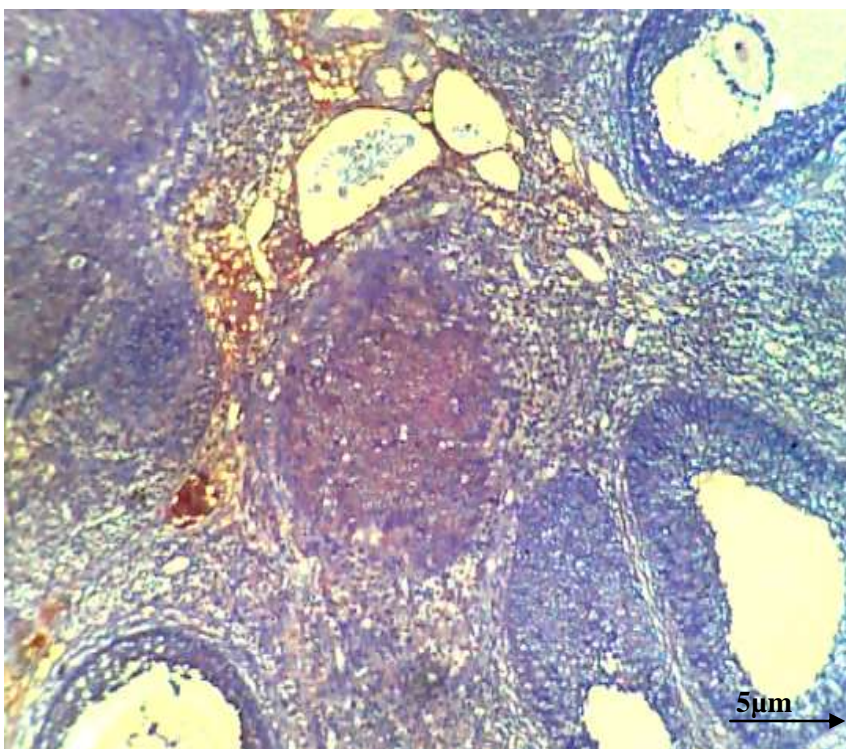
**Plate 31:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days followed by 5 ml/100 g. b. w. of GCW for 8 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 32:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days followed by 10 ml/100 g. b. w. of GCW for 8 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

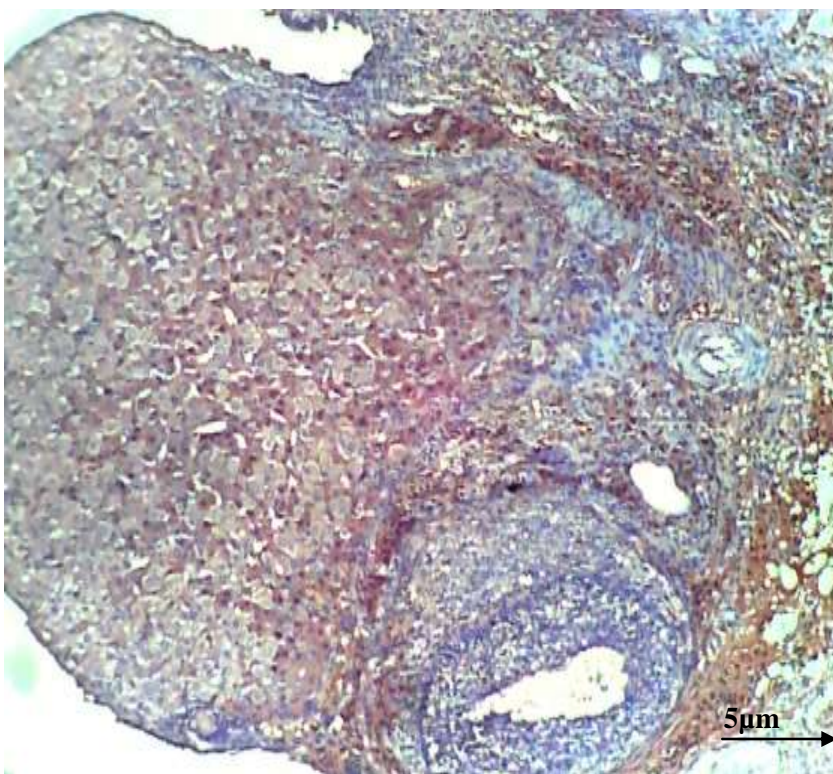
Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 33:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days followed by 5 ml/100 g. b. w. of GCW for 16 days; shows high expression of oestrogen antigen-antibody complex at magnification of X100.

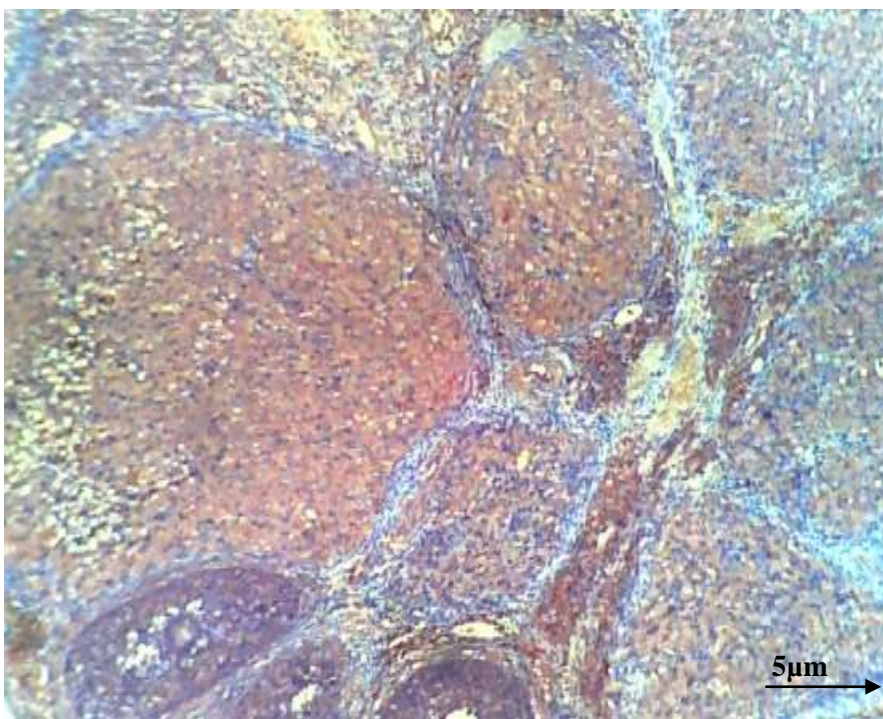
Antibody: Rabbit monoclonal oestrogen receptor antibody





**Plate 34:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days followed by 10 ml/100 g. b. w. of GCW for 16 days; shows high expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 35:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days followed by 5 ml/100 g. b. w. of GCW for 28 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

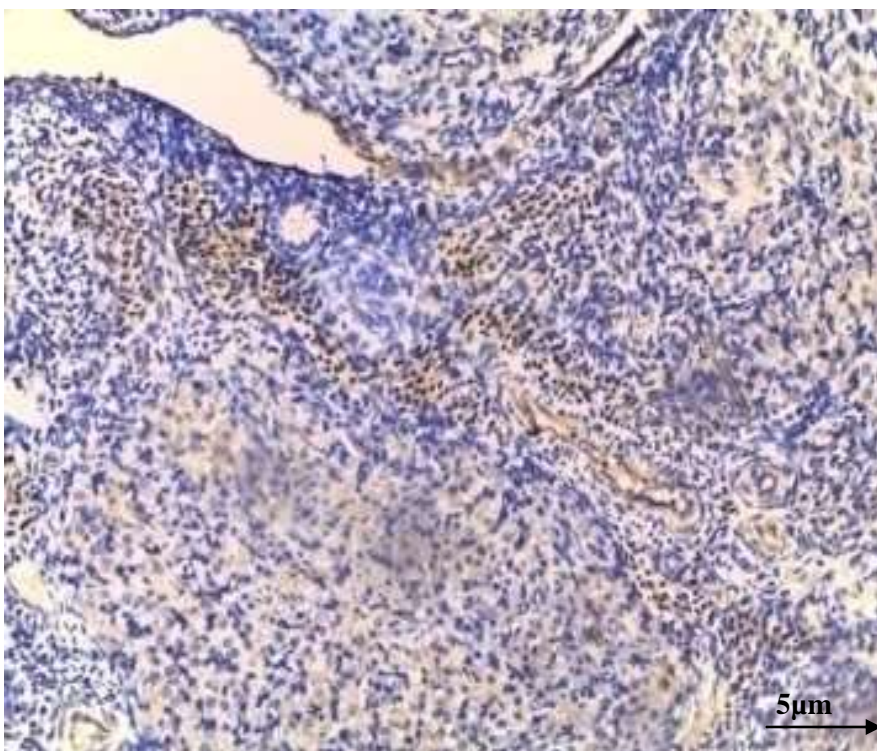
Antibody: Rabbit monoclonal oestrogen receptor antibody





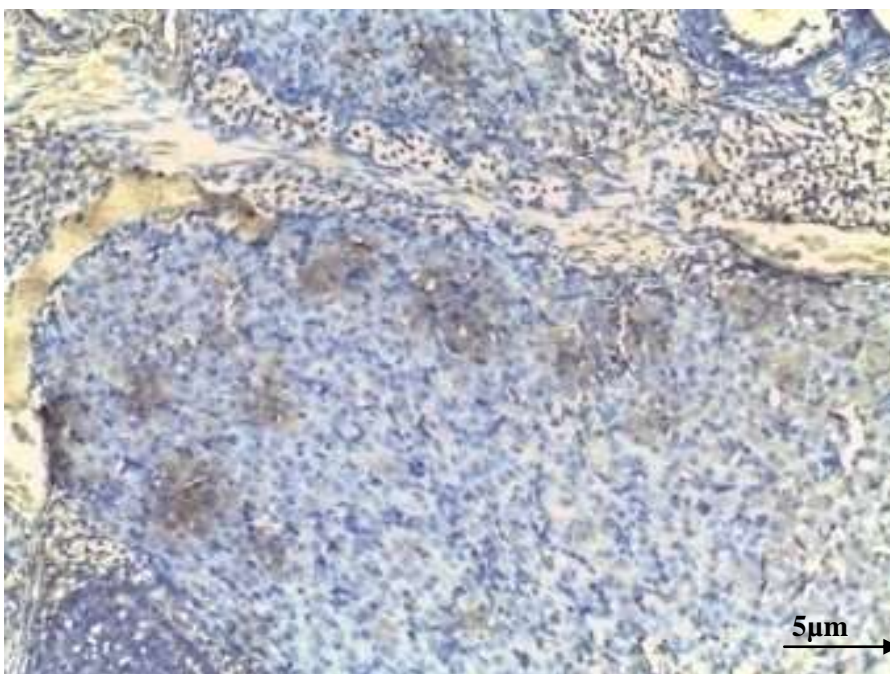
**Plate 36:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 0.2 mg/100 g b. w. metoclopramide hydrochloride for 28 days followed by 10 ml/100 g. b. w. of GCW for 28 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 37:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated concurrently with 0.2 mg/100 g. b. w. metoclopramide hydrochloride and 5 ml/100 g. b. w. of GCW for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

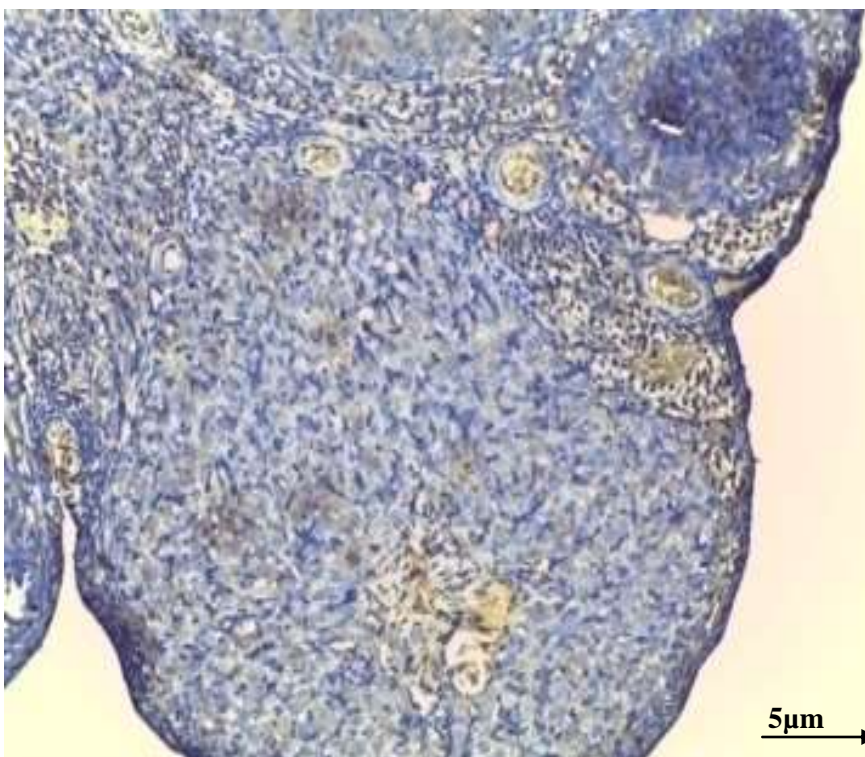
Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 38:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated concurrently with 0.2 mg/100 g. b. w. metoclopramide hydrochloride and 10 ml/100 g. b. w. of GCW for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

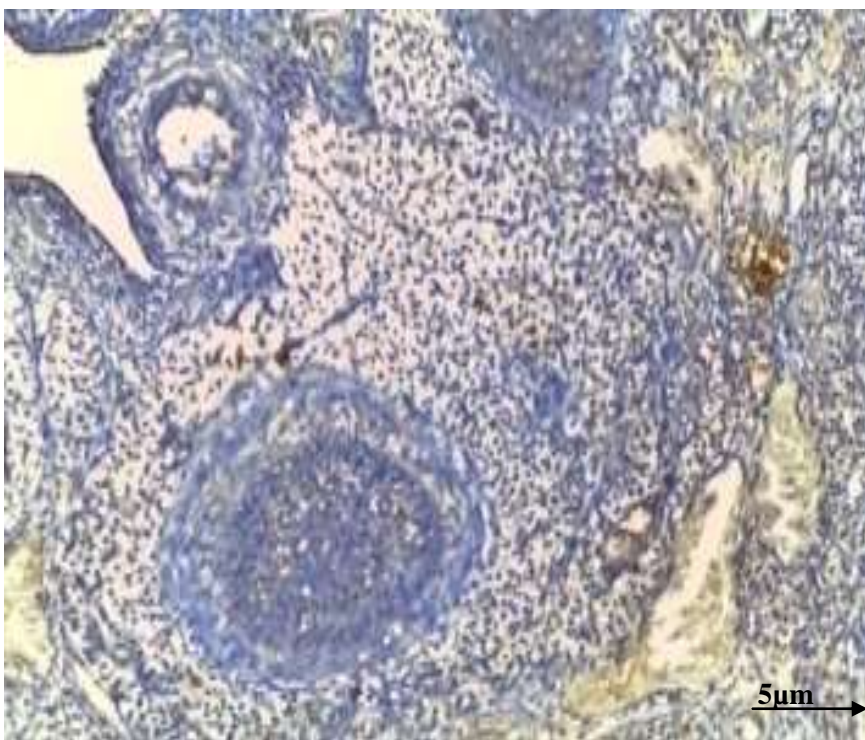
Antibody: Rabbit monoclonal oestrogen receptor antibody





**Plate 39:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 5 ml/100 g. b.w. of GCW for 8 days followed by 0.2 mg/100 g. b.w. metoclopramide hydrochloride for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

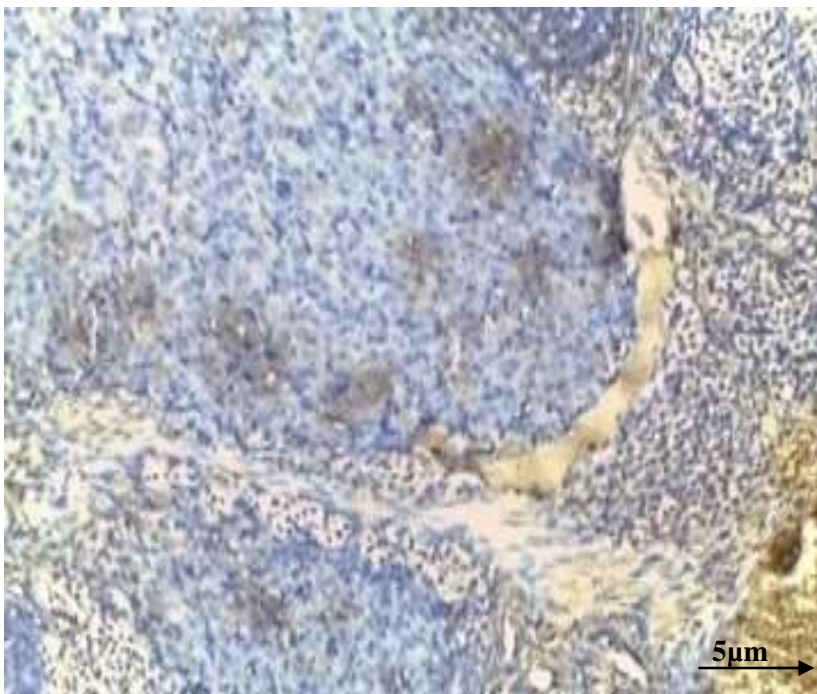
Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 40:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 10 ml/100 g. b. w. of GCW for 8 days followed by 0.2 mg/100 g. b.w. metoclopramide hydrochloride for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

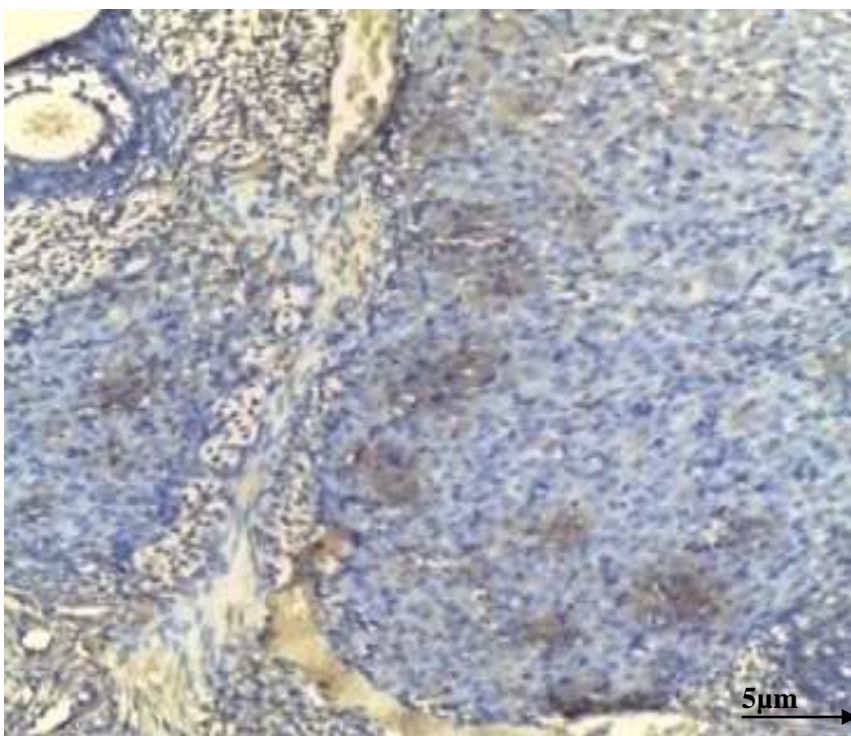
Antibody: Rabbit monoclonal oestrogen receptor antibody





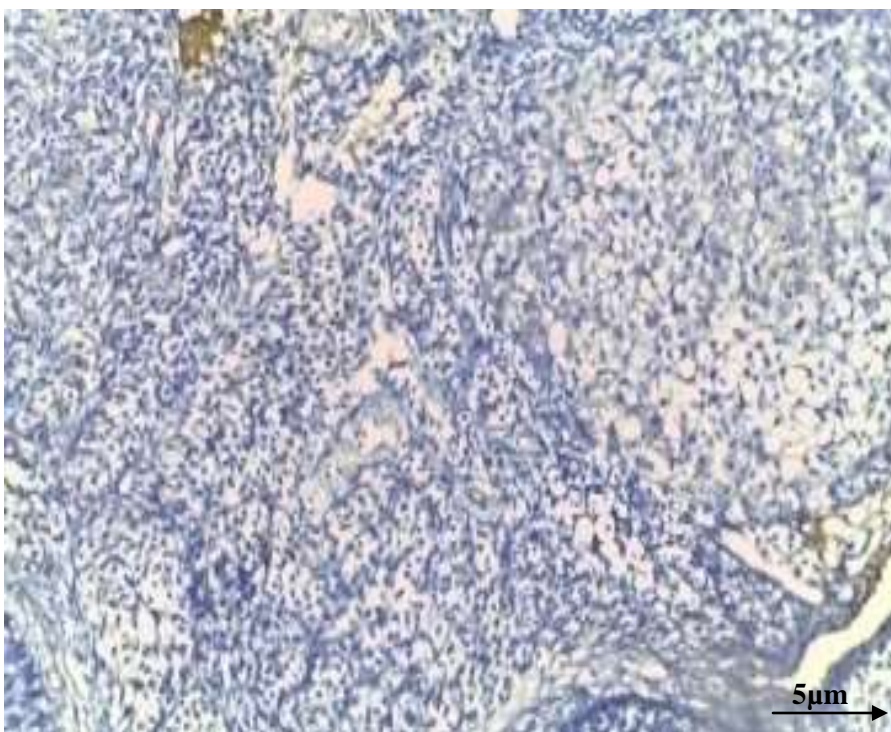
**Plate 41:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 5 ml/100 g. b. w. of GCW for 16 days followed by 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 42:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 10 ml/100 g. b. w. of GCW for 16 days followed by 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

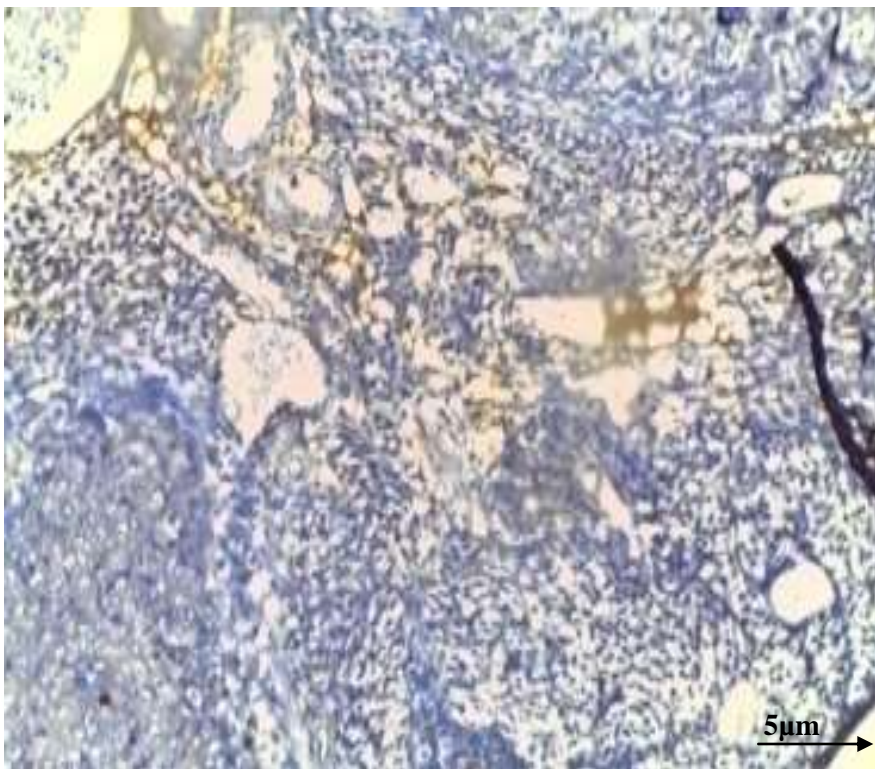
Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 43:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 5 ml/100 g. b. w. of GCW for 28 days followed by 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody

