CHAPTER ONE

1.0 Introduction

Medicinal plants are known to possess substances or agents that have therapeutic effects or be used in producing drugs and they have been in use long before the development of orthodox medicine. Many plants used primitively to tackle illnesses have been investigated and show medical importance, an example of which is *Ocimum gratissimum* (scent leaf) (Idris *et al*., 2011, Odugbemi, 2006). *Ocimum gratissimum* is an herbaceous plant belonging to the Lamiacae family (Singh, 2012). It can be found in tropical areas, in the savannah and coastal areas (Singh, 2012). It is cultivated in India, Nigeria, Bengal, Deccan, South Sea Islands, etc. In Nigeria, it is used in the treatment of miscarriage (Ogbe *et al*., 2009), diarrhea (Sofowora, 1993), and high fever (Oliver, 1960). *Ocimum gratissimum* possesses certain phytochemicals, that is, biologically active plant compounds that have disease fighting capabilities (Jumare, 2018). They include alkaloids, flavonoids, and polyphenols. (Vieira *et al* 2001). *Ocimum gratissimum* has been reported to be useful in many conditions by effecting anti-inflammatory, anti-mycotoxicogenic, antibacterial, anthelminthic, antioxidant qualities and it is also used in vasorelaxation (Dambolena *et al*, 2010; Lee *et al*, 2011; Costa *et al*.,2012; Pires *et al* 2012). *Ocimum gratissimum* is grown for its essential oils which include eugenol, thymol, citral. These oils have been investigated and found to possess antifungal properties as well as anti-nociceptive properties (Dubey *et al*, 2000).

In medical practice, one area that physicians often face difficulty is in the treatment of syndromes that have signs and symptoms connected to each other and with a disease. An example of such syndromes is Polycystic Ovarian Syndrome (PCOS). Polycystic Ovarian Syndrome (PCOS) also referred to as Stein-Leventhal syndrome is a common endocrine system disorder common in 12-21% women of reproductive age (Dey *et al*., 2011). PCOS is a combination of hyper-androgenism, anovulation with polycystic ovaries (Franks, 1989). It is the most common cause of infertility due to the absence of ovulation (Spritzer, 2002). This disorder is associated with the presence of insulin resistance, obesity, type 2 diabetes, metabolic syndrome, hypertension, dyslipidemia amongst others (Azziz, *et al.*, 2006). The persistent hyper-androgenism is associated with poor hypothalamic-pituitary feedback, excess secretion of LH, abnormal oocyte maturation (Palomba *et al*, 2017). Women with PCOS experience abnormalities in their menstrual cycle, acne and excess growth of hair. An *in vivo* and *in vitro* studies on theca

cells showed that ovarian theca cell in PCOS patients were more active in converting androgenic precursors to testosterone than in healthy women (Rosenfield *et al*., 2016). Theca cells produce androgen in response to secretion of luteinizing hormone (Rosenfield *et al*., 2016). The high blood androgen levels, lack of ovulation and disrupted synthesis of endocrine hormones are all progressive factors leading to infertility (Kirilovas *et al.,* 2006). The prevalence rate of this disease was reported to be 5.6-8% in Europe, in Iran based on Rotterdam criteria, 19.5% and based on National Institute of Health (NIH) criteria, 6.8%. Azziz *et al* also reported a prevalence rate of 6.6% in females between the ages of 18-45 years. A 2015 meta-analysis reported a prevalence rate of 6.8-19.5% across the globe (Jalilian, 2015). Lifestyle factors and ethnicities also have an effect on the prevalence of PCOS; urban women have a higher risk of PCOS compared to rural women (Balaji *et al.,* 2015). Currently clomiphene citrate, metformin, and tamoxifen are the drugs used to treat this syndrome (Marx *et al.,* 2003). There is however insufficient literature on the effect of *Ocimum gratissimum* on PCOS in female rats. It is with this background that the study aims at investigating the ameliorative effect of ethanolic extract of *Ocimum gratissimum* leaves in-vivo and in-vitro.