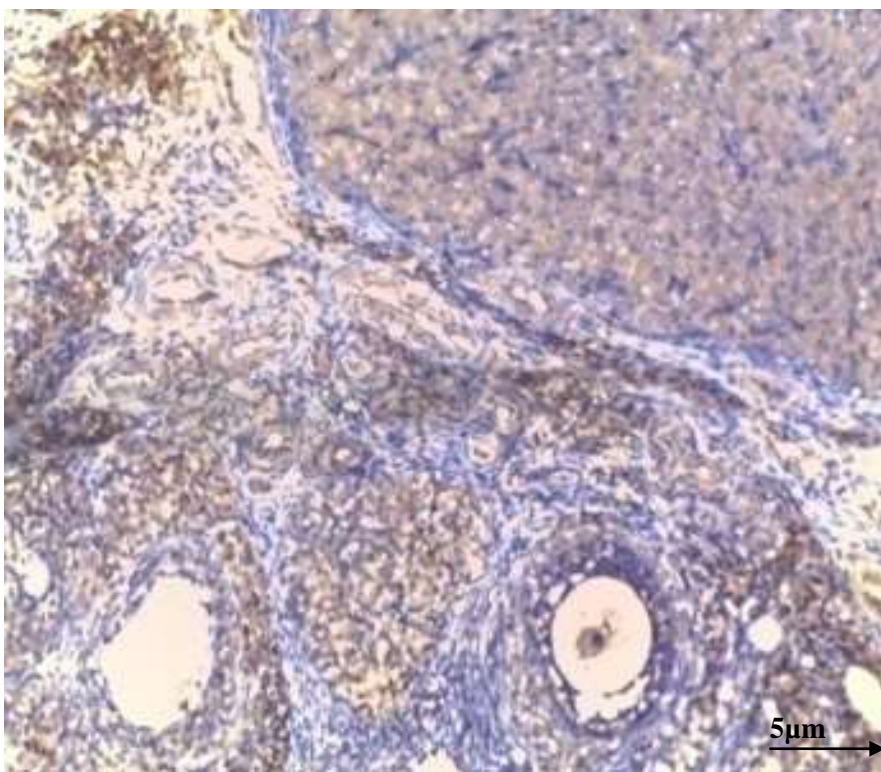




**Plate 44:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 10 ml/100 g. b. w. of GCW for 28 days followed by 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

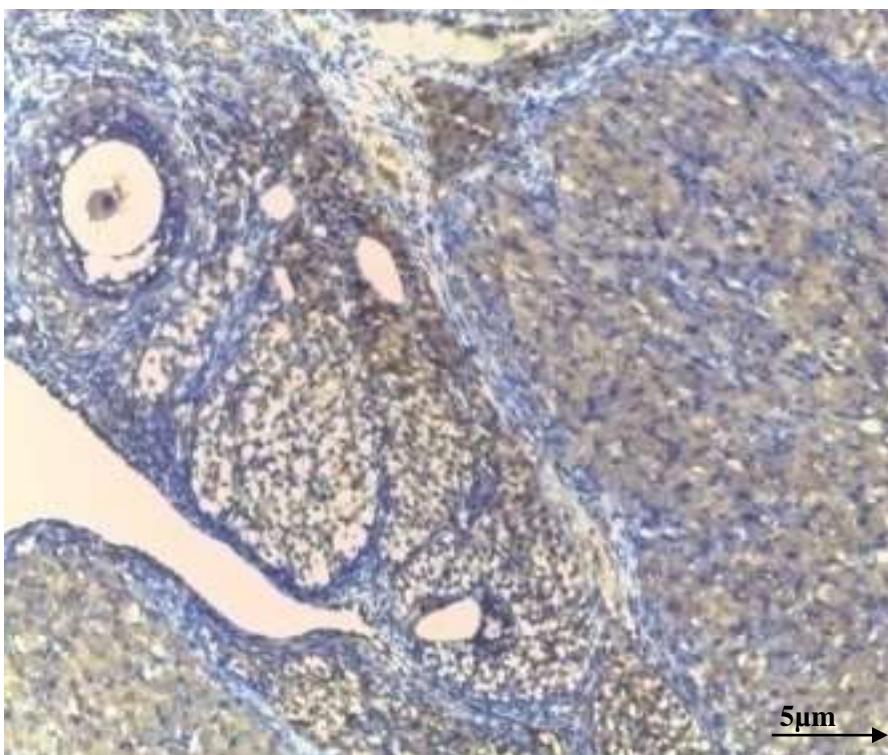
Antibody: Rabbit monoclonal oestrogen receptor antibody





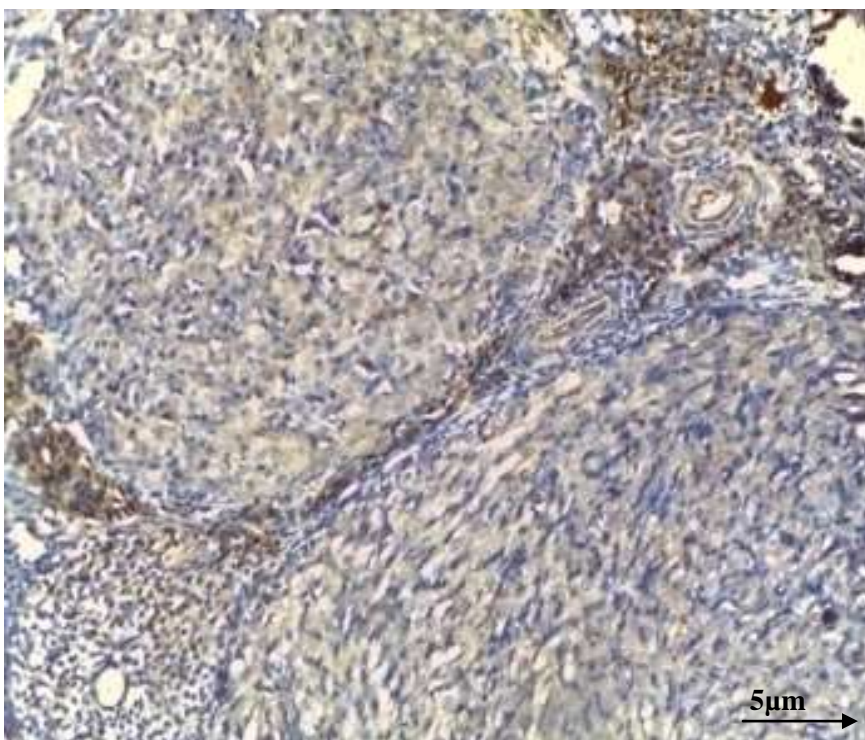
**Plate 45:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 5 ml/100 g. b. w. of GCW for 8 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 46:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 10 ml/100 g. b. w. of GCW for 8 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

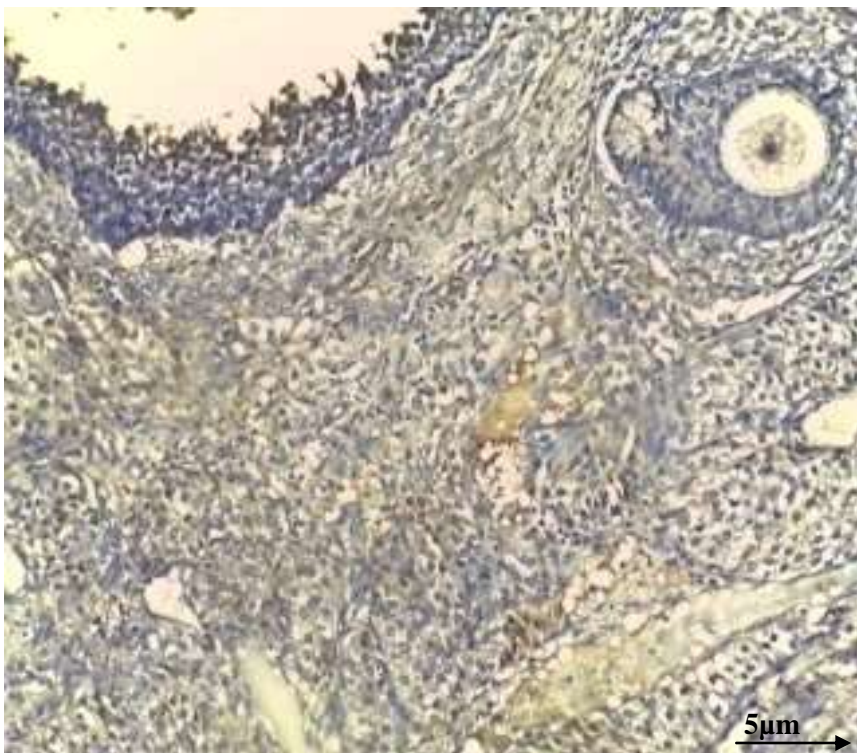
Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 47:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 5 ml/100 g. b. w. of GCW for 16 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody

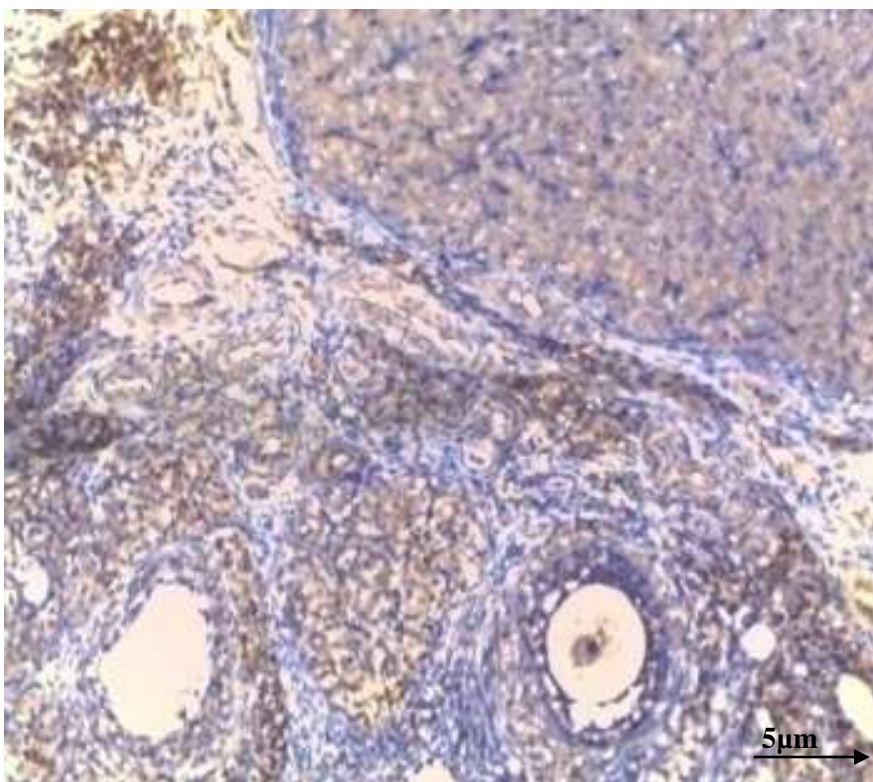




**Plate 48:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 10 ml/100 g. b. w. of GCW for 16 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

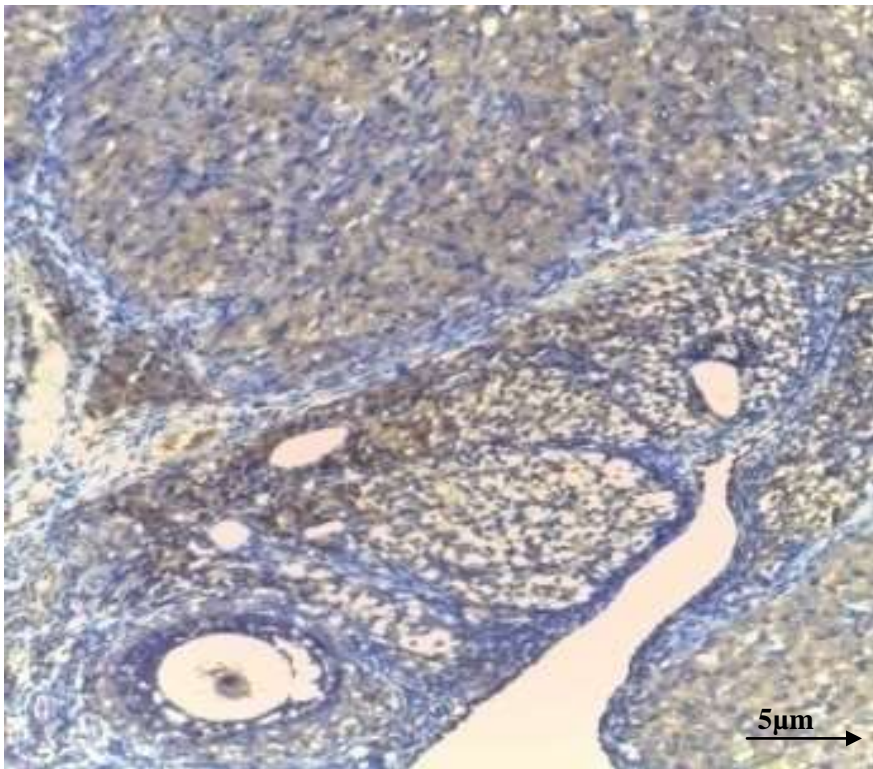
Antibody: Rabbit monoclonal oestrogen receptor antibody





**Plate 49:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 5 ml/100 g. b. w. of GCW for 28 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody



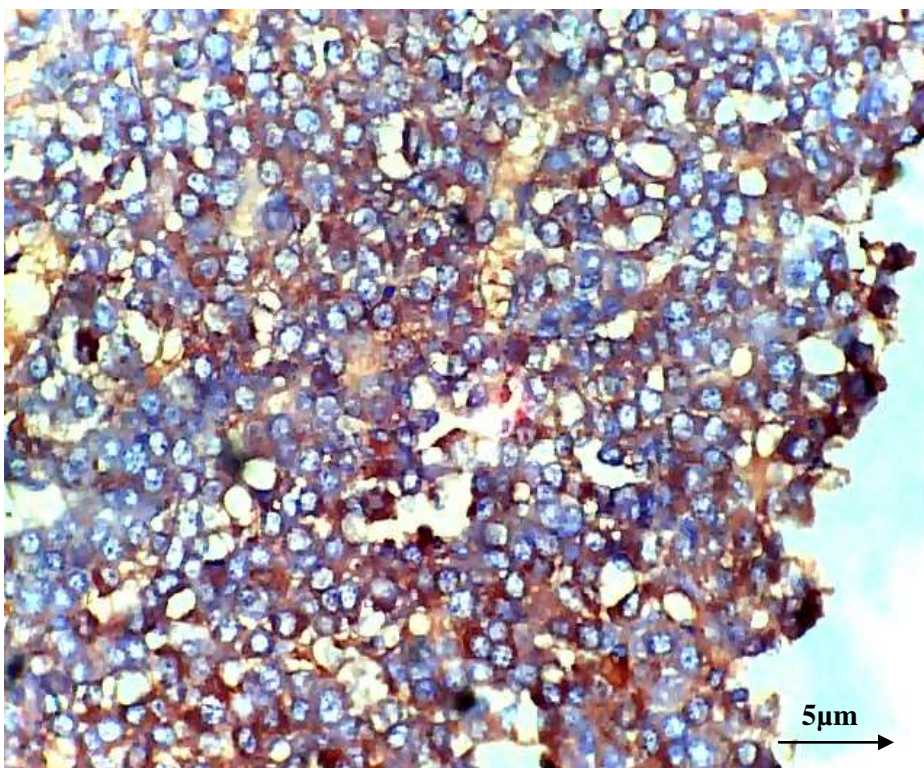
**Plate 50:** Immunohistochemical section of the ovary of Sprague-Dawley rat treated with 10 ml/100 g. b. w. of GCW for 28 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody

#### **4.5.2 In the Pituitary Gland**

The Immunohistochemical sections of the pituitary gland of the control group showed high and extensive expression of oestrogen antigen-antibody complexes (OR-Agbc), this is demonstrated in the high intensity of brown colouration in its section (Plate 51). There were mild expression of OR-Agbc in the section of the pituitary gland of the induced group (Plate 52). The expression of OR-Agbc in the pituitary gland of the group withdrawn for 8 days was mild (Plate e 53). The group withdrawn for 16 days showed moderate expression of OR-Agbc (Plate 54) while 28 days withdrawal group showed high but non-extensive expression of OR-Agbc (Plate 55). The expressions of OR-Agbc in the pituitary gland of the groups post-treated with low and high doses of GCW for 8 days were mild (Plate 56 -57). In the groups post-treated with GCW for 16 and 28 days, the expressions of OR-Agbc were high and extensive (Plate 58 - 61). There were mild expressions of OR-Agbc in the pituitary gland of the co-administered groups (Plate 37 - 38). The immunohistochemical sections of the pituitary gland of the rats pre-treated with GCW at low and high doses for 8, 16 and 28 days showed mild expression of OR-Agbc. (Plate 39 - 44). The GCW- treated groups at low and high doses for 8, 16 and 28 days showed high expression of OR-Agbc (Plate 45 - 50).

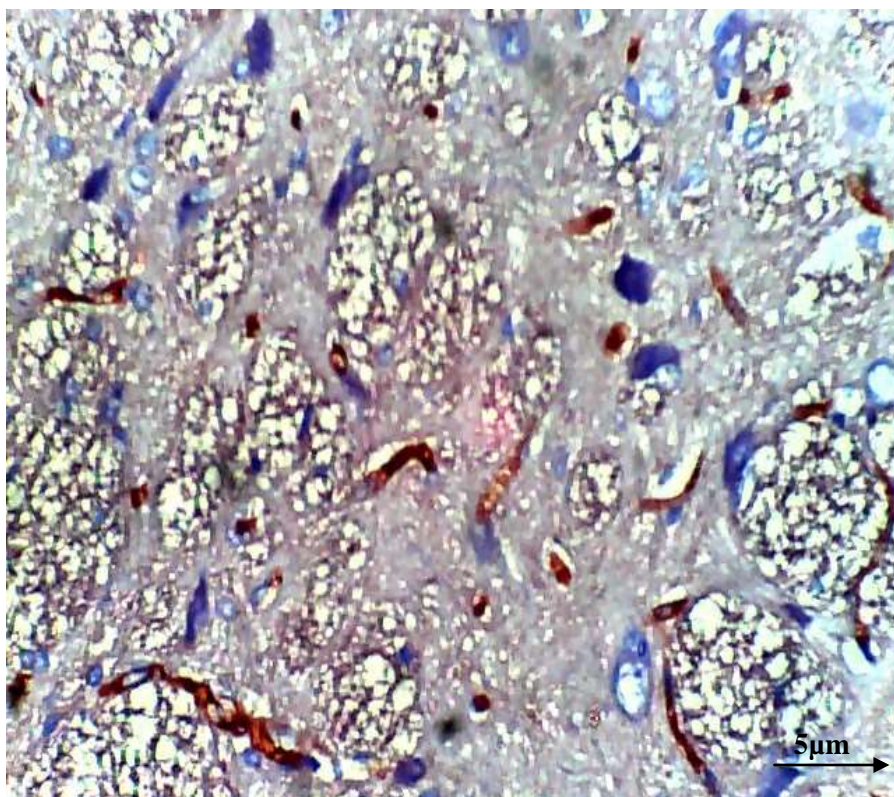




**Plate 51:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with distilled water; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

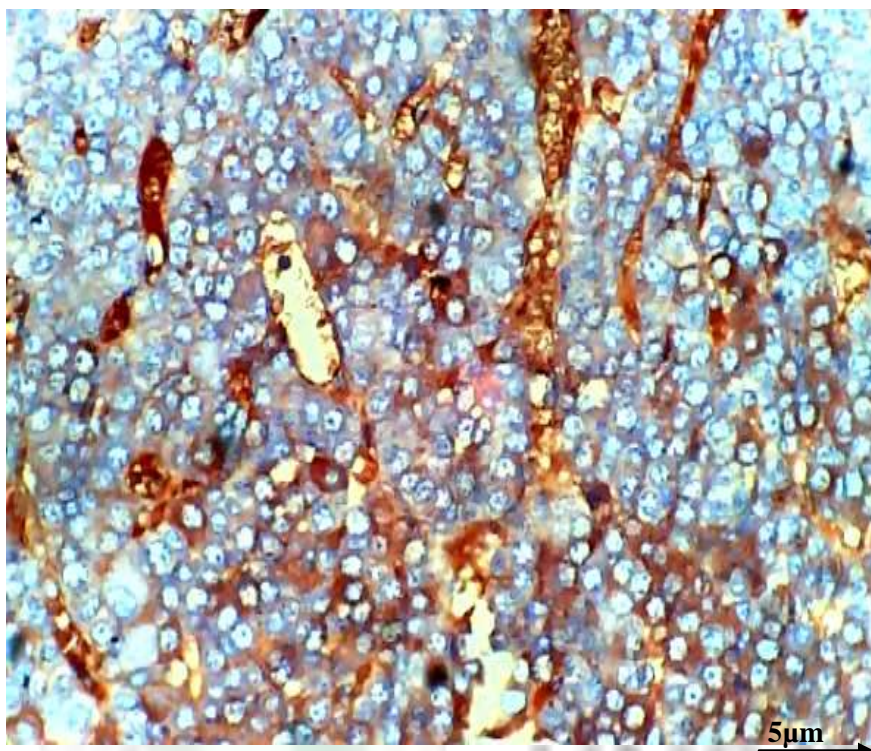
Antibody: Rabbit monoclonal oestrogen receptor antibody





**Plate 52:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

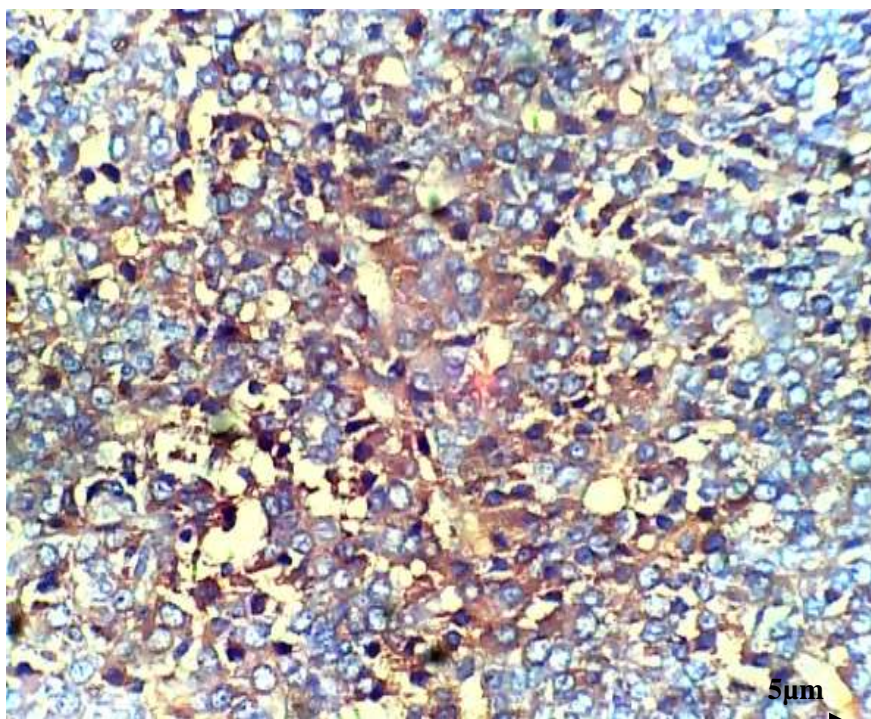
Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 53:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days and withdrawn for 8 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

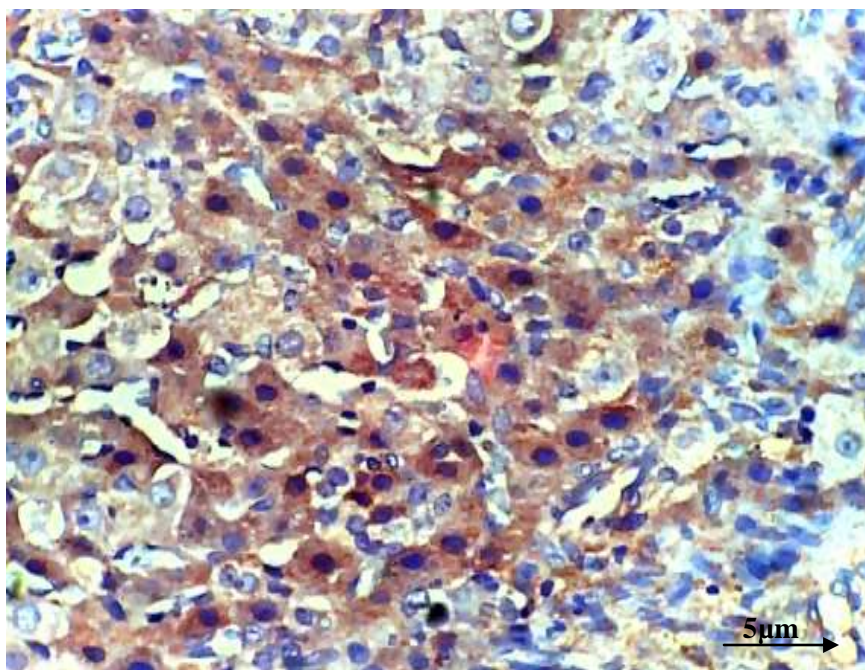
Antibody: Rabbit monoclonal oestrogen receptor antibody





**Plate 54:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days and withdrawn for 16 days; shows moderate expression of oestrogen antigen-antibody complex at magnification of X100.

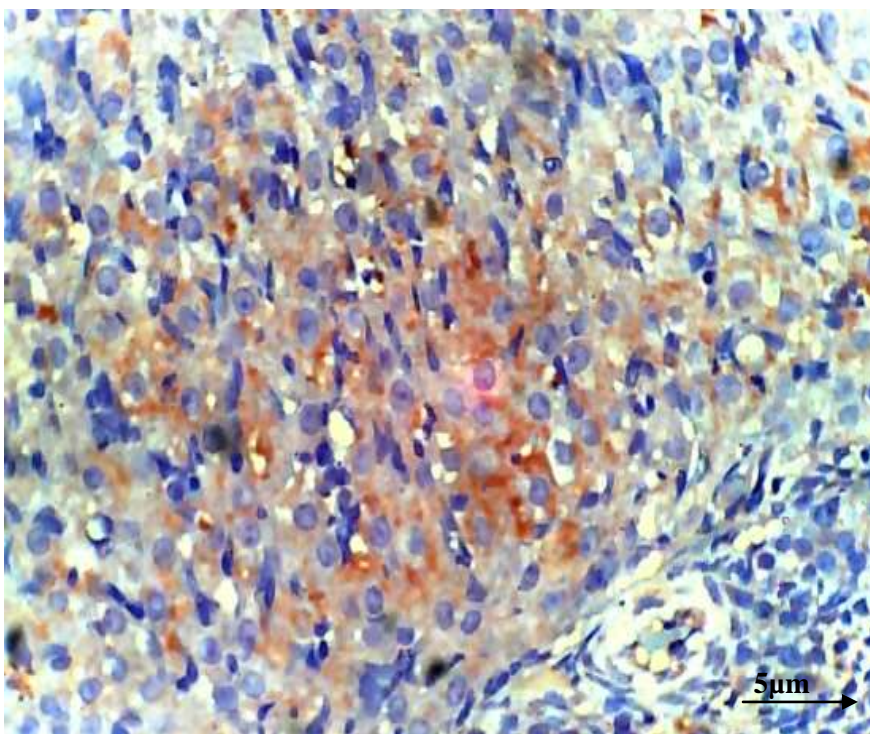
Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 55:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days and withdrawn for 28 days; shows high but non-extensive expression of oestrogen antigen-antibody complex at magnification of X100.

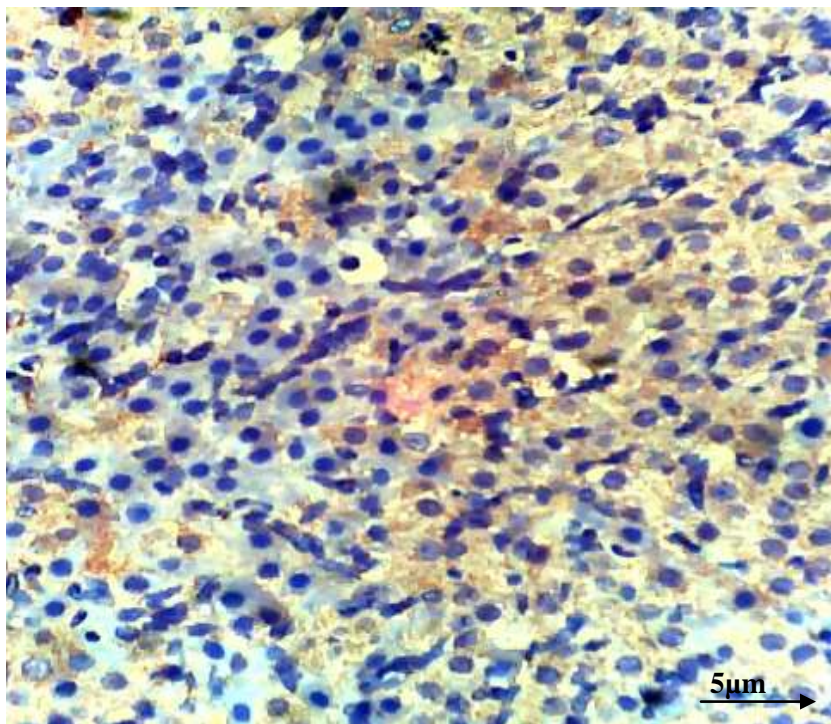
Antibody: Rabbit monoclonal oestrogen receptor antibody





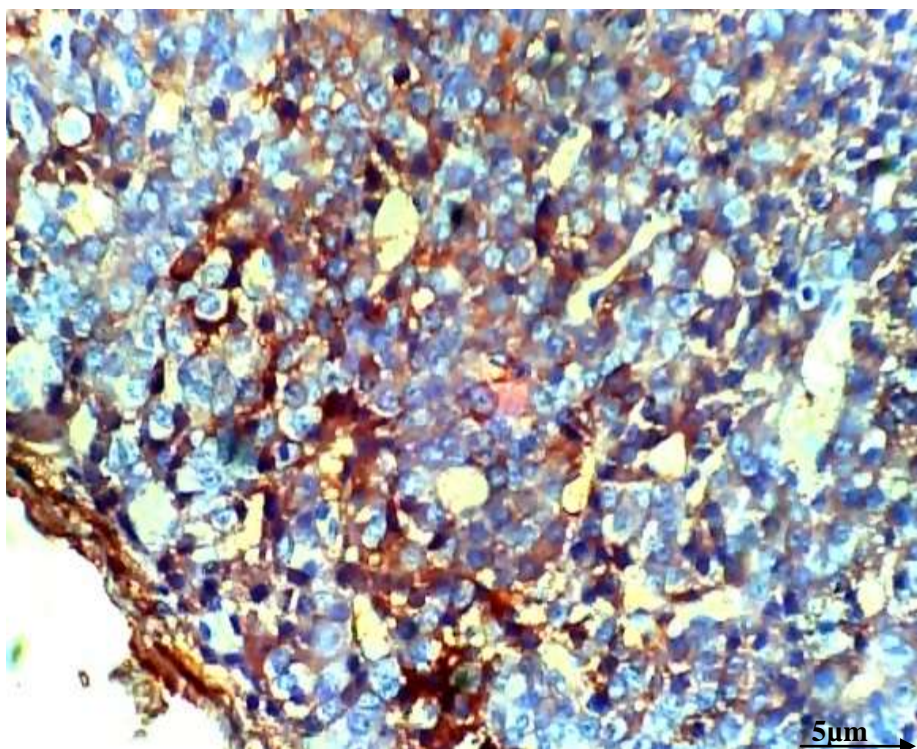
**Plate 56:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days followed by 5 ml/100 g. b. w. of GCW for 8 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 57:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days followed by 10 ml/100 g. b. w. of GCW for 8 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

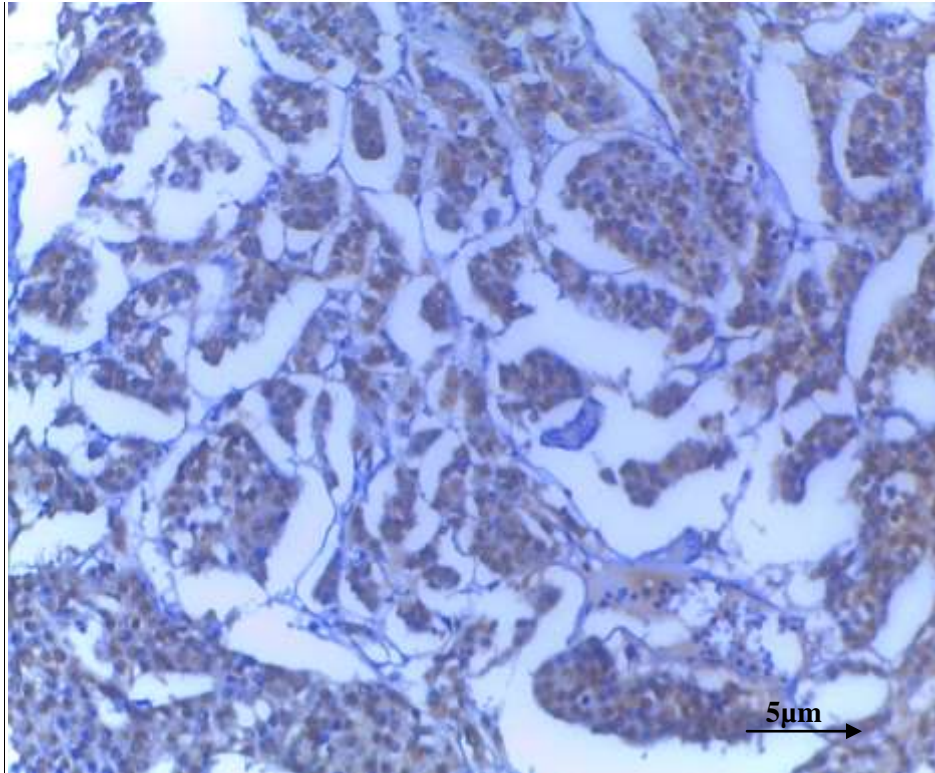
Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 58:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days followed by 5 ml/100 g. b. w. of GCW for 16 days; shows high expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody

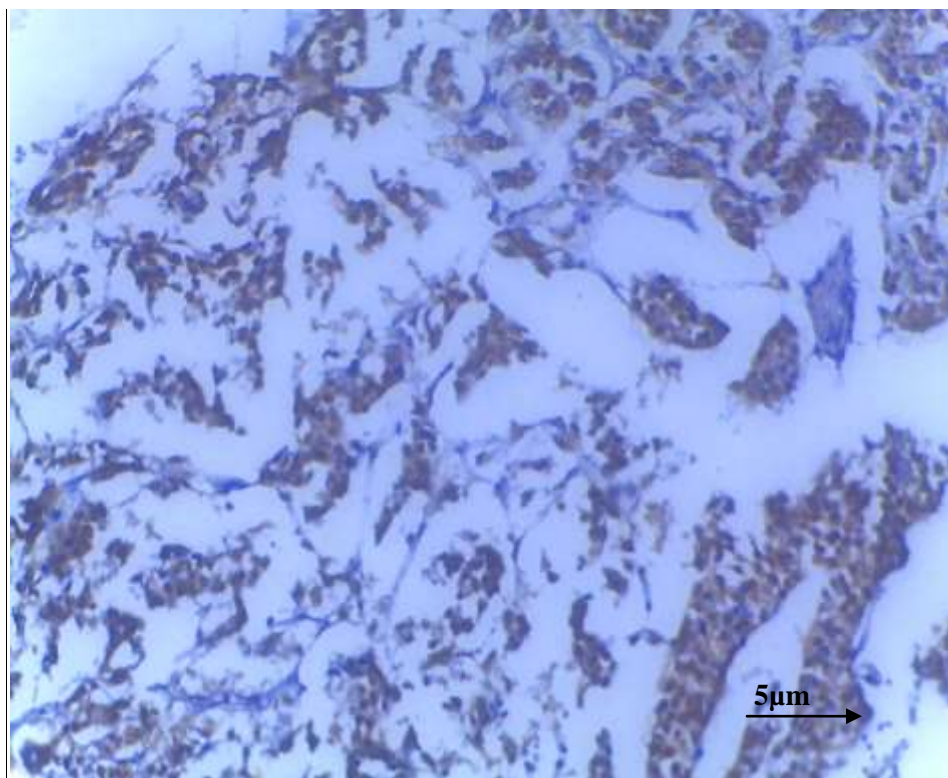




**Plate 59:** Immunohistochemical section of the pituitary of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days followed by 10 ml/100 g. b. w. of GCW for 16 days; shows high expression of oestrogen antigen-antibody complex at magnification of X100.

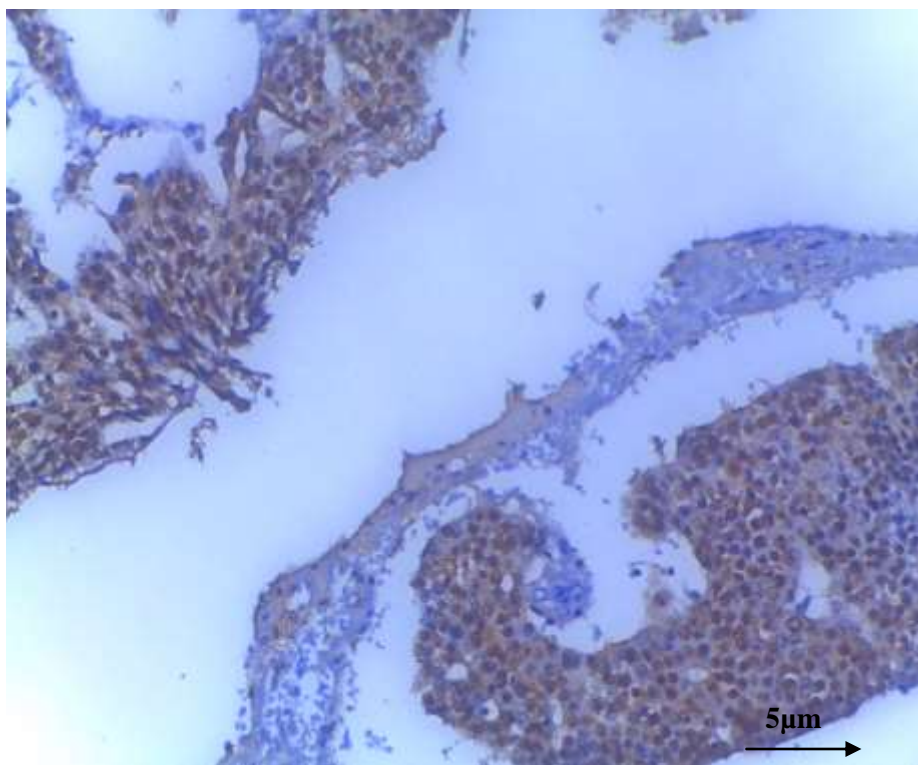
Antibody: Rabbit monoclonal oestrogen receptor antibody





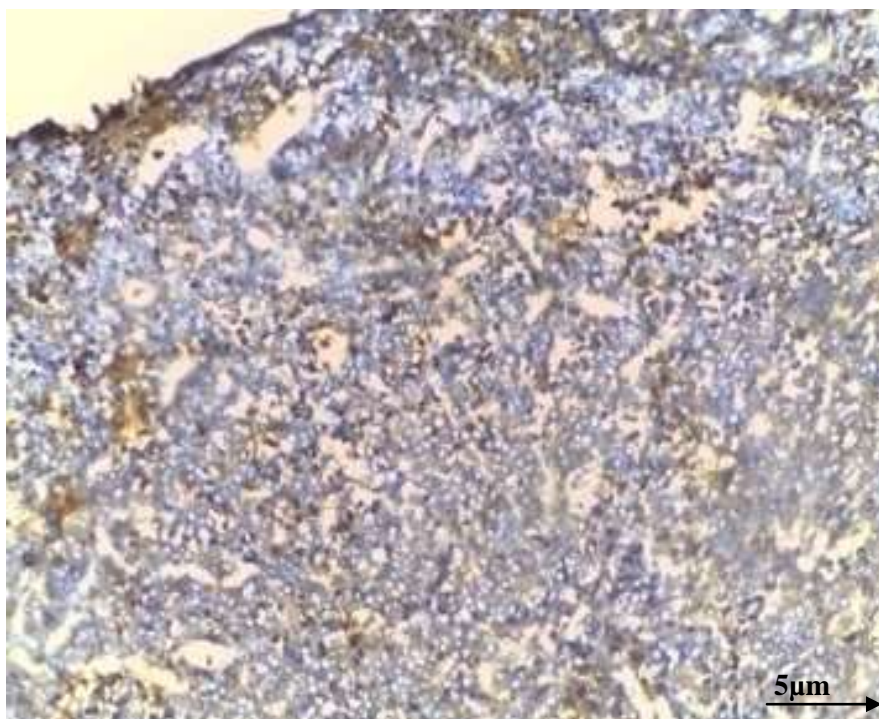
**Plate 60:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days followed by 5 ml/100 g. b. w. of GCW for 28 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody



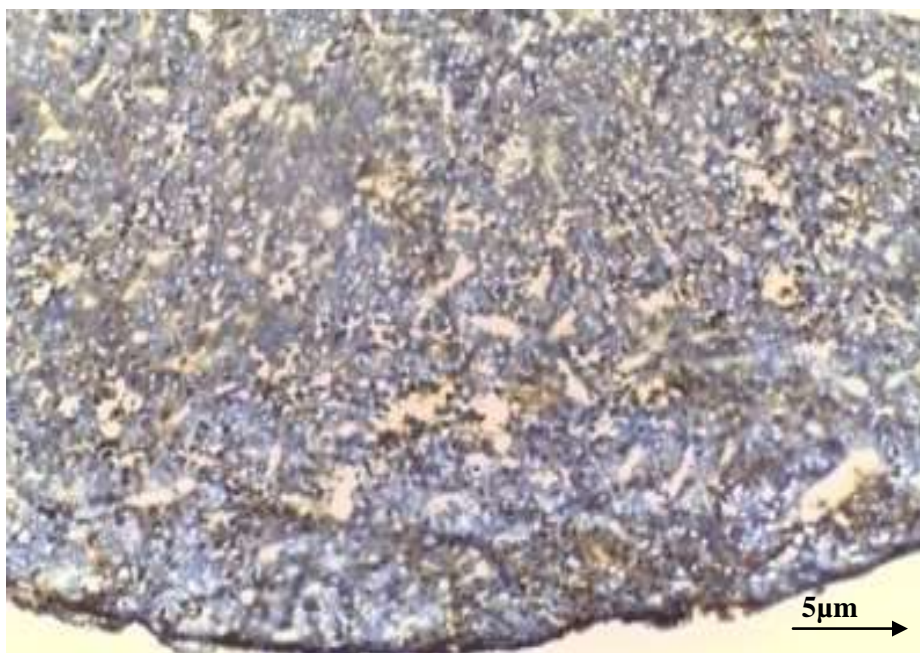
**Plate 61:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 0.2 mg/100 g. b.w. metoclopramide hydrochloride for 28 days followed by 10 ml/100 g. b.w. of GCW for 28 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 62:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated concurrently with 0.2 mg/100 g. b. w. metoclopramide hydrochloride and 5 ml/100 g. b. w. of GCW for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 63:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated concurrently with 0.2 mg/100 g. b. w. metoclopramide hydrochloride and 10 ml/100 g. b. w. of GCW for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

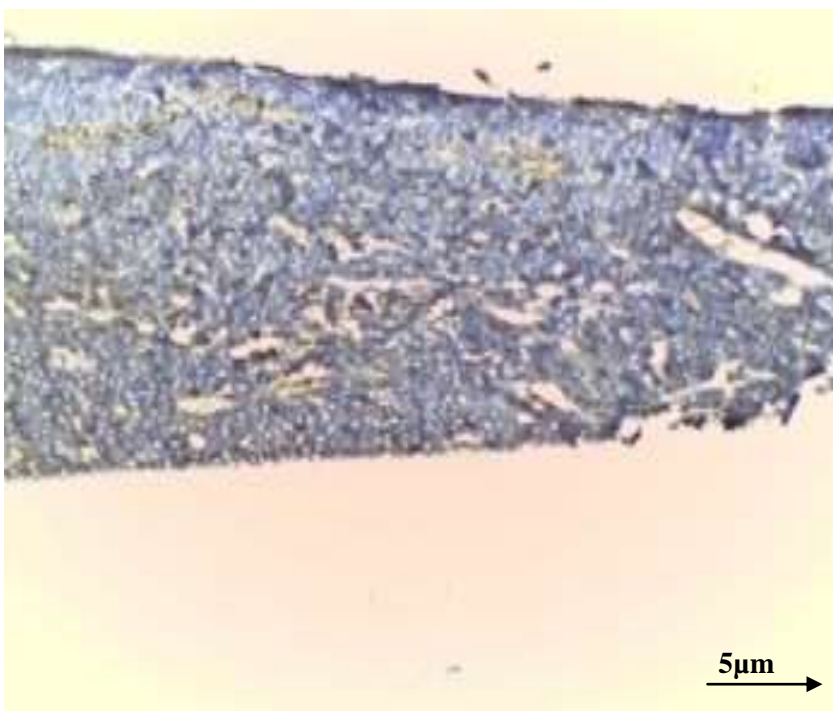
Antibody: Rabbit monoclonal oestrogen receptor antibody