1.1 Statement of the Problem

PCOS is a leading cause of infertility. This study aims to discover effective ways to tackle PCOS and evolve treatment measures for the syndrome. The study is focused on the use of ethanolic extract of *Ocimum gratissimum* leaves in PCOS in vitro and in vivo tests and compare its ameliorative effect to that of drugs used for PCOS treatment such as metformin and clomiphene citrate.

1.2 Justification of the Study

Traditional medicine has laid claims on the use of *Ocimum gratissimum* leaves in treating reproductive conditions such as PCOS; therefore it is important to scientifically validate this claim.

1.3 Aim of the Study

This study aims to evaluate the effect of ethanolic extract of *Ocimum gratissimum* leaves on reproductive functions such as oestrous cycle and hormones associated with letrozole-induced PCOS rats.

1.4 Specific Objectives of Study

The specific objectives of this study were:

1. To evaluate the phytochemical constituents in ethanolic extract of *Ocimum gratissimum* leaves using phytochemical screening, GC-MS and UV-visible spectroscopy.
2. To induce PCOS in rats using letrozole
3. To monitor the oestrous cycle
4. To assay for reproductive hormones such as testosterone, progesterone, luteinizing hormone, and follicle stimulating hormone
5. To determine the lipid profile of PCOS rats
6. To evaluate the associated metabolic disturbances associated with PCOS such as hyperglycemia, increased weight and hyperinsulinemia

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Perspective

Polycystic ovary syndrome is the most common endocrine found in women of reproductive age. (Ehrmann, 2005; Lauritsen *et al.,* 2014). It was first described by Stein and Levanthal as a syndrome of polycystic ovaries and oligo-amenorrhea usually accompanied by various conditions including hirsutism, obesity and acne (Stein *et al* 1935; Azziz *et al* 2016). Although Stein and Leventhal were described as the first investigators of this syndrome, in 1721, Vallisneri an Italian medical scientist described a married infertile woman with shiny ovaries having a white surface and size of pigeon eggs (Vallisneri, 1721). Another report from Chereau and Rokitansky was found in 1844 where they described fibrous and sclerotic lesions in ovaries of a degenerative character with hydrops follicle (Chereau, 1844; Rokitansky, 1855). Bulius and Kretschmar described hyperthecosis (Bulius *et al*, 1897). Lawson Tait in 1879 presented the need for oophorectomy as treatment of symptomatic cystic degeneration of ovaries (Tait, 1879). In 1902, von Kahlden published a review on these ovaries stating their pathophysiology and clinical implications (von Kahlden, 1902). Stein and Leventhal in 1935 presented a group of 7 women with common features including menstruation disorders, hirsutism and enlarged ovaries with the presence of many small follicles (Stein *et al* 1935). They described the lack of menstruation in women with increased volume of ovaries and were the first to suggest ovarian wedge resection. After surgical intervention, all menstrual cycles of the 7 patients became regulated and 2 became pregnant (Stein *et al*, 1948). As medical treatment became available, surgical treatment became used less often (Greenblatt, 1961; Kovacs *et al* 1989; Wang *et al* 1980). PCOS was described as masculinization and theca luteinization syndrome (Geist *et al*, 1942; Culiner *et al*, 1910). Many scientists tried to provide information on the etiology of cystic ovaries. In their original report, Stein and Leventhal thought bilateral cystic ovaries originated due to hormonal abnormalities which was confirmed later (Stein *et al*, 1948; Yen *et al*, 1970). In 1961, following the description of a method of measuring plasma testosterone level, increased circulating level of androgens was demonstrated (Hill, 1937; Deanesly, 1938; Plate, 1951).

Increased LH and testosterone levels were of key importance in diagnosis of PCOS (Yen *et al*, 1970; Rebar *et al*, 1976). It was not until the 1990s that the national institute of health came up with a diagnostic criteria that received acceptance in the research and clinical studies. After many years of research, another criteria was established in 2003 called the Rotterdam criteria. Many years have gone by since PCOS study was first published but the etiology is still puzzling and there is still ongoing research concerning therapy in PCOS women.