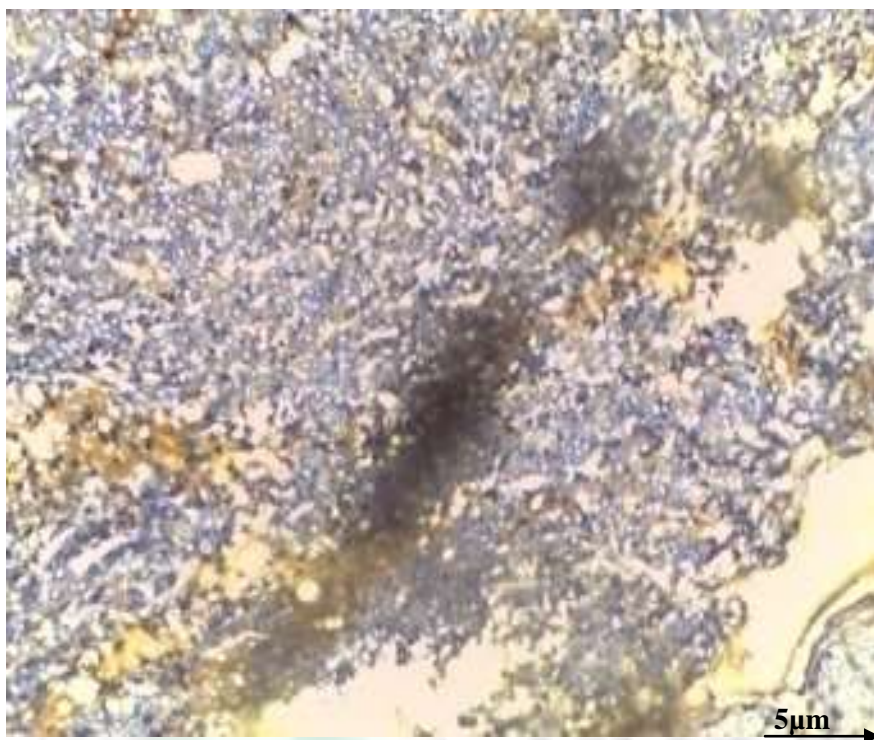
**Plate 64:** Immunohistochemical section of the pituitary of Sprague-Dawley rat treated with 5 ml/100 g. b. w. of GCW for 8 days followed by 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 65:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 10 ml/100 g. b. w. of GCW for 8 days followed by 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 66:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 5 ml/100 g. b. w. of GCW for 16 days followed by 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

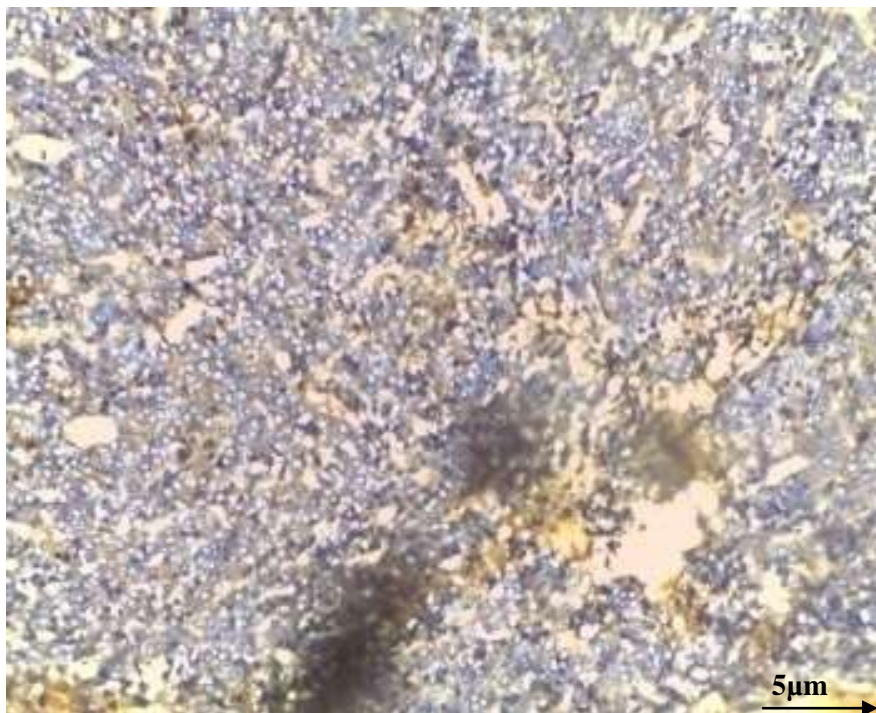
Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 67:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 10 ml/100 g. b. w. of GCW for 16 days followed by 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

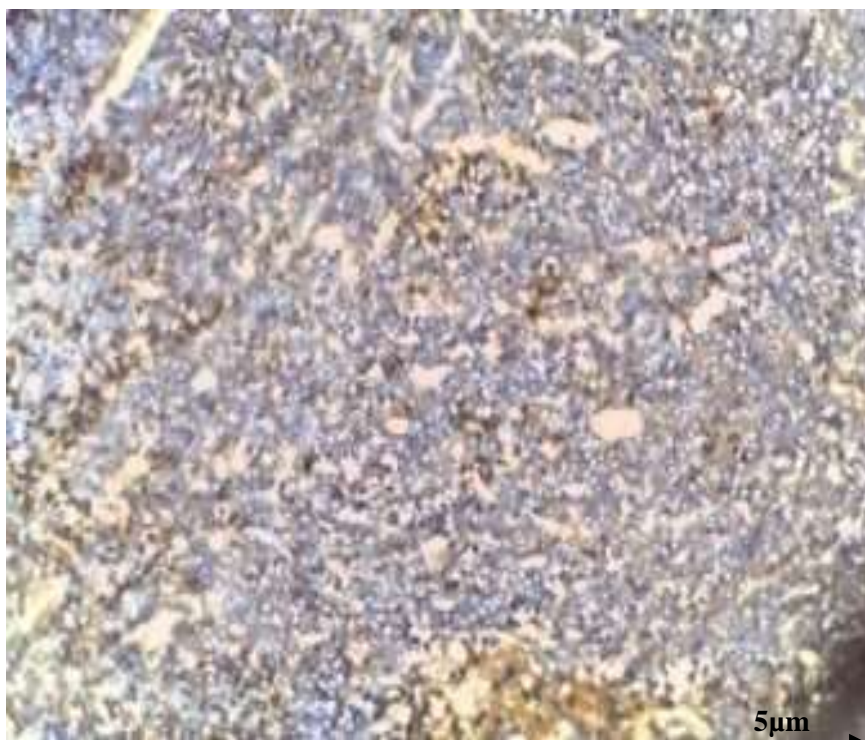
Antibody: Rabbit monoclonal oestrogen receptor antibody





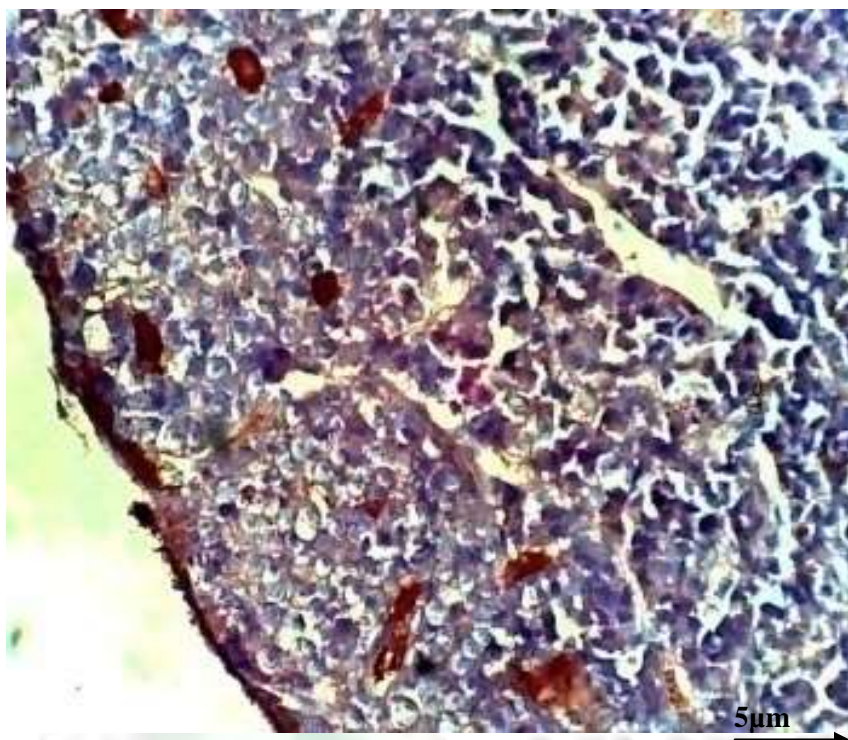
**Plate 68:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 5 ml/100 g. b. w. of GCW for 28 days followed by 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody.



**Plate 69:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 10 ml/100 g. b. w. of GCW for 28 days followed by 0.2 mg/100 g. b. w. metoclopramide hydrochloride for 28 days; shows mild expression of oestrogen antigen-antibody complex at magnification of X100.

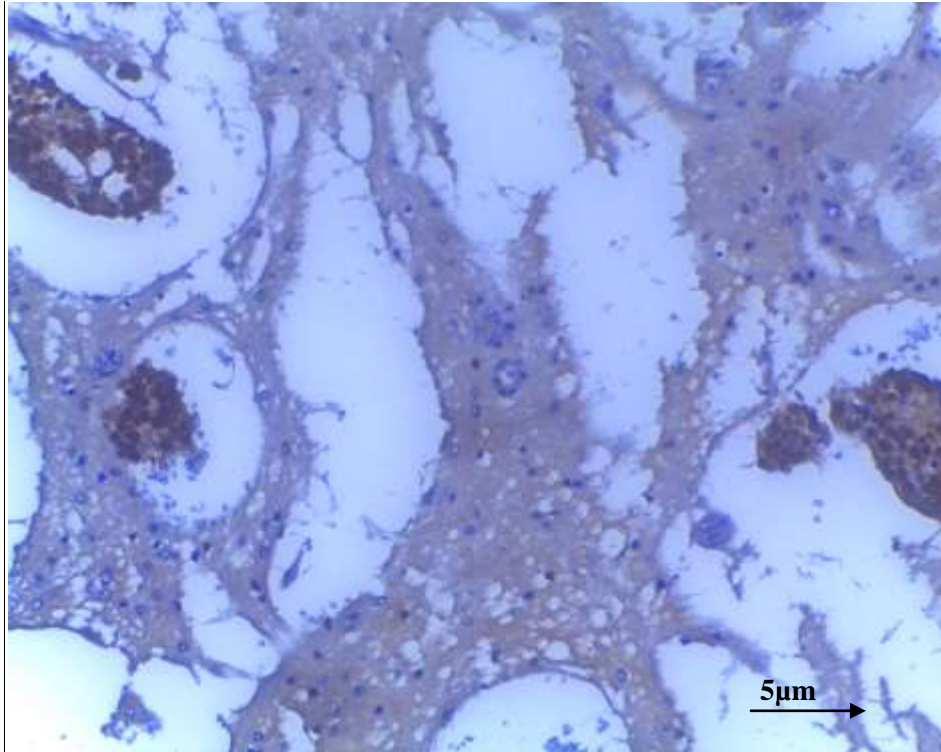
Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 70:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 5 ml/100 g. b. w. of GCW for 8 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody

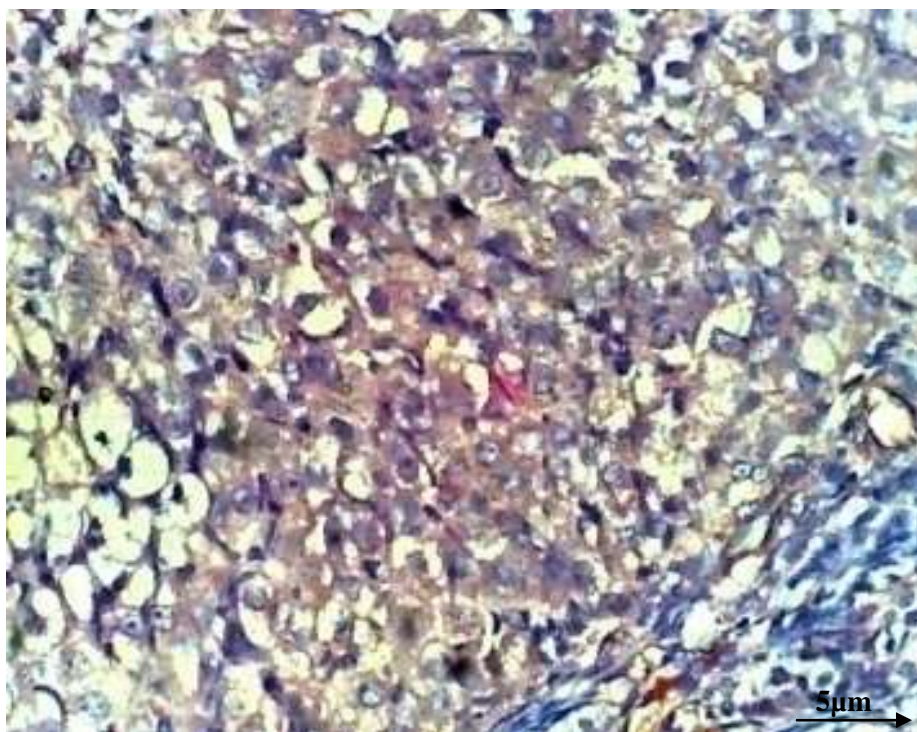




**Plate 71:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 10 ml/100 g. b. w. of GCW for 8 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

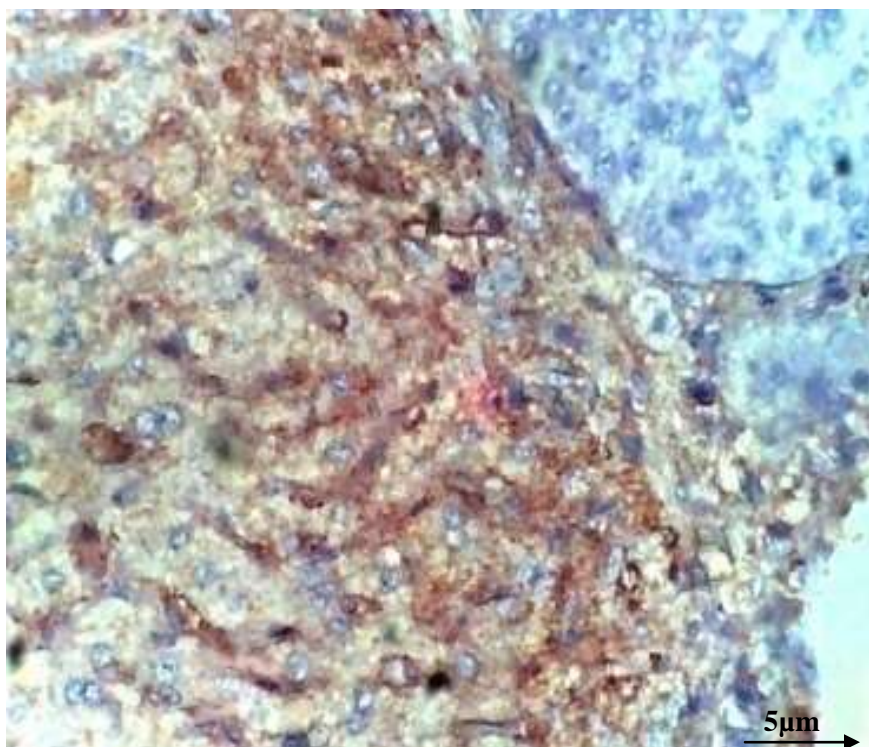
Antibody: Rabbit monoclonal oestrogen receptor antibody





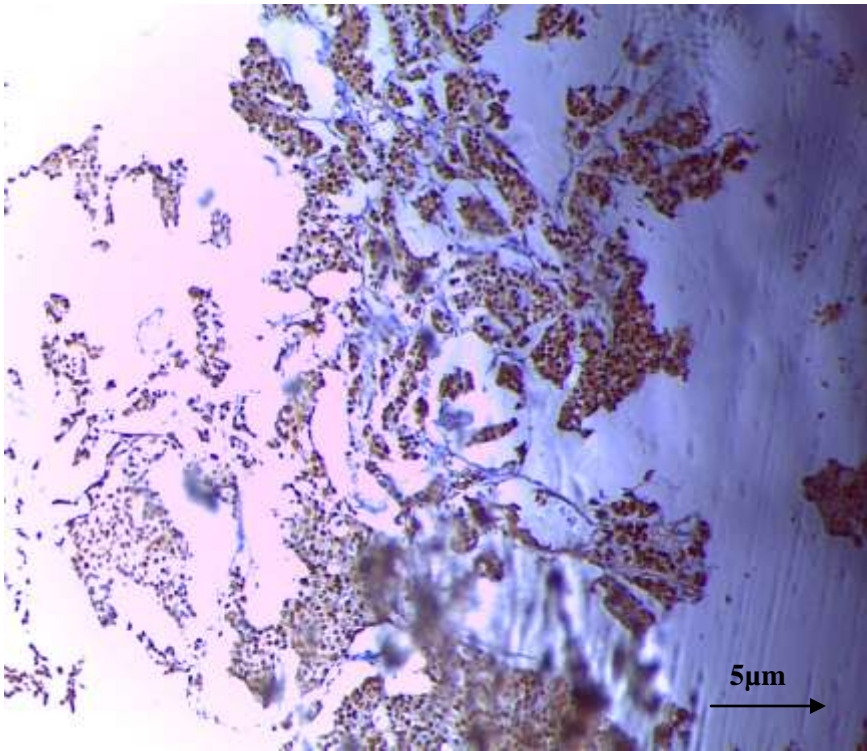
**Plate 72:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 5 ml/100 g. b. w. of GCW for 16 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 73:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 10 ml/100 g. b. w. of GCW for 16 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

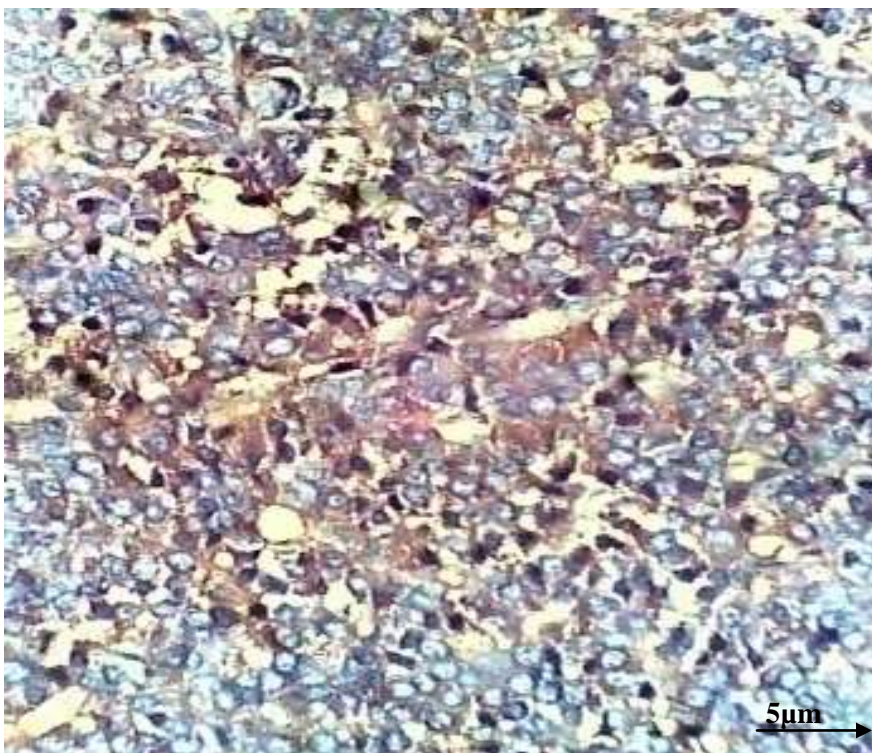
Antibody: Rabbit monoclonal oestrogen receptor antibody



**Plate 74:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 5 ml/100 g. b. w. of GCW for 28 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody





**Plate 75:** Immunohistochemical section of the pituitary gland of Sprague-Dawley rat treated with 10 ml/100 g. b. w. of GCW for 28 days; shows high and extensive expression of oestrogen antigen-antibody complex at magnification of X100.

Antibody: Rabbit monoclonal oestrogen receptor antibody



## 4.6 ESTROUS CYCLE STUDIES

### 4.6.1 The number of estrous cycles

The animals in the control group completed each cycle at an average of 4 days. The number of estrous cycles in the 8, 16 and 28 days experimental durations were  $2 \pm 0.10$ ,  $4 \pm 0.04$  and  $7.38 \pm 0.04$  respectively (Table 3a, b and c). The induced group demonstrated a significant decrease in the number of cycles when compared with the control group ( $3.20 \pm 0.45$  vs  $7.38 \pm 0.04$ ) as the animals spent more number of days in completing a single cycle (Table 3c). There were significant decrease in the number of estrous cycles in the respective days of withdrawal when compared with the control. (Table 3a, b and c). The withdrawal of the administration of GCW for 28 days showed significant decrease in the number of estrous cycles when compared with the group post-treated with GCW for the same experimental duration. In the 8 and 16 days post-treated groups, significant decreases in the number of estrous cycles were demonstrated when compared with the control (Table 3a and b). However, the groups post-treated with GCW for 28 days demonstrated comparable number of cycles with the control group of the same experimental duration ( $6.01 \pm 0.01$  and  $6.40 \pm 0.55$  vs  $7.38 \pm 0.04$ ). This shows that by the 28<sup>th</sup> day of post-treatment with GCW, a reversal of normal cyclic pattern was achieved (Table 3c). There were significant decrease in the number of estrous cycles in the co-administered and pre-treated groups when compared with the control (Table 3c). The administration of GCW for 8, 16 and 28 days demonstrated value in the number of estrous cycles that were statistically comparable with the control. The animals completed a cycle in an average duration of 4 days (Table 3a, b and c).

**TABLE 3a:** The Number of Estrous Cycles in the 8 Days Experimental Groups in Female Sprague-Dawley Rats.

GROUP	SUB-GROUP DETAIL	NUMBER OF ESTROUS CYCLES
Control	DSTL <sub>8 days</sub>	2.00 ± 0.10
Withdrawal	MCH <sub>28 days</sub> -WD <sub>8 days</sub>	0.00 ± 0.00*
Post-treated	MCH <sub>28 days</sub> -GCW <sub>L8 days</sub>	0.00 ± 0.00*
	MCH <sub>28 days</sub> -GCW <sub>H8 days H/d</sub>	0.00 ± 0.00*
GCW treated	GCW <sub>L8 days</sub>	2.00 ± 0.06
	GCW <sub>H 8 days</sub>	2.00 ± 0.20

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5.

Significant differences; \*  $p < 0.05$

Key for table 3a: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; –: treated followed by.

**TABLE 3b:** The Number of Estrous Cycles in the 16 Days Experimental Groups in Female Sprague-Dawley Rats.

GROUP	SUB-GROUP DETAIL	NUMBER OF ESTROUS CYCLES
Control	DSTL <sub>16 days</sub>	4.00 ± 0.04
Withdrawal	MCH <sub>28 days</sub> -WD <sub>16 days</sub>	0.00 ± 0.00
Post-treated	MCH <sub>28 days</sub> - GCW <sub>L16 days</sub>	1.80 ± 0.45*
	MCH <sub>28 days</sub> - GCW <sub>H16 days</sub>	2.00 ± 0.78*
GCW treated	GCW <sub>L16 days</sub>	4.00 ± 0.10
	GCW <sub>H16 days</sub>	3.80 ± 0.45

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5.

Significant differences; \*  $p < 0.05$

Key for table 3b: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; —: treated followed by.

**TABLE 3c:** The Number of Estrous Cycles in the 28 Days Experimental Groups in Female Sprague-Dawley Rats.

GROUP	SUB-GROUP DETAIL	NUMBER OF ESTROUS CYCLES
Control	DSTL <sub>28 days</sub>	7.38 ± 0.04
Induction	MCH <sub>28 days</sub>	3.20 ± 0.45**
Withdrawal	MCH <sub>28 days</sub> -WD <sub>28 days</sub>	4.40 ± 0.55*
Post-treated	MCH <sub>28 days</sub> - GCW <sub>L28 days</sub>	6.01 ± 0.01
	MCH <sub>28 days</sub> - GCW <sub>H28 days</sub>	6.40 ± 0.55
Co-administered	MCH <sub>28 days</sub> + GCW <sub>L28 days</sub>	5.60 ± 0.55*
	MCH <sub>28 days</sub> + GCW <sub>H28 days</sub>	4.80 ± 0.45*
Pre-treated	GCW <sub>L8 days</sub> -MCH <sub>28 days</sub>	5.40 ± 0.55*
	GCW <sub>H8 days</sub> -MCH <sub>28 days</sub>	5.00± 0.00*
	GCW <sub>L16 days</sub> -MCH <sub>28 days</sub>	5.40 ± 0.55*
	GCW <sub>H16 days</sub> -MCH <sub>28 days</sub>	5.20± 0.45*
	GCW <sub>L28 days</sub> -MCH <sub>28 days</sub>	4.40 ± 0.55*
	GCW <sub>H28 days</sub> -MCH <sub>28 days</sub>	5.20 ± 0.45*
GCW treated	GCW <sub>L28 days</sub>	7.00 ± 0.10
	GCW <sub>H28 days</sub>	7.20 ± 0.11

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5. Significant differences; \*  $p < 0.05$ , \*\* 0.01.

**Key for table 3c:** DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g b.w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by; +: treated concurrently.



#### **4.6.2 The number of days spent on each phase of estrous cycle**

The animals in the control group exhibited an average of 4 days of cycling with phases changing from metestrus, diestrus, proestrus to estrus leading to an approximate equal number of days spent on each phase of the estrous cycle (Table 4a, b and c). The induced group demonstrated significant differences in the number of days spent on each phase of the cycle when compared with the control. There was a significant increase in the number of days spent on the diestrus phase and decrease in the number of days spent on metestrus, proestrus and estrus (Table 4c). In the withdrawal groups, significant decreases in the number of days spent on metestrus, proestrus and estrus and increase in the number of days spent on the diestrus were demonstrated (Table 4a, b and c). The groups post-treated with GCW for 8 and 16 days demonstrated significant decreases in the number of days spent on metestrus, proestrus and estrus and increase in the number of days spent on the diestrus phase (Table 4a and b). The group post-treated with GCW for 28 days demonstrated comparable values in the number days spent on each phase of estrous cycle with the control group (Table 4c). This shows that by the 28<sup>th</sup> day of post-treatment with GCW, a complete reversal of acyclicity was achieved. The Co-administered and pre-treated groups demonstrated significant increase in the number of days spent on the diestrus phase and decreases in the number of days spent on metestrus, proestrus and estrus (Table 4c). The animals in the GCW-treated groups spent an approximately equal number of days on each phase of estrous cycle. The administration of GCW for 8, 16 and 28 days demonstrated comparable values in the number of days spent on each phase of estrous cycle when compared with the control. (Table 4a, b and c).

**TABLE 4a:** The Number of Days Spent on Each Phase of Estrous Cycle in the 8 Days Experimental Groups in Female Sprague-Dawley Rats

GROUP	SUB-GROUP DETAIL	METESTRUS	DIESTRUS	PROESTRUS	ESTRUS
Control	DSTL <sub>8 days</sub>	2.00 ± 0.01	2.00 ± 0.50	2.00 ± 0.22	2.00 ± 0.10
Withdrawal	MCH <sub>28 days</sub> -WD <sub>8 days</sub>	0.00 ± 0.00	8.00 ± 0.00**	0.00 ± 0.00	0.00± 0.00
Post-treated	MCH <sub>28 days</sub> -GCW <sub>L8 days</sub>	0.00 ± 0.00	8.00 ± 0.00 **	0.00 ± 0.00	0.00± 0.00
	MCH <sub>28 days</sub> -GCW <sub>H8 days</sub>	0.00 ± 0.00	7.40 ± 0.55 **	0.60 ± 0.55*	0.00± 0.00
GCW treated	GCW <sub>L8 days</sub>	1.80 ± 0.56	2.00 ± 0.00	2.20 ± 0.55	2.00± 0.00
	GCW <sub>H 8 days</sub>	1.80 ± 0.55	2.00 ± 0.00	2.20 ± 0.75	2.00± 0.00

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5.

Significant differences; \*  $p < 0.05$ , \*\*  $P < 0.001$ .

Key for table 4a: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b.w. of green coconut water; –: treated followed by.

**TABLE 4b:** The Number of Days Spent on Each Phase of Estrous Cycle in the 16 Days Experimental Groups in Female Sprague-Dawley Rats

GROUP	SUB-GROUP DETAIL	METESTRUS	DIESTRUS	PROESTRUS	ESTRUS
Control	DSTL <sub>16 days</sub>	4.00 ± 0.01	4.00 ± 0.40	4.00 ± 0.06	4.00 ± 0.30
Withdrawal	MCH <sub>28 days</sub> -WD <sub>16 days</sub>	0.00 ± 0.00	15.40 ± 0.55**	0.60 ± 0.55**	0.00 ± 0.00
Post-treated	MCH <sub>28 days</sub> - GCW <sub>L16 days</sub>	1.00 ± 0.00*	11.40 ± 0.89**	2.20 ± 0.45*	1.20 ± 0.45*
	MCH <sub>28 days</sub> - GCW <sub>H16 days</sub>	1.80 ± 0.45*	10.00 ± 1.22**	2.40 ± 0.55*	0.80 ± 0.45*
GCW treated	GCW <sub>L16 days</sub>	3.80 ± 0.45	4.00 ± 0.00	4.20 ± 0.45	4.00 ± 0.00
	GCW <sub>H16 days</sub>	3.70 ± 0.84	4.40 ± 0.55	4.10 ± 0.55	3.80 ± 0.45

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5.

Significant differences; \*  $p < 0.05$ , \*\*  $P < 0.001$ .

Key for table 4b: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by.

**TABLE 4c:** The Number of Days Spent on Each Phase of Estrous Cycle in the 28 Days Experimental Groups in Female Sprague-Dawley Rats

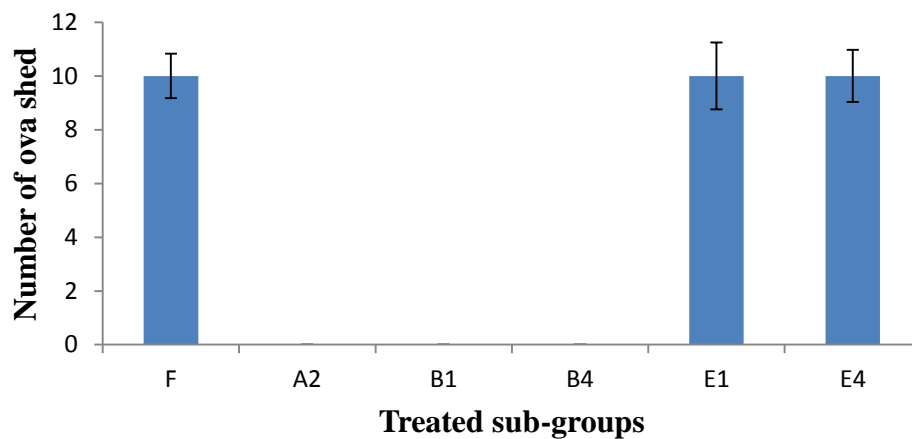
GROUP	SUB-GROUP DETAIL	METESTRUS	DIESTRUS	PROESTRUS	ESTRUS
Control	DSTL <sub>28 days</sub>	7.00 ± 0.03	7.00 ± 0.41	7.00 ± 0.20	7.00 ± 0.10
Induction	MCH <sub>28 days</sub>	2.40 ± 0.55 *	18.00 ± 1.00**	3.00 ± 0.00*	3.60 ± 0.55*
Withdrawal	MCH <sub>28 days</sub> -WD <sub>28 days</sub>	4.80 ± 0.84*	14.40 ± 1.14**	4.40 ± 0.55*	4.40 ± 0.55*
Post-treated	MCH <sub>28 days</sub> - GCW <sub>L28 days</sub>	6.20 ± 0.45	9.60 ± 0.55	6.00 ± 0.01	6.80 ± 0.45
	MCH <sub>28 days</sub> - GCW <sub>H28 days</sub>	6.80 ± 0.45	9.20 ± 0.45	6.00 ± 0.03	6.00 ± 0.01
Co-administered	MCH <sub>28 days</sub> + GCW <sub>L28 days</sub>	5.60 ± 0.89*	11.40 ± 1.67**	5.40 ± 0.55*	5.60 ± 0.55*
	MCH <sub>28 days</sub> + GCW <sub>H28 days</sub>	4.40 ± 0.54*	14.40 ± 1.14**	4.60 ± 0.54*	4.80 ± 0.45*
Pre-treated	GCW <sub>L8 days</sub> -MCH <sub>28 days</sub>	4.40± 0.89*	12.00±1.22**	5.40± 0.55*	5.60±0.54*
	GCW <sub>H8 days</sub> -MCH <sub>28 days</sub>	5.00± 0.04*	13.20±1.30**	5.00± 0.010*	5.40±0.54*
	GCW <sub>L16 days</sub> -MCH <sub>28 days</sub>	5.20± 0.84*	12.60±0.89**	5.40± 0.54*	5.60±0.55*
	GCW <sub>H16 days</sub> -MCH <sub>28 days</sub>	5.40± 0.55*	12.00±1.22**	5.20± 0.45*	5.40±0.55*
	GCW <sub>L28 days</sub> -MCH <sub>28 days</sub>	5.00 ± 0.00*	13.20 ± 1.30**	4.60 ± 0.89*	5.20 ± 0.45*
GCW treated	GCW <sub>L28 days</sub>	6.60 ± 0.45	7.10 ± 0.45	7.20 ± 0.84	7.10 ± 0.55
	GCW <sub>H28 days</sub>	6.90 ± 0.45	6.90 ± 0.45	7.40 ± 0.55	7.01 ± 0.00

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5. Significant differences; \*  $P < 0.05$ , \*\*  $P < 0.01$   
Key for table 4c: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; – : treated followed by; +: treated concurrently.



#### 4.7 NUMBER OF OVA SHED

The animals in the control group showed normal value (10.00) in the number of ova count in the upper part of the oviduct (Figure 6a-c). There were no ova seen in the oviducts of the animals in the induced group and the group withdrawn for 8 days (Figure 6a and c). In the oviduct of the 16 and 28 days withdrawal groups, there were decrease in the number of ova count when compared with the value of the control group. This implies that by the 28<sup>th</sup> day of withdrawal, the number of ova count were lower than the values of the control group (Figure 6a-c). There were no ova seen in the groups post-treated with GCW for 8 days while decrease in the number of ova count were seen in 16 days post-treated groups when compared with the control. However, in the 28 days post-treated groups the numbers of ova count were normal and comparable with the value of the control. This implies that by the 28<sup>th</sup> day of post-treatment, the number of ova had increased and were comparable to the value obtained from the control group (Figure 6a-c). There were no ova seen in the oviducts of the animals in the co-administered and pre-treated groups which were comparable with the expression in the induced group (Figure 6c). The numbers of ova count in all GCW- treated groups were of normal values and were comparable with the control (Figure 6a-c).



**Figure 6a:** The Number of Ova Count in the 8 Days Experimental Groups in Female Sprague-Dawley rats.

Sub-group Detail: F- DSTL<sub>8 days</sub>

A2 - MCH<sub>28 days</sub>-WD<sub>8 days</sub>

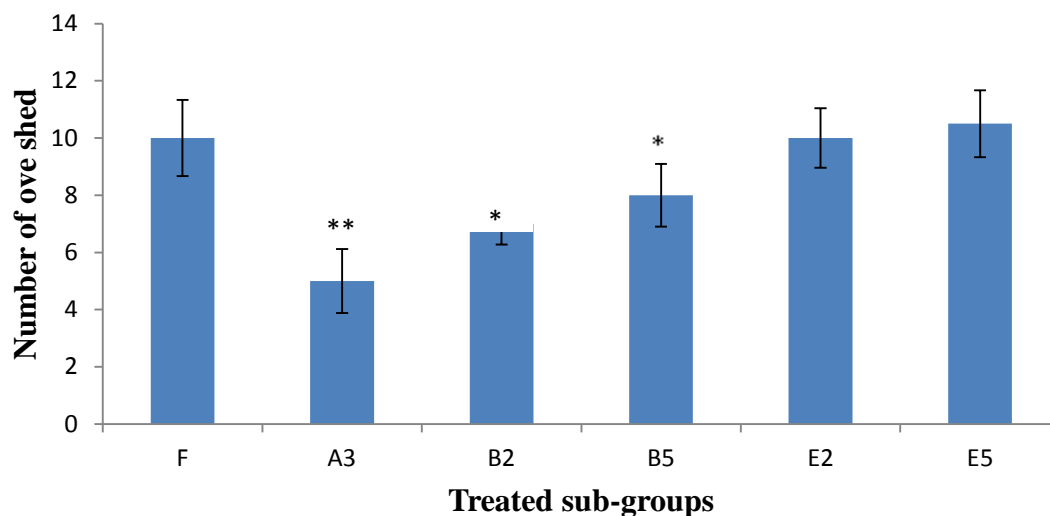
B1 - MCH<sub>28 days</sub>-GCW<sub>L8 days</sub>

B4 - MCH<sub>28 days</sub>-GCW<sub>H8 days</sub>

E1 - GCW<sub>L8 days</sub>

E4 - GCW<sub>H8 days</sub>

Key for Figure 6a: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by.



**Figure 6b:** The Number of Ova Count in the 16 Days Experimental Groups in Female Sprague-Dawley rats. Significant differences; \*\*  $P < 0.01$ , \*  $P < 0.05$ .

Sub-group Detail: F- DSTL<sub>16 days</sub>

A3 - MCH<sub>28days</sub>-WD<sub>16days</sub>

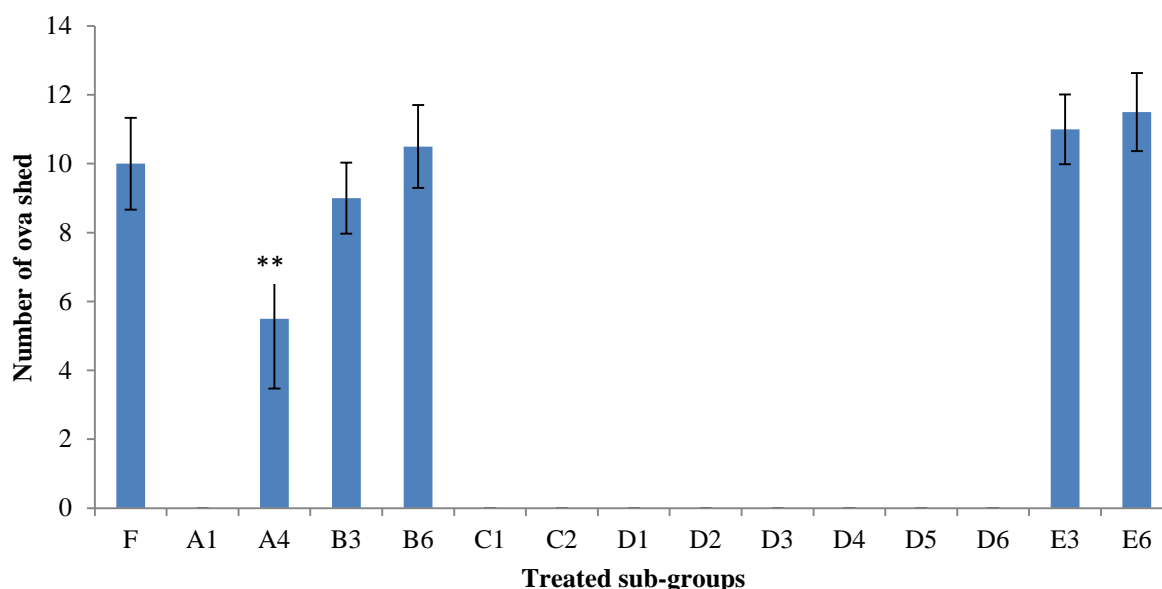
B2 - MCH<sub>28days</sub>-GCW<sub>L16 days</sub>

B5 - MCH<sub>28 days</sub>-GCW<sub>H16 days</sub>

E2 - GCW<sub>L16 days</sub>

E5 - GCW<sub>H16 days</sub>

Key for Figure 6b: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>M/d</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H/d</sub>: 10 ml/100 g. b. w. of green coconut water; —: treated followed by.



**Figure 6c:** The Number of Ova Count in the 28 Days Experimental Groups in Female Sprague-Dawley rats. Significant differences; \*\*  $P < 0.01$

Sub-group Detail: F- DSTL<sub>28 days</sub>; A1 – MCH<sub>28 days</sub>; A4 - MCH<sub>28 days</sub>- WD<sub>28 days</sub>; B3 - MCH<sub>28 days</sub>- GCW<sub>L28 days</sub>; B6 - MCH<sub>28 days</sub>-GCW<sub>H28 days</sub>; C1- MCH<sub>28 day</sub> + GCW<sub>L28 day</sub>; C2- MCH<sub>28 day</sub> + GCW<sub>H28 day</sub>; D1- GCW<sub>L8 days</sub> - MCH<sub>28 days</sub>; D2- GCW<sub>H8 days</sub> - MCH<sub>28 days</sub>; D3- GCW<sub>L16 days</sub> - MCH<sub>28 days</sub>; D4- GCW<sub>H16 days</sub> - MCH<sub>28 days</sub>; D5- GCW<sub>L28 days</sub> - MCH<sub>28 days</sub>; D6- GCW<sub>H28 days</sub> - MCH<sub>28 days</sub>; E3 - GCW<sub>L28 days</sub>; E6- GCW<sub>H28 days</sub>

Key for Figure 6c: DSTL: Distilled water; MCH: 0.2 mg/100 g b.w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by; +: treated concurrently.



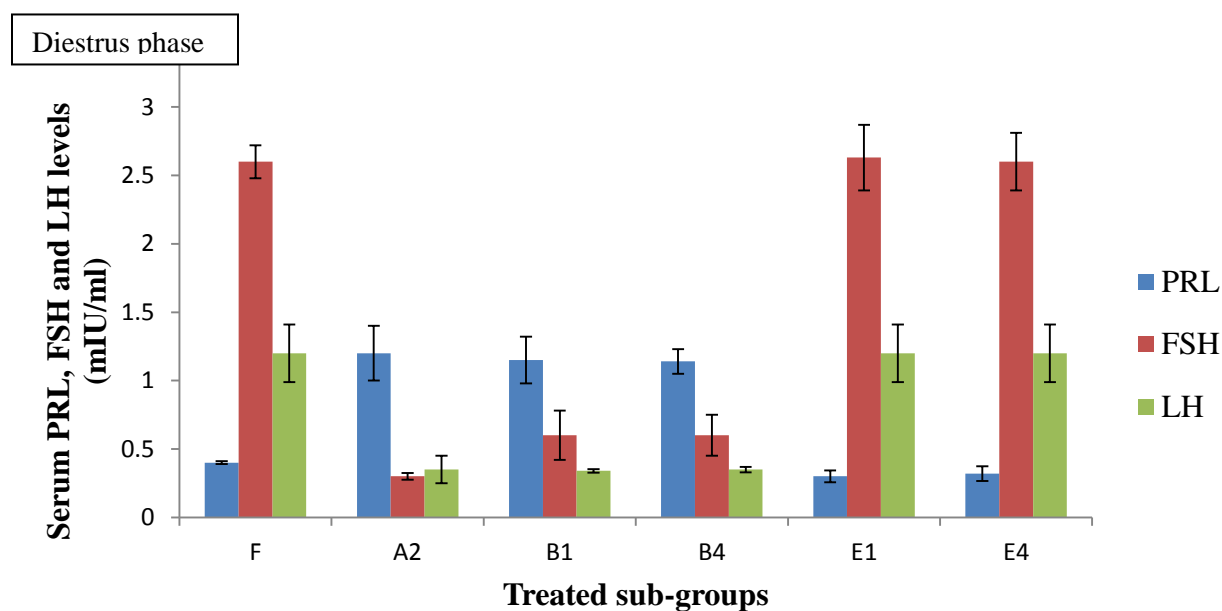
## 4.8 HORMONAL MILIEU

### 4.8.1. Serum Prolactin, Luteinizing and Follicle Stimulating Hormone

In the control group, there was low serum prolactin level on the diestrus and estrus phase of the estrous cycle (Figure 7a- c). The serum value of FSH was higher on the diestrus phase than the estrus phase while the serum level of LH was higher on the estrus phase than the value of the diestrus phase (Figure 7a-b). This implies that there was a high serum level of FSH during the period of follicular development and high LH level during the period of ovulation and the formation of corpus luteum. The induced group demonstrated significant increase in serum PRL level and decrease in serum LH and FSH levels when compared with the control group (Figure 7c). There were gradual decrease in serum PRL levels as the number of days of withdrawal increases from 8, 16 to 28 days (Figure 7a- c), despite the gradual decrease, a higher value of serum PRL was seen after 28 days of withdrawal when compared with the control value (Figure 7c). There were gradual increases in the serum values of FSH and LH with increase in the number of days of withdrawal (Figure 7a- c). However, significant lower values of FSH and LH were obtained in the 28 days withdrawal group when compared with the value of the control (Figure 7c). The post-treated groups demonstrated gradual decrease in serum PRL level with increase in the number of days of post-treatment with green coconut water from 8, 16 to 28 days (Figure 7a-c), and by the 28<sup>th</sup> day of post-treatment, the serum level of PRL was low and comparable with the value of the control (0.5 and 0.38 mIU/ml versus 0.4 mIU/ml ) (Figure 7c). Furthermore, there were increases in the serum values of FSH and LH with increase in the number of days of post-treatment with green coconut water from 8, 16 to 28 days (Figure 7a-c). The levels of FSH (0.5 and 0.38 mIU/ml versus 0.4 mIU/ml) and LH (1.25 and 1.25 mIU/ml versus 1.225 mIU/ml) in the 28 days post-treated groups were high and comparable with the level

in the control group (Figure 7c). There were significant increase in serum PRL level and decrease in serum LH and FSH levels in the co-administered and pre-treated groups when compared with the control group (Figure 7c). The administration of GCW for 8, 16 and 28 days demonstrated low value in serum PRL level and high levels of FSH and LH (Figure 7a-c).