2.2 Polycystic Ovarian Syndrome (PCOS)

Polycystic ovarian syndrome is the most common endocrinopathy in women of reproductive age and it is associated with metabolic disorder and reproductive dysfunction (Wood *et al*., 2007; Spritzer, 2014; Agapova *et al*., 2014; Azziz *et al*., 2004). It is also regarded as the most common cause of infertility due to its presence of ovarian dysfunction as its main feature (Baker *et al*., 2007; Hamilton-Fairley and Taylor, 2003). This syndrome can be defined through the use of clinical and biochemical criteria and ultrasonography (Lujan *et al*., 2008). PCOS is commonly associated with insulin resistance, hyperinsulinaemia, metabolic syndrome and oligo anovulatory cycles (Wood *et al*., 2007; Baker *et al*., 2007; Hamilton-Fairley and Taylor, 2003; March *et al*., 2010; Franks, 1995, Ehrmann, 2005; Legro, 2001; Tsikouras *et al*., 2015). Some of the symptoms are age-dependent but characteristics such as ovarian failure and hyperandrogenism are common to any age (Tsikouras *et al*., 2015). Women suffering from PCOS often show forth a series of series associated with menstrual dysfunction and hyperandrogenism. Such patients also are prone to a set of multiple morbidities such as obesity, insulin resistance, diabetes, cardiovascular disease (CVD), infertility and other psychological disorders (Hung *et al*., 2014)like anxiety, depression and sexual dysfunction which may affect the patient's quality of life. The increasing prevalence rate of PCOS can be attributed to genetic, environmental factors (Brady *et al*., 2009) but it is also considered as a combination of insulin resistance, hyperandrogenism and factors causing follicular abnormalities (Dumesic *et al*., 2016). Elevated luteinizing hormone levels have been reported to be a significant factor in PCOS-associated ovulation disorders (Akram *et al*., 2015).

2.2.1 Ovarian Dysfunction

Ovarian dysfunction also known as primary ovarian deficiency is a condition characterized by primary amenorrhea or premature depletion of ovarian follicles, that is, arrested folliculogenesis before the age of 40 years (Santoro, 2003).

2.2.2 Metabolic Disturbances

Women with PCOS have a higher prevalence rate of the metabolic syndrome. The metabolic syndrome is a combination of metabolic abnormalities that often manifest together and increase risk of type 2 diabetes and cardiovascular disease (Ehrmann *et al*; 2006). These conditions include impaired glucose tolerance, high blood pressure, abdominal obesity and high blood cholesterol (Panidis *et al*; 2012).

2.2.2.1 Insulin Resistance

In PCOS a lot of attention has been given to the metabolic disturbances and effects of these disturbances later in life among such disturbances is insulin resistance. Insulin resistance is regarded as the main pathogenic factor when considering increased metabolic disturbances in PCOS patients (Siklar *et al*., 2015). Burghen *et al* (1980) first noted the relationship between hyperinsulinaemia and PCOS in 1980. Recent studies show that hyperinsulinemia is present in 85% of PCOS patients including 95% obese patients, 65% lean affected women (Teede *et al*., 2010, 2011; Stepto *et al*., 2013). Insulin acts synergistically with LH to increase androgen production in the ovarian theca cells (Tsilchorozidou *et al*., 2004). Hyperinsulinemia also alters the gonadotropin releasing hormone (GnRH) pulse secretion pattern, suppresses sex hormone binding globulin (SHGB) and enhances ovarian androgen production in PCOS patients (Bhattacharya and Jha, 2011; Hart *et al*., 2011; Huang *et al*., 2011; Lass *et al*., 2011; Rathsman *et al*., 2012; Wedin *et al*., 2012). Many studies have supported a correlation between hyperinsulinemia and PCOS showing that insulin resistance promotes hyperandrogenemia (Lungu *et al*., 2012). This led to the consideration of the use of insulin-sensitizing or insulin-mimetic drugs as part of management mechanisms for the disease.

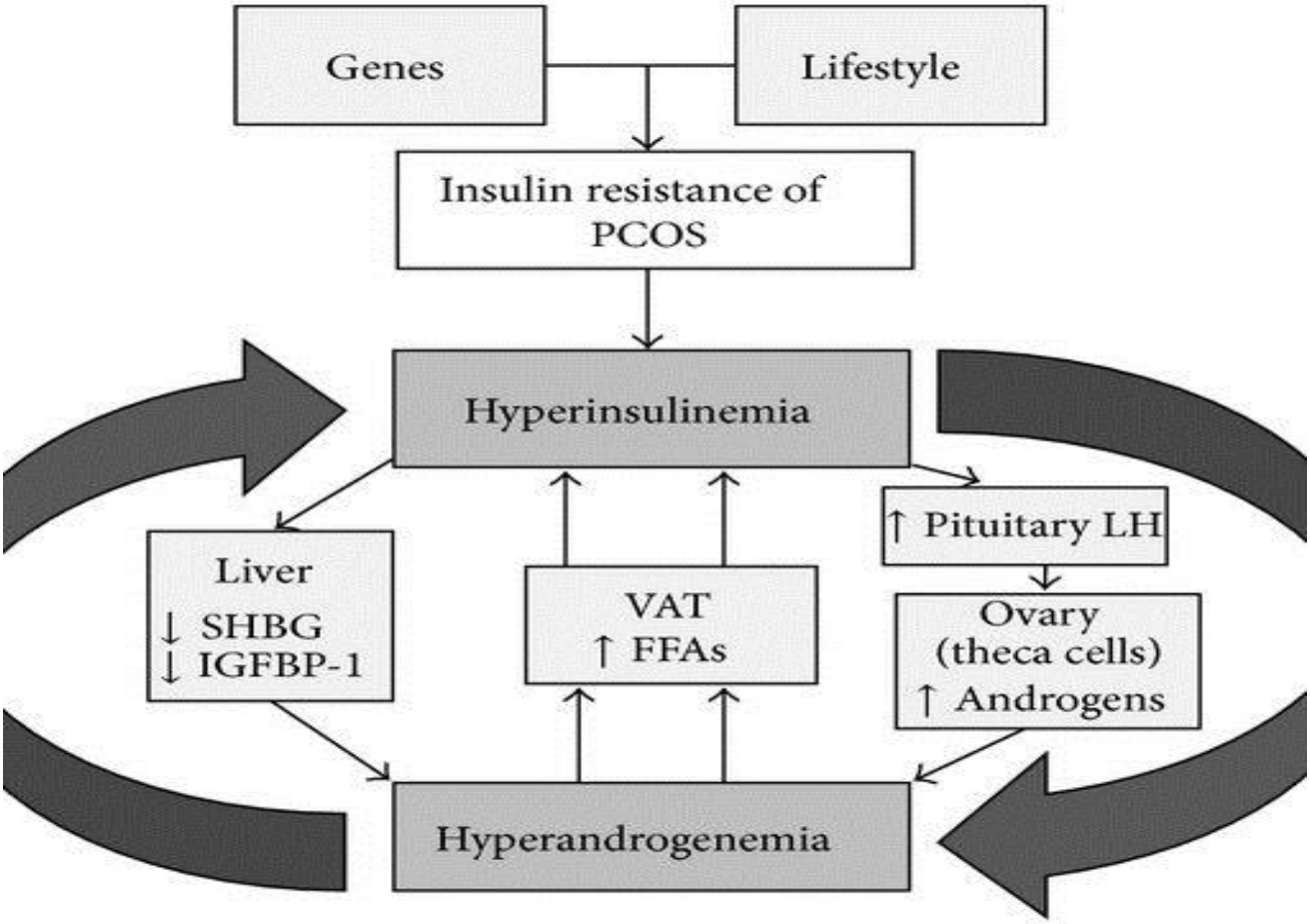


Figure 1: Relationship between hyperinsulinemia in PCOS

Source: Amjal *et al*., 2019

2.2.2.2 Obesity

Obesity is also one of the most important features of PCOS. Its prevalence is affected by environmental factors, ethnic backgrounds and lifestyle not on just the presence of PCOS. Childhood obesity is a risk factor for PCOS, obese girls are also at a higher risk of developing metabolic syndrome and then PCOS later in life(Pasquali *et al*., 2011).. Adults who are PCOS patients face a great risk of developing obesity (Randeva *et al*., 2012). Many studies show that females with PCOS have increased body fat distribution due to increased androgen production (Kirschner *et al*., 1990). Obese women with PCOS have increased prevalence of glucose intolerance and T2DM (Pasquali *et al*., 1985). In a study, dysregulation of lipolysis was identified in PCOS patients (Ingvar *et al*., 2002), as an increase in lipolysis of visceral fats results in an increase of free fatty acids in circulation. This levels of free fatty acids in circulation are major modulators of hepatic gluconeogenesis (Montague *et al*., 2000). Women with PCOS have atherogenic lipid profiles, combined with high LDL, triglycerides and total cholesterol levels and low HDL levels (Hart and Norman, 2006). They also face the risk of developing atherosclerosis, altered vascular endothelium and arterial stiffness (Hart and Norman, 2006). However obesity is not the main reason for these features. Kamangar *et al*., (2015) reported that the inter correlation between obesity and PCOS is still debatable.

2.2.2.3 Type 2 Diabetes Mellitus

PCOS poses a great risk of type 2 diabetes and gestational diabetes from early ages (Randeva *et al*., 2012). Many studies have constantly showed that women with PCOS face a greater risk of developing type 2 diabetes or impaired glucose tolerance later in life especially in young and middle aged women due to the high prevalence rate of obesity and insulin resistance. (Ehrmann *et al*., 1999; Legro *et al*., 1999, 2005; Boudreaux *et