**Figure 7a:** Serum Levels of Prolactin, Luteinizing and Follicle Stimulating Hormone in the 8 Days Experimental Groups in Female Sprague-Dawley rats; \*\*  $P < 0.01$

Sub-group Detail: Sub-group Detail: F- DSTL

A2 - MCH<sub>28 days</sub>-WD<sub>8 days</sub>

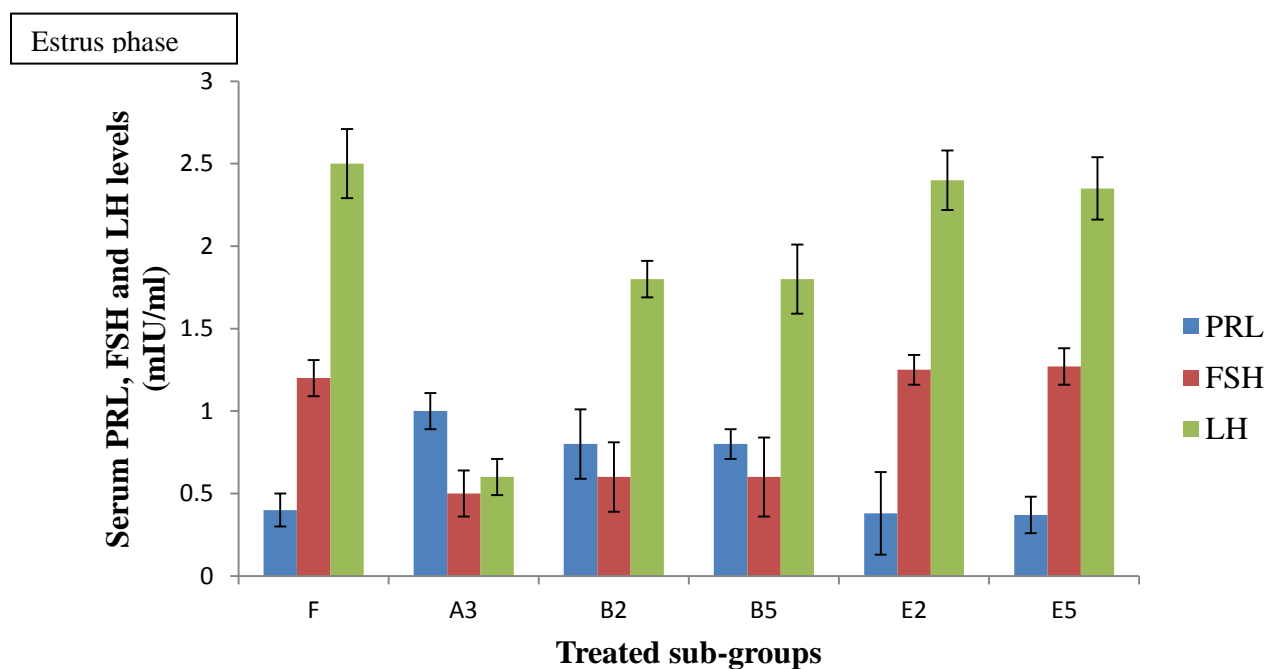
B1 - MCH<sub>28 days</sub>-GCW<sub>L8 days</sub>

B4 - MCH<sub>28 days</sub>-GCW<sub>H8 days</sub>

E1 - GCW<sub>L8 days</sub>

E4 - GCW<sub>H8 days</sub>

Key for Figure 7a: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; —: treated followed by.



**Figure 7b:** Serum Levels of Prolactin, Luteinizing and Follicle Stimulating Hormone in the 16 Days Experimental Groups in Female Sprague-Dawley rats; \*  $P < 0.05$ , \*\*  $P < 0.01$

Sub-group Detail: F- DSTL

A3 - MCH<sub>28days</sub>-WD<sub>16days</sub>

B2 - MCH<sub>28days</sub>-GCW<sub>L16 days</sub>

B5 - MCH<sub>28 days</sub>-GCW<sub>H16 days</sub>

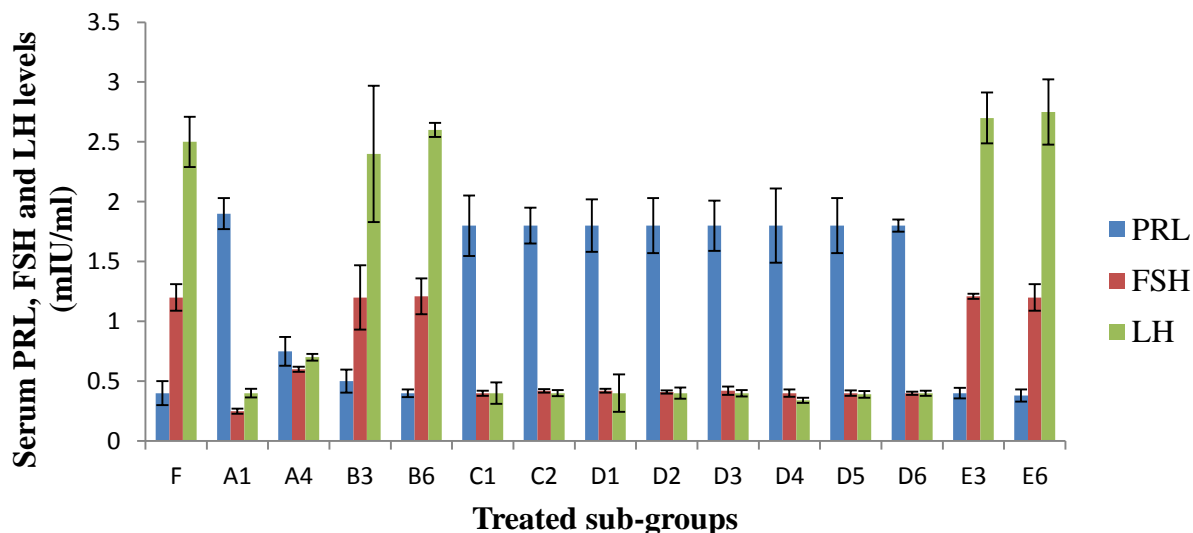
E2 - GCW<sub>L16 days</sub>

E5 - GCW<sub>H16 days</sub>

Key for Figure 7b: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>M/d</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H/d</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by.



**Diestrus** -A1, C1-2, D1-6  
**Estrus** – F, A4,B3, B6, E3 and E6



**FIGURE 7c:** Serum Levels of Prolactin, Luteinizing and Follicle Stimulating Hormone in the 28 Days Experimental Groups in Female Sprague-Dawley rats; \*\*  $P < 0.01$ , \*  $P < 0.05$ .

Sub-group Detail: F- DSTL; A1 – MCH<sub>28</sub> days; A4 - MCH<sub>28</sub> days- WD<sub>28</sub> days; B3 - MCH<sub>28</sub> days-GCW<sub>L28</sub> days; B6 - MCH<sub>28</sub> days-GCW<sub>H28</sub> days; C1- MCH<sub>28</sub> day + GCW<sub>L28</sub> day; C2- MCH<sub>28</sub> day + GCW<sub>H28</sub> day; D1- GCW<sub>L8</sub> days - MCH<sub>28</sub> days; D2- GCW<sub>H8</sub> days - MCH<sub>28</sub> days; D3- GCW<sub>L16</sub> days - MCH<sub>28</sub> days; D4- GCW<sub>H16</sub> days - MCH<sub>28</sub> days; D5- GCW<sub>L28</sub> days - MCH<sub>28</sub> days; D6- GCW<sub>H28</sub> days - MCH<sub>28</sub> days; E3 - GCW<sub>L28</sub> days; E6- GCW<sub>H28</sub> days

Key for Figure 7c: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by; +: treated concurrently.

#### 4.8.2 Serum Oestrogen

The control group demonstrated normal serum oestrogen level of  $28.76 \pm 0.51$  and  $27.15 \pm 0.23$  on the diestrus and estrus phase respectively (Table 5a-b). There was a significant decrease in the value of serum oestrogen level in induced group when compared with the value of the control (Table 5c). There was gradual increase in the value of serum oestrogen level with increase in the number of days of withdrawal (Table 5a-c). Despite the gradual increase, significant lower value of oestrogen was obtained in the 28 days withdrawal group when compared with the control (Table 5c). The post-treated groups demonstrated gradual increase in the values of serum oestrogen levels with increase in days of post-treatment with GCW and by the 28<sup>th</sup> day of post-treatment, the serum level of oestrogen both at low and high doses were comparable with control group;  $28.66 \pm 0.50$  and  $30.96 \pm 2.54$  versus  $28.76 \pm 0.51$  mg/ml (Table 5c). The Co-administered and the pre-treated groups demonstrated significant lower values of serum oestrogen when compared with the control group (Table 5c). The administration of GCW showed values of serum oestrogen that were comparable with the control (Table 5c).

**Table 5a:** The Serum Levels of Oestrogen in the 8 Days Experimental Groups in Female Sprague-Dawley Rats (Diestrus phase).

Diestrus phase		
GROUP	SUB-GROUP DETAIL	OESTROGEN (mg/ml)
Control (F1)	DSTL <sub>8 days</sub>	28.76 ± 0.51
Withdrawal (A2)	MCH <sub>28 days</sub> -WD <sub>8 days</sub>	6.67 ± 0.42**
Post-treated (B1)	MCH <sub>28 days</sub> -GCW <sub>L8 days</sub>	8.96 ± 0.92**
(B4)	MCH <sub>28 days</sub> -GCW <sub>H8 days</sub>	10.06 ± 0.16**
GCW treated (E1)	GCW <sub>L8 days</sub>	29.21 ± 4.31
(E4)	GCW <sub>H8 days</sub>	29.12 ± 0.26

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5.

Significant differences; \*\* $P < 0.001$

Key for table 5a: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; –: treated followed by.

**TABLE 5b:** The Serum Levels of Oestrogen in the 16 Days Experimental Groups in Female Sprague-Dawley Rats.

Estrus phase		
GROUP	SUB-GROUP DETAIL	OESTROGEN (mg/ml)
Control (F2)	DSTL <sub>16 days</sub>	27.15 ± 0.23
Withdrawal (A3)	MCH <sub>28 days</sub> -WD <sub>16 days</sub>	9.30 ± 0.52**
Post-treated (B2)	MCH <sub>28 days</sub> - GCW <sub>L16 days</sub>	22.60 ± 0.37*
(B4)	MCH <sub>28 days</sub> - GCW <sub>H16 days</sub>	23.04 ± 0.35*
GCW treated (E2)	GCW <sub>L16 days</sub>	28.28 ± 0.04
(E5)	GCW <sub>H16 days</sub>	28.08 ± 3.89

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5.

Significant differences; \*\* $P < 0.001$

Key for table 5b: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by.



**TABLE 5c:** The Serum Levels of Oestrogen in the 26 Days Experimental Groups in Female Sprague-Dawley Rats

Diestrus -A1, C1-2, D1-6, F1; Estrus – F2, A4,B3, B6, E3, E6, F2		
GROUP	SUB-GROUP DETAIL	OESTROGEN (mg/ml)
Control (F1)	DSTL <sub>28 days</sub>	28.76 ± 0.51
(F2)	DSTL <sub>28 days</sub>	27.15 ± 0.23
Induction (A1)	MCH <sub>28 days</sub>	3.91 ± 0.29**
Withdrawal (A4)	MCH <sub>28 days</sub> -WD <sub>28 days</sub>	19.30 ± 2.54*
Post-treated (B3)	MCH <sub>28 days</sub> - GCW <sub>L28 days</sub>	28.66 ± 0.50
(B6)	MCH <sub>28 days</sub> - GCW <sub>H28 days</sub>	30.96 ± 2.54
Co-administered (C1)	MCH <sub>28 days</sub> + GCW <sub>L28 days</sub>	4.17 ± 0.31**
(C2)	MCH <sub>28 days</sub> + GCW <sub>H28 days</sub>	4.17 ± 0.57**
Pre-treated (D1)	GCW <sub>L8 days</sub> -MCH <sub>28 days</sub>	4.19± 0.32**
(D2)	GCW <sub>H8 days</sub> -MCH <sub>28 days</sub>	3.93± 0.66**
(D3)	GCW <sub>L16 days</sub> -MCH <sub>28 days</sub>	4.09± 0.49**
(D4)	GCW <sub>H16 days</sub> -MCH <sub>28 days</sub>	4.25± 1.034**
(D5)	GCW <sub>L28 days</sub> -MCH <sub>28 days</sub>	4.13 ± 1.41**
(D6)	GCW <sub>H28 days</sub> -MCH <sub>28 days</sub>	4.41± 0.03**
GCW treated (E3)	GCW <sub>L28 days</sub>	29.96 ± 5.35
(E6)	GCW <sub>H28 days</sub>	30.04 ± 4.88

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5. Significant differences; \*\*  $P < 0.001$ , \*  $P < 0.005$

Key for table 5c: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by; +: treated concurrently.

### 4.8.3 Serum Dopamine Levels

The values of serum dopamine in all experimental groups were comparable with the values of the control (Table 6a-c).

**TABLE 6a:** The Serum Levels of Dopamine in the 8 Days Experimental Groups in Female Sprague-Dawley Rats.

GROUP	SUB-GROUP DETAIL	DOPAMINE (ng/ml)
Control	DSTL <sub>8 days</sub>	8.58 ± 0.791
Withdrawal	MCH <sub>28 days</sub> -WD <sub>8 days</sub>	8.58 ± 0.094
Post-treated	MCH <sub>28 days</sub> -GCW <sub>L8 days</sub>	8.55 ± 0.125
	MCH <sub>28 days</sub> -GCW <sub>H8 days</sub>	8.80 ± 0.054
		8.48 ± 0.891
GCW treated	GCW <sub>L8 days</sub>	8.54 ± 0.790
	GCW <sub>H8 days</sub>	

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5.

Key for table 6a: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by.

**TABLE 6b:** The Serum Levels of Dopamine in the 16 Days Experimental Groups in Female Sprague-Dawley Rats.

GROUP	SUB-GROUP DETAIL	DOPAMINE (ng/ml)
Control	DSTL <sub>16 days</sub>	8.58 ± 0.791
Withdrawal	MCH <sub>28 days</sub> -WD <sub>16 days</sub>	8.81 ± 0.054
Post-treated	MCH <sub>28 days</sub> - GCW <sub>L16 days</sub>	8.80 ± 0.055
	MCH <sub>28 days</sub> - GCW <sub>H16 days</sub>	8.81± 0.054
GCW treated	GCW <sub>L16 days</sub>	8.58 ± 0.870
	GCW <sub>H16 days</sub>	8.68 ± 0.890

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5.

Key for table 6b: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b.w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by.

**TABLE 6c:** The Serum Levels of Dopamine in the 28 Days Experimental Groups in Female Sprague-Dawley Rats.

GROUP	SUB-GROUP DETAIL	DOPAMINE (ng/ml)
Control	DSTL <sub>28days</sub>	8.76 ± 0.509
Induction	MCH <sub>28 days</sub>	8.46 ± 0.048
Withdrawal	MCH <sub>28 days</sub> -WD <sub>28 days</sub>	8.92 ± 0.039
Post-treated	MCH <sub>28 days</sub> - GCW <sub>L28 days</sub>	8.92 ± 0.038
	MCH <sub>28 days</sub> - GCW <sub>H28 days</sub>	8.91 ± 0.038
Co-administered	MCH <sub>28 days</sub> + GCW <sub>L28 days</sub>	8.42 ± 0.048
	MCH <sub>28 days</sub> + GCW <sub>H28 days</sub>	8.41 ± 0.047
Pre-treated	GCW <sub>L8 days</sub> -MCH <sub>28 days</sub>	8.41 ± 0.047
	GCW <sub>H8 days</sub> -MCH <sub>28 days</sub>	8.42 ± 0.048
	GCW <sub>L16 days</sub> -MCH <sub>28 days</sub>	8.46 ± 0.048
	GCW <sub>H16 days</sub> -MCH <sub>28 days</sub>	8.42 ± 0.048
	GCW <sub>L28 days</sub> -MCH <sub>28 days</sub>	8.42 ± 0.407
	GCW <sub>H28 days</sub> -MCH <sub>28 days</sub>	8.42 ± 0.048
GCW treated	GCW <sub>L28 days</sub>	8.42 ± 0.048
	GCW <sub>H28 days</sub>	8.78 ± 0.691

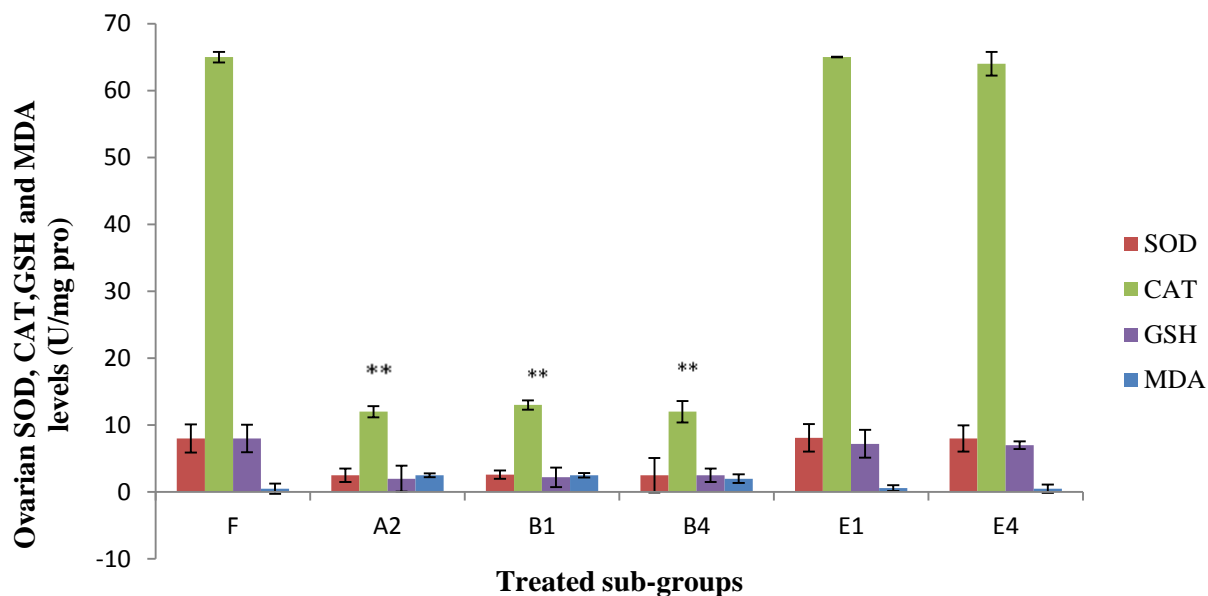
All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5.

Key for table 6c: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by; +: treated concurrently.



#### 4.9 OVARIAN OXIDATIVE STRESS MARKERS

There were high levels of SOD, CAT and GSH and low concentration of MDA in the ovarian tissues of the control group (Figure 8a-c). In the induced group, there were significant lower levels of SOD, CAT and GSH and higher level of MDA in the ovarian tissues when compared with the control (Figure 8c). The withdrawal groups demonstrated significant lower levels of SOD, CAT and GSH and higher value in the concentration of MDA in the ovarian tissues when compared with the control (Figure 8a-c). There was gradual increase in the concentrations of SOD, CAT and GSH with increase with the number of days of post-treatment and by the 28<sup>th</sup> day, the concentrations of SOD, CAT and GSH were comparable with the control group; SOD: 7.52 and 7.52 U/mgpro versus 7.12 U/mgpro, CAT: 63.67 and 65.87 U/mgpro versus 66.87 U/mgpro and GSH: 6.91 and 7.17 U/mgpro versus 7.47 U/mgpro (Figure 8a-c). Additionally, there was gradual decrease in the concentration of MDA as the number of days of post-treatment increases and by the 28<sup>th</sup> day, the concentration of ovarian MDA was comparable with the control group; 0.59 and 0.64 U/mgpro versus 0.62 U/mgpro (Figure 8a-c). The Co-administered groups demonstrated higher levels of SOD, CAT and GSH and lower level of MDA when compared with the values of the control group (Figure 8c). However, there were significant lower levels of SOD, CAT and GSH and higher concentration of MDA in the ovarian tissues of the pre-treated group when compared with the control (Figure 8c). The administration of GCW in all experimental duration demonstrated high levels of SOD, CAT and GSH and low level of MDA in the ovarian tissues (Figure 8a-c).



**FIGURE 8a:** Ovarian Concentrations of SOD, CAT, GSH AND MDA Concentrations in 8 Days Experimental Groups in Female Sprague-Dawley Rats. Significant differences; \*\*  $P < 0.001$

Sub-group Detail: Sub-group Detail:

F- DSTL<sub>8 days</sub>

A2 - MCH<sub>28 days</sub>-WD<sub>8 days</sub>

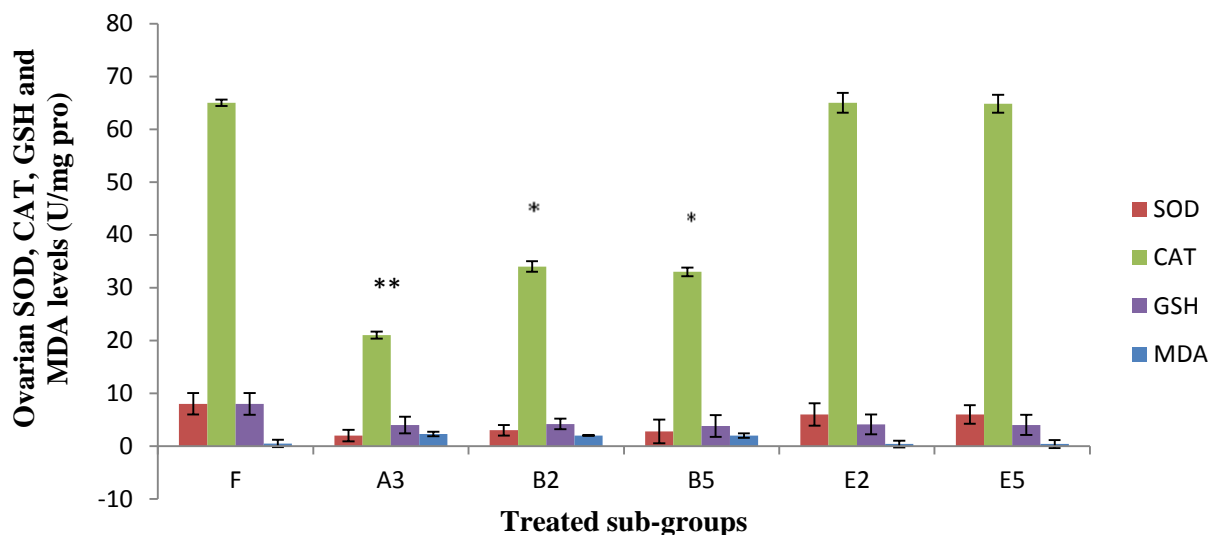
B1 - MCH<sub>28 days</sub>-GCW<sub>L8 days</sub>

B4 - MCH<sub>28 days</sub>-GCW<sub>H8 days</sub>

E1 - GCW<sub>L8 days</sub>

E4 - GCW<sub>H8 days</sub>

Key for Figure 8a: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b.w. of green coconut water; —: treated followed by.



**FIGURE 8b:** Ovarian Concentrations of SOD, CAT, GSH AND MDA Concentrations in 16 Days Experimental Groups in Female Sprague-Dawley Rats.

Significant differences; \*\* $P < 0.001$ ; \* $P < 0.05$

Sub-group Detail: F- DSTL<sub>16 days</sub>

A3 - MCH<sub>28days</sub>-WD<sub>16days</sub>

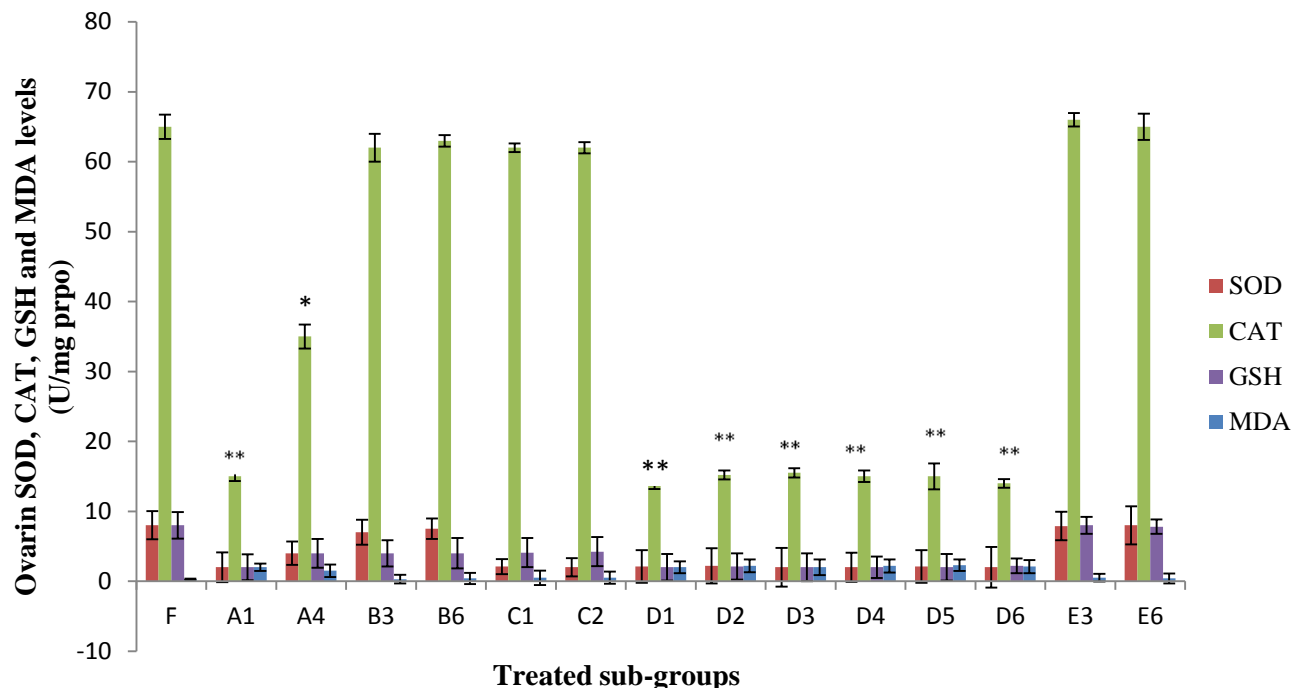
B2 - MCH<sub>28days</sub>-GCW<sub>L16 days</sub>

B5 - MCH<sub>28 days</sub>-GCW<sub>H16 days</sub>

E2 - GCW<sub>L16 days</sub>

E5 - GCW<sub>H16 days</sub>

Key for Figure 8b: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by.



**FIGURE 8c:** Ovarian Concentrations of SOD, CAT, GSH AND MDA Concentrations in 28 Days Experimental Groups in Female Sprague-Dawley Rats. Significant differences; \*\* $P < 0.001$ ; \* $P < 0.05$

Sub-group Detail: F- DSTL<sub>28 days</sub>; A1 – MCH<sub>28 days</sub>; A4 - MCH<sub>28 days</sub>- WD<sub>28 days</sub>; B3 - MCH<sub>28 days</sub>- GCW<sub>L28 days</sub>; B6 - MCH<sub>28 days</sub>-GCW<sub>H28 days</sub>; C1- MCH<sub>28 day</sub> + GCW<sub>L28 day</sub>; C2- MCH<sub>28 day</sub> + GCW<sub>H28 day</sub>; D1- GCW<sub>L8 days</sub> - MCH<sub>28 days</sub>; D2- GCW<sub>H8 days</sub> - MCH<sub>28 days</sub>; D3- GCW<sub>L16 days</sub> - MCH<sub>28 days</sub>; D4- GCW<sub>H16 days</sub> - MCH<sub>28 days</sub>; D5- GCW<sub>L28 days</sub> - MCH<sub>28 days</sub>; D6- GCW<sub>H28 days</sub> - MCH<sub>28 days</sub>; E3 - GCW<sub>L28 days</sub>; E6- GCW<sub>H28 days</sub>

Key for Figure 8c: DSTL: Distilled water; MCH: 0.2 mg/100 g b.w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by; +: treated concurrently.



#### 4.10 FERTILITY ASSESSMENT

All the rats in the control group were pregnant and carried their individual pregnancy to full term with typical number of fetuses;  $10.24 \pm 1.41$  (Table 7a-c). There was no fetus seen in the uterus of the induced animals after 20 days of assumed gestation period after mating was confirmed (Table 7c). This implies that pregnancy did not occur in the group induced with hyperprolactinaemia. Additionally, no pregnancy occurred in the 8 days withdrawal group (Table 7a). However, in the 16 and 28 days groups, pregnancy occurred but with a lower number of fetuses when compared with the control;  $5.60 \pm 0.79$  and  $5.76 \pm 0.65$  vs  $10.24 \pm 1.41$  respectively (Table 7b-c). There were no fetuses seen in the uterus of the animals of 8 days post-treated groups (Table 7a). In the 16 days post-treated groups at both low and high doses, pregnancy occurred but with a lower number of fetuses when compared with the control;  $8.10 \pm 0.49$  and  $8.98 \pm 0.74$  vs  $10.24 \pm 1.41$  (Table 7b). However, in the group post-treated with GCW for 28 days demonstrated comparable values in the number of pregnancies and fetuses with the control group;  $9.20 \pm 0.84$  and  $10.60 \pm 1.67$  fetuses versus  $10.24 \pm 1.41$  fetuses (Table 7c). There was no fetus seen in the uterus of the animals of the co-administered and pre-treated groups after the 20 days of assumed gestation period after mating was confirmed (Table 7c). The administration of GCW for 8, 16 and 28 days demonstrated comparable values in the number of pregnancies and fetuses when compared with the control. (Table 7a-c).

**TABLE 7a:** The Number of Pregnancies and Fetuses in 8 Days Experimental Groups in Female Sprague-Dawley Rats.

GROUP	SUB-GROUP DETAIL	NO OF PREGNANT RATS	MEAN NO OF FETUS
Control	DSTL <sub>8 days</sub>	5	10.24 ± 1.41
Withdrawal	MCH <sub>28 days</sub> - WD <sub>8 days</sub>	0	0.00 ± 0.00**
Post-treated	MCH <sub>28 days</sub> - GCW <sub>L8 days</sub>	0	0.00 ± 0.00**
	MCH <sub>28 days</sub> - GCW <sub>H8 days</sub>	0	0.00 ± 0.00**
GCW treated	GCW <sub>L8 days</sub>	5	10.31 ± 1.33
	GCW <sub>H8 days</sub>	5	9.98 ± 1.92

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5.

Significant differences; \*\* $P < 0.001$

Key for table 7a: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; —: treated followed by.

**TABLE 7b:** The Number of Pregnancies and Fetuses in 16 Days Experimental Groups in Female Sprague-Dawley Rats.

GROUP	SUB-GROUP DETAIL	NO OF PREGNANT RATS	MEAN NO OF FETUS
Control	DSTL <sub>16 days</sub>	5	10.24 ± 1.41
Withdrawal	MCH <sub>28 days</sub> -WD <sub>16 days</sub>	3	5.60 ± 0.79**
Post-treated	MCH <sub>28 days</sub> -GCW <sub>L16 days</sub>	4	8.10 ± 0.49*
	MCH <sub>28 days</sub> -GCW <sub>H16 days</sub>	5	8.98 ± 0.74*
GCW treated	GCW <sub>L16 days</sub>	5	10.50 ± 1.56
	GCW <sub>H16 days</sub>	5	10.44 ± 0.68

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5.

Significant differences; \*\*  $P < 0.001$ , \*  $P < 0.005$

Key for table 7b: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b.w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; –: treated followed by.

**TABLE 7c:** The Number of Pregnancies and Fetuses in 28 Days Experimental Groups in Female Sprague-Dawley Rats.

GROUP	SUB-GROUP DETAIL	NO OF PREGNANT RATS	MEAN NO OF FETUS
Control	DSTL <sub>28 days</sub>	5	10.24 ± 1.41
Induction	MCH <sub>28 days</sub>	0	0.00 ± 0.00**
Withdrawal	MCH <sub>28 days</sub> -WD <sub>28 days</sub>	4	5.76 ± 0.65*
Post-treated	MCH <sub>28 days</sub> -GCW <sub>L28 days</sub>	5	9.20 ± 0.84
	MCH <sub>28 days</sub> -GCW <sub>H28 days</sub>	5	10.60 ± 1.67
Co-administered	MCH <sub>28 days</sub> +GCW <sub>L28 days</sub>	0	0.00 ± 0.00**
	MCH <sub>28 days</sub> +GCW <sub>H28 days</sub>	0	0.00 ± 0.00**
Pre-treated	GCW <sub>L8 days</sub> -MCH <sub>28 days</sub>	0	0.00 ± 0.00**
	GCW <sub>H8 days</sub> -MCH <sub>28 days</sub>	0	0.00 ± 0.00**
	GCW <sub>L16 days</sub> -MCH <sub>28 days</sub>	0	0.00 ± 0.00**
	GCW <sub>H16 days</sub> -MCH <sub>28 days</sub>	0	0.00 ± 0.00**
	GCW <sub>L28 days</sub> -MCH <sub>28 days</sub>	0	0.00 ± 0.00**
	GCW <sub>H28 days</sub> -MCH <sub>28 days</sub>	0	0.00 ± 0.00**
GCW treated	GCW <sub>L28 days</sub>	5	11.21 ± 0.65
	GCW <sub>H28 days</sub>	5	11.82 ± 1.10

All values are expressed as mean ± standard error and ANOVA with Scheffe's post hoc test was used to check the level of significance where n is 5.

Significant differences; \*\*  $P < 0.001$ , \*  $P < 0.005$

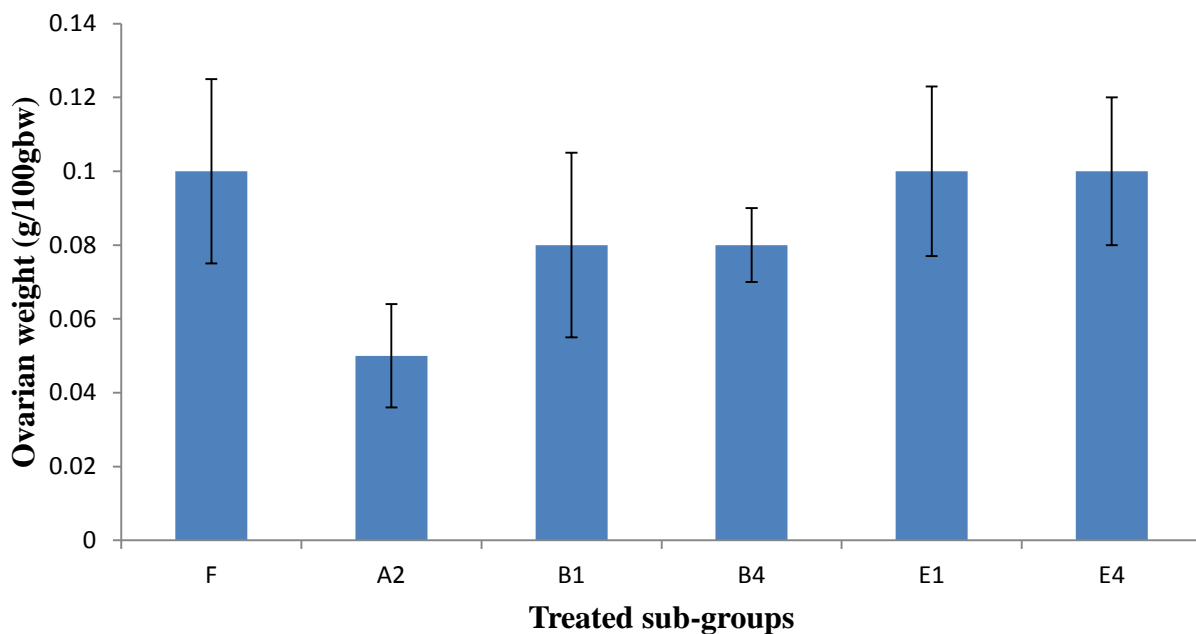
Key for table 6c: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; –: treated followed by; +: treated concurrently.



## **4.11 ASSESSMENT OF WEIGHT CHANGES**

### **4.11.1 Ovarian Weight Evaluation**

The control group demonstrated normal value in ovarian weight (Figure 9a- c). There was a significant lower value of ovarian weight in the induced group when compared with the value of the control group (Figure 9c). There was gradual increase in ovarian weight with increase in the number of days of withdrawal. However, by the 28<sup>th</sup> day of withdrawal, a significant lower value of ovarian weight was measured when compared with the control groups (Figure 9a-c). The group post-treated with GCW for 8 days at low and high doses demonstrated significant lower values in the ovarian weight when compared with the control (Figure 9a). However, in the groups post-treated with GCW for 16 and 28 days demonstrated comparable values in the weight of the ovaries with the control groups (Figure 9b-c). The co-administered and the post-treated groups demonstrated significant lower values of ovarian weights when compared with the values of the control group (Figure 9c). The administration of GCW for 8, 16 and 28 days demonstrated comparable values in the weight of the ovaries when compared with the control (Figure 9a-c).



**Figure 9a:** The Weight of the Ovary in the 8 Days Experimental Groups in Female Sprague-Dawley Rats. Significant differences;  $*P < 0.05$

Sub-group Detail: Sub-group Detail: F- Control

A2 - MCH<sub>28 days</sub>-WD<sub>8 days</sub>

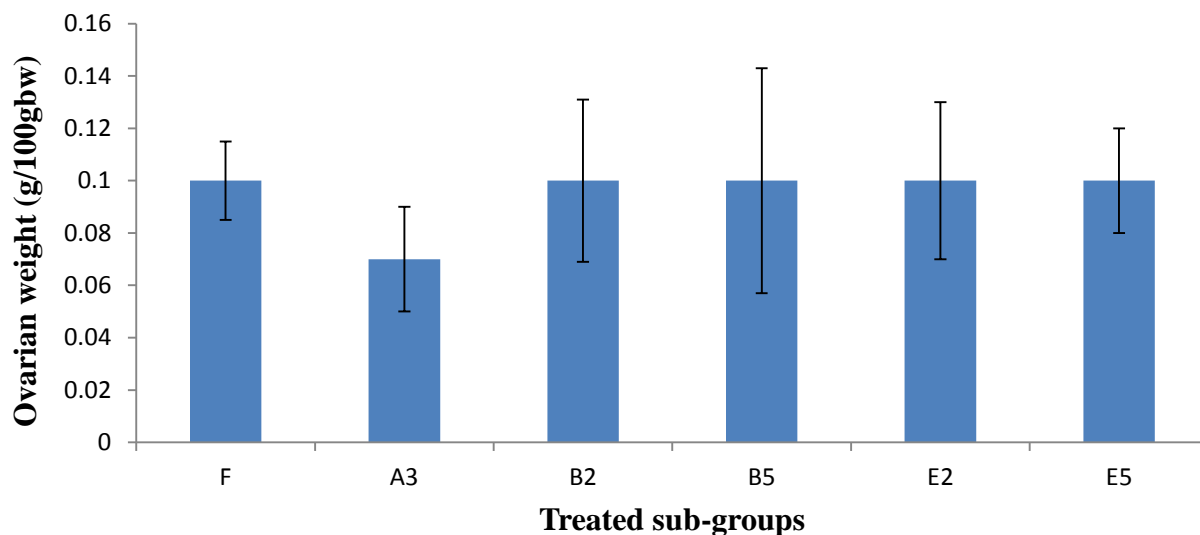
B1 - MCH<sub>28 days</sub>-GCW<sub>L8 days</sub>

B4 - MCH<sub>28 days</sub>-GCW<sub>H8 days</sub>

E1 - GCW<sub>L8 days</sub>

E4 - GCW<sub>H8 days</sub>

Key for Figure 9a: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b.w. of green coconut water; —: treated followed by.



**Figure 9b:** The Weight of the Ovary in the 16 Days Experimental Groups in Female Sprague-Dawley Rats. Significant differences; \* $P < 0.05$

Sub-group Detail: F- Control

A3 - MCH<sub>28days</sub>-WD<sub>16days</sub>

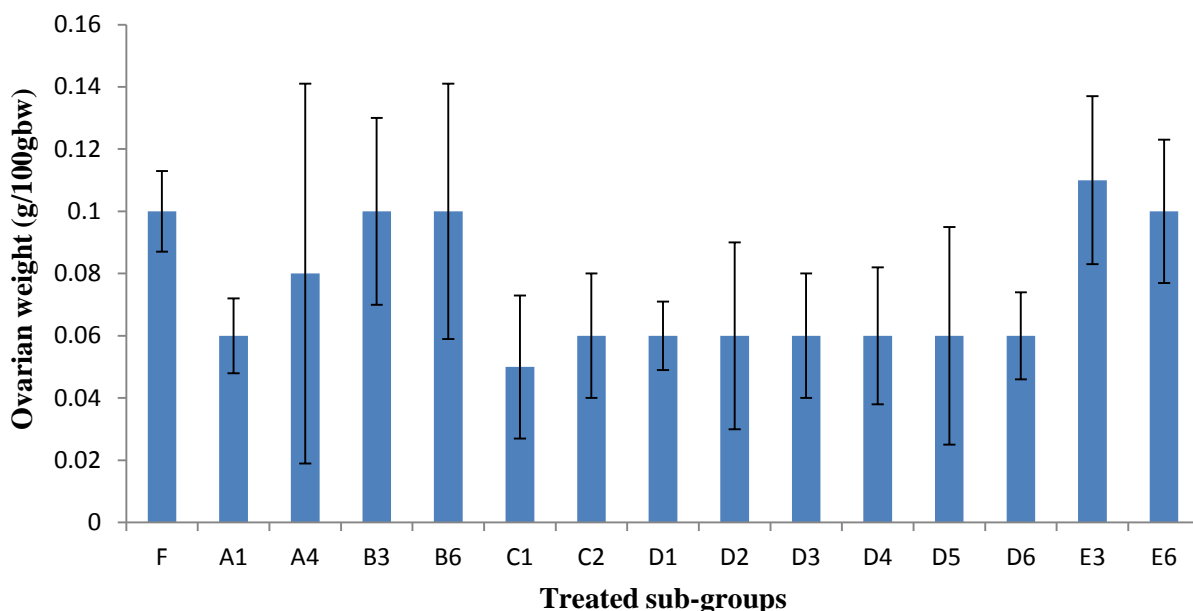
B2 - MCH<sub>28days</sub>-GCW<sub>L16 days</sub>

B5 - MCH<sub>28 days</sub>-GCW<sub>H16 days</sub>

E2 - GCW<sub>L16 days</sub>

E5 - GCW<sub>H16 days</sub>

Key for Figure 9b: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by.



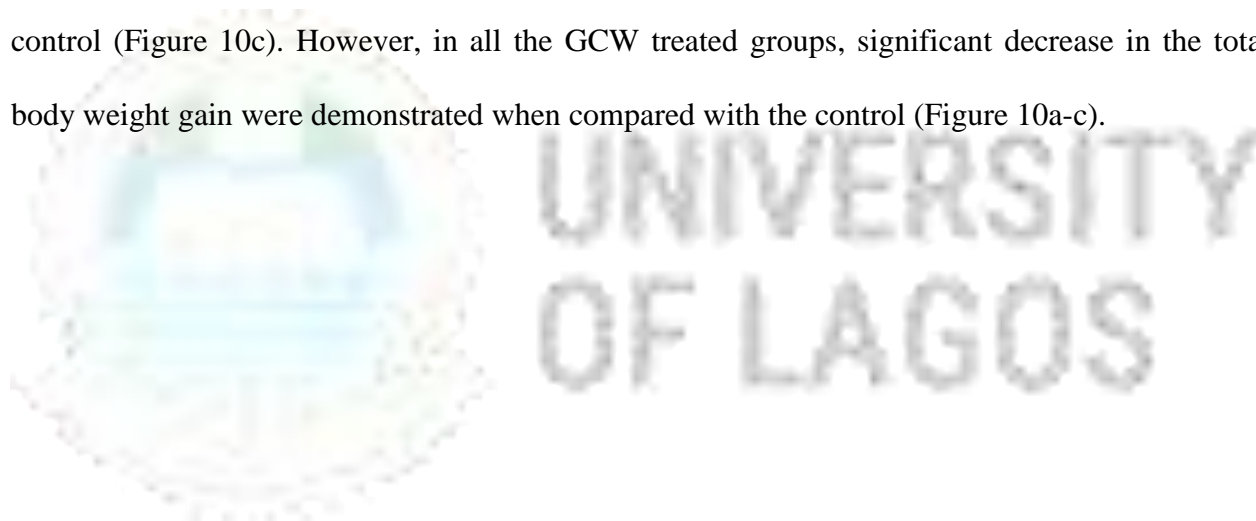
**Figure 9c:** The Weight of the Ovary in the 28 Days Experimental Groups in Female Sprague-Dawley Rats. Significant differences; \* $P < 0.05$

Sub-group Detail: F- Control; A1 – MCH<sub>28</sub> days; A4 - MCH<sub>28</sub> days- WD<sub>28</sub> days; B3 - MCH<sub>28</sub> days- GCW<sub>L28</sub> days; B6 - MCH<sub>28</sub> days- GCW<sub>H28</sub> days; C1- MCH<sub>28</sub> day + GCW<sub>L28</sub> day; C2- MCH<sub>28</sub> day + GCW<sub>H28</sub> day; D1- GCW<sub>L8</sub> days - MCH<sub>28</sub> days; D2- GCW<sub>H8</sub> days - MCH<sub>28</sub> days; D3- GCW<sub>L16</sub> days - MCH<sub>28</sub> days; D4- GCW<sub>H16</sub> days - MCH<sub>28</sub> days; D5- GCW<sub>L28</sub> days - MCH<sub>28</sub> days; D6- GCW<sub>H28</sub> days - MCH<sub>28</sub> days; E3 - GCW<sub>L28</sub> days; E6- GCW<sub>H28</sub> days

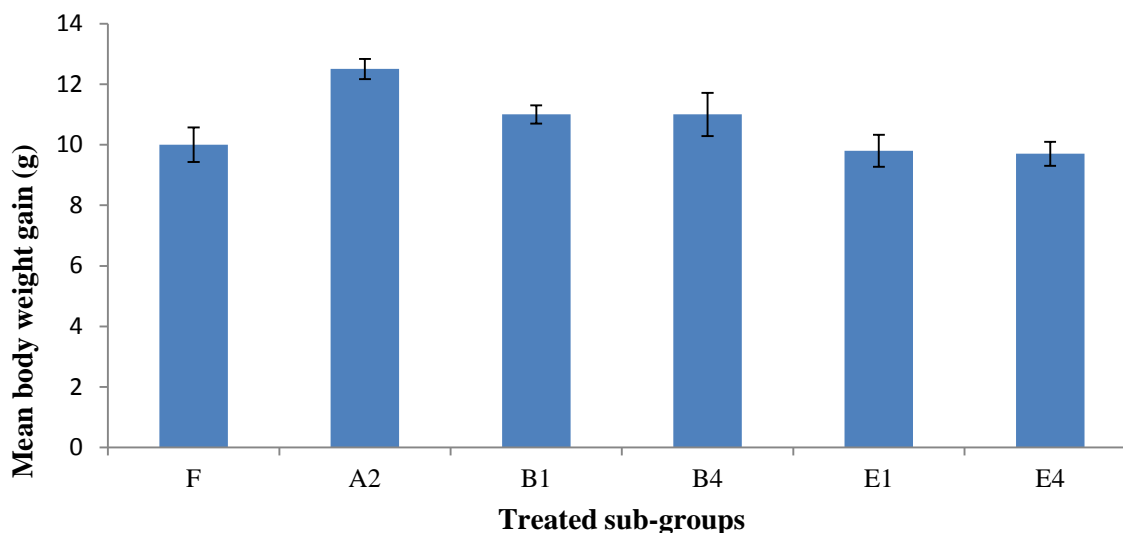
Key for Figure 9c: DSTL: Distilled water; MCH: 0.2 mg/100 g b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g b. w. of green coconut water; -: treated followed by; +: treated concurrently.

#### **4.11.2 Total Body Weight Gain**

There was a normal value in total body weight gain in the control group (Figure 10a-c). In the induced group, a significant increase in the total body weight gain was demonstrated when compared with the values of the control (Figure 10c). There was gradual decrease in the total body weight gain as the number of days of withdrawal increased (Figure 10a-c). In the Post-treated groups, gradual decrease in the total body weight gain with increase in the number of days of post-treatment with GCW were demonstrated (Figure 10a-c). The co-administered and pre-treated groups showed significant increase in the total body weight gain when compared with the control (Figure 10c). However, in all the GCW treated groups, significant decrease in the total body weight gain were demonstrated when compared with the control (Figure 10a-c).







**Figure 10a:** The Total Body Weight Gain in 8 days Experimental Groups in Female Sprague-Dawley Rats. Significant differences;  $*P < 0.05$

\*

Sub-group Detail: Sub-group Detail:

F-DSTL

A2 - MCH<sub>28 days</sub>-WD<sub>8 days</sub>

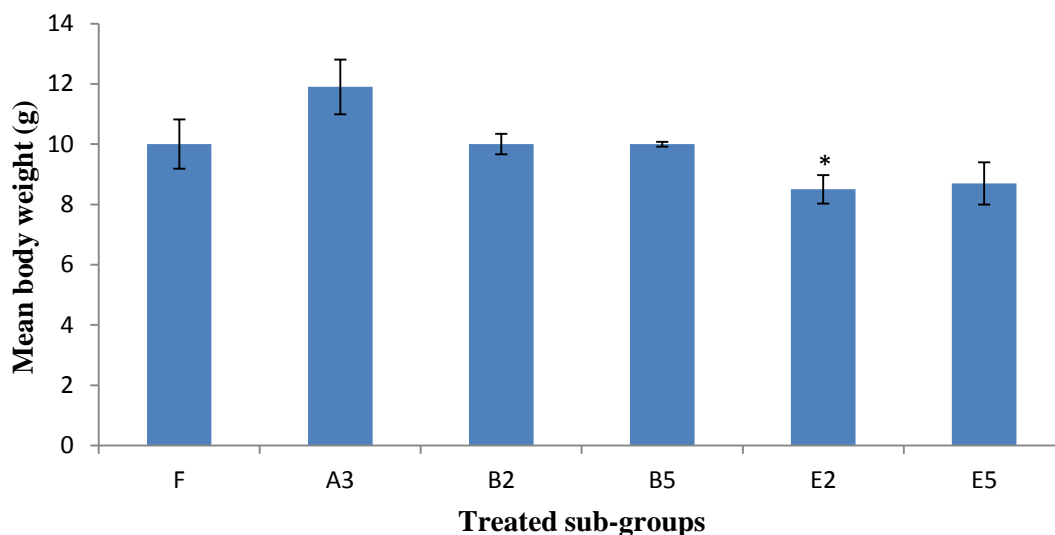
B1 - MCH<sub>28 days</sub>-GCW<sub>L8 days</sub>

B4 - MCH<sub>28 days</sub>-GCW<sub>H8 days</sub>

E1 - GCW<sub>L8 days</sub>

E4 - GCW<sub>H8 days</sub>

Key for Figure 10a: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b.w. of green coconut water; -: treated followed by.



**Figure 10b:** The Total Body Weight Gain in 16 days Experimental Groups in Female Sprague-Dawley Rats. Significant differences; \* $P < 0.05$

Sub-group Detail: F- DSTL

A3 - MCH<sub>28days</sub>-WD<sub>16days</sub>

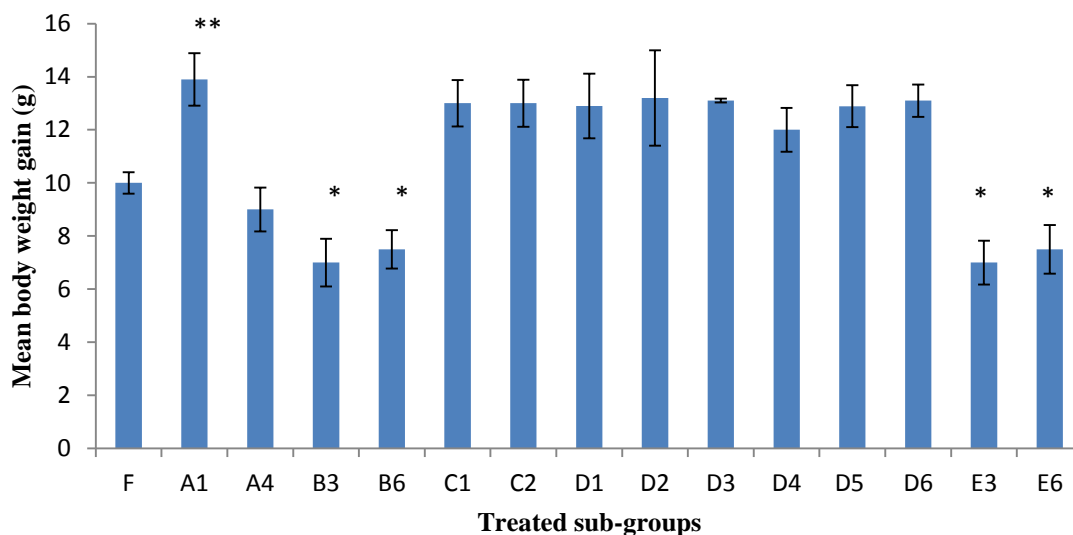
B2 - MCH<sub>28days</sub>-GCW<sub>L16 days</sub>

B5 - MCH<sub>28 days</sub>-GCW<sub>H16 days</sub>

E2 - GCW<sub>L16 days</sub>

E5 - GCW<sub>H16 days</sub>

Key for Figure 10b: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g. b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g. b. w. of green coconut water; -: treated followed by.



**Figure 10c:** The Total Body Weight Gain in 28 days Experimental Groups in Female Sprague-Dawley Rats. Significant differences; \*\*  $P < 0.001$ , \*  $P < 0.005$

Sub-group Detail: F- DSTL; A1 – MCH<sub>28 days</sub>; A4 - MCH<sub>28 days</sub>- WD<sub>28 days</sub>; B3 - MCH<sub>28 days</sub>-GCW<sub>L28 days</sub>; B6 - MCH<sub>28 days</sub>-GCW<sub>H28 days</sub>; C1- MCH<sub>28 day</sub> + GCW<sub>L28 day</sub>; C2- MCH<sub>28 day</sub> + GCW<sub>H28 day</sub>; D1- GCW<sub>L8 days</sub> - MCH<sub>28 days</sub>; D2- GCW<sub>H8 days</sub> - MCH<sub>28 days</sub>; D3- GCW<sub>L16 days</sub> - MCH<sub>28 days</sub>; D4- GCW<sub>H16 days</sub> - MCH<sub>28 days</sub>; D5- GCW<sub>L28 days</sub> - MCH<sub>28 days</sub>; D6- GCW<sub>H28 days</sub> - MCH<sub>28 days</sub>; E3 - GCW<sub>L28 days</sub>; E6- GCW<sub>H28 days</sub>

Key for Figure 10c: DSTL: Distilled water; MCH: 0.2 mg/100 g b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW<sub>L</sub>: 5 ml/100 g b. w. of green coconut water; GCW<sub>H</sub>: 10 ml/100 g b. w. of green coconut water; -: treated followed by; +: treated concurrently.

## **CHAPTER FIVE**

### **DISCUSSION**

#### **5.1 GENERAL DISCUSSION**

Many lines of evidence in human and experimental model indicate that changes in the secretion and action of prolactin are sufficient to affect the integrity of the hypothalamic-pituitary-gonadal axis. Hyperprolactinaemia has been shown to suppress the release of gonadotropin releasing hormone by the hypothalamus which consequently inhibit the secretion of follicle stimulating hormone and luteinizing hormone by the pituitary gland. The decrease in pituitary FSH and LH through integrated central and peripheral mechanisms suppress the physiological activities in the gonad thus leading to infertility (Matsuzaki *et al.*, 1994).

#### **5.2 EFFECTS ON ESTROUS CYCLE**

The hypothalamic–pituitary axis regulates all of the reproductive changes along the estrous cycle. Therefore, estrous cycle is controlled by reproductive hormones in cycling female mammals, hence imbalance in reproductive hormonal profile will consequently cause estrous acyclicity (Marcondes *et al.*, 2002). Estrous cycling animal changes phases progressively from metestrus, diestrus, proestrus to estrus phase. Each phase of estrous cycle is attributed to certain hormonal event. The metestrus phase designated by the formation of corpus luteum which occurs as a result in the decrease of progesterone synthesis because of the failure of implantation following previous ovulation. In the diestrus phase, further decline in progesterone release causes the inhibition of LH secretion; hence the functional regression of the corpus luteum occurs during late diestrus.

During the proestrus phase, antral follicles grow exponentially under the influence of FSH and LH. This is characterized by increase in oestrogen affecting the hypothalamus in a positive feedback manner to stimulate the release of gonadotropins. When follicles have fully developed, there is suppression in the release of FSH and LH. Prior to the time of ovulation in the late proestrus phase, a sudden spike of LH peaks occurs under the stimulation of oestrogen to initiate the release of egg (Caligion, 2009). In the estrus phase, the lining in the uterus (endometrium) starts to develop so as to prepare the uterus for implantation, under the influence of oestrogen and progesterone ( Geoffrey *et al.*, 2007).

Elevated PRL level has been shown to cause reproductive acyclicity by disrupting ovarian steroidogenesis. Oestrogen is primarily produced by the ovarian follicle. As the follicle grows, more oestrogen is produced. As increasing amounts of oestrogen is released into the blood stream, it travels to the anterior pituitary to stimulate the release of FSH and LH. Therefore, any interruption in ovarian steroidogenesis as it relates to oestrogen release will lead to hormonal imbalance; hence disrupt estrous cyclicity (Laura *et al.*, 2012). This study reported that animals with high serum prolactin had prolonged cycle with animals spending more number of days on the diestrus phase of the estrous cycle. GCW was able to cause increase in serum oestrogen which is in conformity with the study that reported that GCW has oestrogen-like characteristics when administered to menopausal rats (Nisaudah *et al.*, 2009). This is due to the fact that oestrogen was able to re-launch normal hormonal profile by stimulating the pituitary gland, it acts in a positive feedback fashion to release gonadotropins. This causes the reversal of estrous acycling pattern in the group post-treated with GCW for long term following the induction of hyperprolactinaemia. The phases of estrous cycle progressively change daily from one phase of estrous cycle to the other and the lengthened diestrus phases demonstrated in the induced rats



were gradually shortened during post-treatment with GCW. It is also confirmed in this study that GCW maintained the pattern of estrous cycling. There were non- disruption of cyclic pattern in all GCW-treated groups as the animal progressively transit from one phase of the cycle to the other.

### **5.3 EFFECTS ON OVULATION**

The process of ovulation is controlled by the hypothalamus through the release of FSH and LH in the anterior lobe of the pituitary gland. Ovulation occurs about midway through the menstrual cycle, after the pre-ovulatory follicular phase. In the pre-ovulatory phase of the menstrual cycle, the ovarian follicles undergo transformations from simple primary ovarian follicle to complex Graafian or mature follicle, which is mediated by FSH (Wilcox *et al.*, 1999). Ovulation is triggered by a spike in the amount of LH released from the pituitary gland. Oestrogen levels peak towards the end of the follicular phase, which causes a surge in levels of luteinizing hormone (LH) and this results in the rupture of the ovarian follicles, causing the oocyte to be released from the ovary (Watson & Stacy 2010). Histomorphological studies of the ovary such as; the developmental stages of ovarian follicles and ovarian weight have been used as sensitive parameters for assessing follicular development and thus ovulation (Shivalingappa *et al.*, 2002; Monsefi *et al.*, 2006).

Hyperprolactinaemia is associated with hypogonadotropic hypogonadism that results from low gonadotropin secretion. Anovulation is thus implicated by a decrease in circulating LH and FSH levels. This leads to poor follicular growth and a consequent decrease in gonadal oestrogen secretion; hence loss of LH-surge which is necessary to cause the releases of egg (Ursula and Kaiser, 2012). In this study, histological analysis revealed that GCW stimulate follicular growth

in the ovary. The increase in follicular growth is attributed to the oestrogenic characteristics of GCW proven in this study. This is supported by previous study where GCW was administered to menopausal rats and the oestrogen level in the menopausal rats was comparable to the level in ovulating rats (Nisaudah *et al.*, 2009). Oestrogens are known to exert positive effects on granulosa cell growth and differentiation in association with the release of gonadotropins. The increase in FSH and LH secretion stimulated by oestrogen causes increase in the developmental process of the ovarian follicles and thus ovulation (Ann, 2006).

Evaluation of ovarian weight has been employed as a critical tool in accessing follicular development in the ovary. Folliculogenesis is achieved by increase in cell proliferation and formation of follicular fluid, hence increase in the number of granulosa and theca cells and corresponding increase in the volume of the antrum have been characterized in folliculogenesis. These activities is controlled by FSH under the influence of oestrogen (Erickson, 2008). Decrease in ovarian weight has clearly been indicated in hyperprolactin rats. The loss of ovarian weight has been attributed to decrease in the structural developmental activities in the ovary due to low level of steroidal hormones and gonadotropins (Shivalinagappa, *et al.*, 2002). In this study, morphometric analysis of ovarian weight demonstrated a duration dependent increase in ovarian weight with increase in the number of days of post-treatment with GCW. This implies that significant prevention in the loss of weight of ovaries may be due to the increase in the releases of gonadotropins. These explain that more follicles ascended to the mature Graafian stage due to oestrogen- induced pituitary release of LH and FSH which are essential for follicular growth and development (Ehlers and Halvorson, 2013). The result of the ovarian weight correlates with the values of ova count and the number of fetuses in the fertility test result. The highest value of ovarian weight in all experimental groups was seen in the long term post-treated groups which

demonstrated the highest number of mature follicles. Stereological evaluations of volume densities of follicular components are vital in accessing cell proliferation. Individual follicle consists of an innermost oocyte and a fluid filled cavity called the antrum, surrounded by granulosa cells and outer layers of theca cells. In this study, stereological evaluations of follicular components revealed increase in the volume densities of granulosa cells. This implies that GCW stimulates granulosa cell proliferation. It has been reported that granulosa cell has physiological effects on follicular growth, it is responsible for the production of oestrogen and growth factors (Ashkenazi, 2005).

#### **5.4 EFFECT ON OESTROGEN RECEPTORS**

In this study, the fertility enhancing properties has been directed toward the oestrogenic characteristic of green coconut water. Immunohistochemistry was thus undertaken to examine the cellular activities of oestrogen receptors in the ovarian and pituitary tissues and consequently determining the site of action of GCW in hyperprolactin-induced infertility (Ren *et al.*, 2001). Oestrogen receptors is a ligand-activated transcription factor that modulate the effects of oestrogen, hence the action of oestrogen is mediated at its receptor sites. A feedback mechanism exists upon oestrogen binding to its receptor to induce the activation of oestrogen receptor which in turn activates oestrogen to amplify its signal (Driggers and Segars, 2002). In this study, high and extensive expressions of oestrogen antigen-antibody complexes were demonstrated in the anterior pituitary gland and ovaries following treatment with green coconut water. The result clearly demonstrates the presence of oestrogen receptor immunopositive cells in ovarian and pituitary tissues. This explains that oestrogen is sensitive to its receptors which cause its action to be activated; the interaction between oestrogen and its receptor causes intra-follicular stimulation in the ovary which leads to granulosa cell proliferation due to increase oestrogen activities.

The interaction also stimulates the pituitary gland to secrete gonadotropins which are essential for follicular development (Ehlers and Halvorson, 2013).

## **5.5 BIOCHEMICAL EVALUATION**

### **5.5.1 Reproductive hormonal profile**

The activities of the female reproductive system are controlled by hormones released from the brain and the ovaries. The interactions between these hormones control the reproductive cycle. The gonadotropin-releasing hormone (GnRH) is produced in the hypothalamus. GnRH travels to the pituitary gland where it controls the release of FSH and LH. FSH is carried by the bloodstream to the ovaries where it stimulates the growth of immature ova while LH triggers ovulation and promotes the formation of corpus luteum. Oestrogen is produced by the growing follicle and corpus luteum. In moderate amounts oestrogen helps to control the levels of GnRH, FSH and LH. Hence any factor that causes imbalance in hormonal profile disrupts reproductive processes. In hyperprolactinaemia, it has been reported that the inhibition of follicular maturation and the prevention of the production of mature egg is attributed to a decrease in the secretion of FSH and LH (Nawroth, 2005). More so, this disrupts luteinizing activities of the ovarian follicles which leads to luteal phase defect thus preventing preovulatory LH- surge (Wuttke *et al.*, 2001). This study confirmed that GCW stimulates ovarian steroidogenesis as demonstrated in the increase of granulosa cell proliferation and consequent increase in the concentration of follicular oestrogen production. The increase in serum oestrogen through feedback mechanism sends signal to the pituitary gland to release FSH and LH. The increase in oestrogen causes negative feedback on the lactotrophic cells of the anterior pituitary gland to suppress the release of prolactin (Craven *et al.*, 2006).

### 5.5.2 Oxidative stress markers

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or repair the resulting damage. Oxidative stress is said to be induced when the manifestation of oxygen species outweighs the ability to repair. In the female reproductive system, oxidative stress is induced through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA; thus, causing disruptions in the normal mechanisms of cellular signalling. Oxidant status of the reproductive cell is critical for follicular growth, corpus luteum formation, endometrial differentiation and embryonic growth (Agarwal *et al.*, 2005). Prolactin is well established as a stress-induced hormone, its secretion follows the induction of stress in reproductive organs (Franci *et al.*, 1992; Mohan *et al.*, 2011).

Antioxidants are compounds that delay auto-oxidation by inhibiting formation of free radicals or by interrupting propagation of the free radical. In recent years, there has been considerable interest in finding sources of natural antioxidants which give protection against the process of oxidative damage. This is due to the fact that the natural antioxidant substances demonstrate anti-oxidative activity that is better than the synthetic antioxidants, which is as result the presence of two or more aromatic rings. The synthetic antioxidants have one aromatic ring. The effectiveness of these aromatic antioxidants is generally proportional to the number of hydroxyl-groups present on the aromatic ring (Brewer, 2011). The results from this study demonstrated that GCW has anti-oxidant property by increasing SOD, CAT and GSH concentrations and decreasing the concentration of MDA in the ovarian tissue. Several studies also conformed to the anti-oxidant property of coconut products. The tender coconut water has been reported to inhibit lipid peroxidation and up-regulation of antioxidant activities on oxidative stress in fructose fed insulin



resistant hypertensive rats (Bhagya *et al.*, 2012). More so, virgin coconut oil has been shown to improve antioxidant status by decreasing the level of MDA and bringing lipid status to normal in alcohol-induced oxidative stress (Dosumu *et al.*, 2012).

## **5.6 EFFECT ON BODY WEIGHT**

The understanding of the physiological mechanism behind the regulation of body weight can facilitate the alleviation of some disease conditions including infertility (Betsy and Tsu-Shuen, 2007). Natural weight loss has been used in restoring reproductive hormonal profiles and fertility (Stephanie *et al.*, 2010). The decrease in body weight gain in GCW-treated groups conforms to the report that the administration of immature coconut for 28 days demonstrated a statistically significant decrease in total body weight in mice (Eze and Chukwuemeka, 2011). Clinical studies also confirmed that coconut water is natural electrolyte and isotonic beverage that enhances weight loss in a completely natural form by increasing the metabolic rate and fat burning process in the body (Jones, 1997).

## **5.7 SITES OF ACTION OF GREEN COCONUT WATER**

High prolactin has been reported to cause reduction in granulosa cell proliferation and hence reduction in granulosa cell estradiol production. The negative oestrogen feedback on FSH and LH inhibits the normal process of ovulation. Functionally, the hormones oestrogen and prolactin opposes each other (Craven *et al.*, 2006). GCW- oestrogenic property was demonstrated in this study with increase in granulosa cell volume density and consequent rise in serum oestrogen concentration. It has been reported that sterols present in GCW facilitate the synthesis of endogenous oestrogen due to increase in granulosa cell proliferation (Punghmatharith, 1998). The interaction between oestrogen and oestrogen receptors in the ovary and pituitary gland expressed

in this study explains the fact that oestrogen activates its receptors. In the pituitary gland, the interaction between oestrogen and its receptor stimulates the secretion of gonadotropins which are essential for follicular growth and ovulation. In the ovary, it causes intra-follicular stimulation which leads to granulosa cell proliferation and follicular differentiation. The increase in the growth of ovarian follicle leads to increase in the number of ova count and corresponding increase in the number of foetus. In summary, this result supports the hypothesis that the interaction between oestrogen and oestrogen receptor infers with the secretion of gonadotropins and hence reproductive capacity (Stefan and Jan-Ake 2002). Therefore, GCW offers it pro-fertility effect by increasing serum oestrogen level and activating oestrogen receptors for the initiation of its action.

The antioxidant property demonstrated by GCW in this study is favourable in enhancing fertility. Oxidative stress is a confounding factor in infertility as free oxygen radicals may react with cellular components of the reproductive tract to produce destructive effects (Van, 2009). This study demonstrated that green coconut water is a good antioxidant with results shown in the oxidative stress markers. Its phytochemical analysis revealed substances such as kinetin, tannin, phenol and flavonoid which have been reported to have strong antioxidant properties (Verbeke *et al.*, 2000).

## **CHARPET SIX**

### **SUMMARY OF FINDINGS AND CONCLUSION**

#### **6.1 SUMMARY OF FINDINGS**

1. Green coconut water reverses estrous acycling to normal cycling pattern in the long term post-treated groups
2. Green coconut water produces continuous increases in follicular growth and differentiation, granulosa cell proliferation, ovarian weight, number of ova shed and number of fetuses from medium to long term treatment.
3. Green coconut water activates oestrogen receptors in the ovary and pituitary tissues which causes high and extensive expression of oestrogen antigen-antibody complex
4. Green coconut water causes significant decrease in serum PRL level and increases in serum oestrogen, LH and FSH levels, and decrease in ovarian MDA and increases in ovarian SOD, CAT and GSH concentrations following long term treatment.

#### **6.2 CONTRIBUTIONS TO KNOWLEDGE**

The study establishes that green coconut water in its natural form:

1. Is an effective pro-fertility agent in the management of hyperprolactin-induced infertility.
2. Sensitizes oestrogen receptors in the ovary and pituitary gland to form antigen-antibody complexes.
3. Is an effective antioxidant in metoclopramide-induced oxidative stress

### 6.3 CONCLUSION

There is quantitative evidence that GCW demonstrates ameliorative effects on infertility induced by hyperprolactinaemia following long term administration. These effects may be subjected to the fact that there was an established status of low serum oestrogen due to the induction of hyperprolactinaemia. However, there were no positive changes in the parameters measured in the pre-treated and co-administered group when compared with the induced group. It is indicated in these groups that GCW did not offer it fertility enhancing properties as a prophylactic and modulating substance in hyperprolactin-induced infertility in rat but as an ameliorative agent in this regard.

In the present study, oestrogenic property of green coconut is believed to be the main target to which infertility was reversed in the hyperprolactin animals. This is established in the increase in serum oestrogen level and the sensitivity of oestrogen receptors. Although, the usage of oestrogen has been used to treat hyperprolactinaemia clinically but its usage has not been recommended. A clinical study reported that oestrogen treatment in standard contraceptive dosage usually leads to only moderate and non-progressive stimulation of pituitary activity in women with hyperprolactinaemia. In addition, moderate reduction was reported even after long term of treatment and radiological evidence of pituitary tumor growth during treatment was also reported, hence treatment needs to be severely monitored (Fahy *et al.*, 1999). The findings from this study depict clearly that green coconut water is a promising substance in reversing infertility caused as a result of high prolactin in female S-D rat. The results from this study would serve as a preliminary template for further comparative studies and subsequent research work on hyperprolactin-induced infertility in higher female animals and in human.

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