al*., 2006; Talbott *et al*., 2007; Lerchbaum *et al*., 2013). Family history of diabetes also increases prevalence of type 2 diabetes in PCOS patients but even though family history and obesity are major factors in development of diabetes in PCOS patients, insulin resistance allows for it to occur in lean PCOS patients especially those without a family history of diabetes.

2.2.2.4 Cardiovascular Disease

In 1992, Dahlgren *et al*., 1992 identified a 7 times higher risk of myocardial infarction in PCOS patients compared to healthy patients. However Pierpoint *et al*., 1998 carried out an epidemiological study that showed no difference in risk between the two groups. Some data showed PCOS patients to have an elevated level of CVD biomarkers such as C-reactive protein (Bahceci *et al*., 2004; Meyer *et al*., 2005) and lipoprotein A (Yilmaz *et al*., 2005; Bahceci *et al*., 2007; Berneis *et al*., 2009; Rizzo *et al*., 2009), when compared to their respective controls.

In 2010, The Androgen Excess-PCOS Society provided a consensus statement regarding increased risk of CVD in PCOS patients and presented a guideline to prevent complications. But despite the increased CVD risk markers and risk factors, uncertainty remains in regards to the increased morbidity and mortality rate in patients with PCOS (Legro, 2003; Wild *et al*., 2010; Schmidt *et al*., 2011; Sathyapalan and Atkin, 2012).

2.3 Diagnostic Criteria

In 1990, the National Institute of Health (NIH) proposed a criteria for diagnosis. It defined PCOS as a condition of unexplainable hyperandrogenic anovulation (Franks, 1991). It supposed that PCOS can be diagnosed in women if the following criteria were met; Symptoms of excess of androgens (clinical and biochemical), Rare to absent ovulations, Exclusion of other disorders with similar clinical symptoms (Franks, 1991)

Later in 2003, the Rotterdam criteria used polycystic ovarian morphology on ultrasound as a new criterion to be added to the previous NIH criteria. The European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine Rotterdam consensus (ESHRE/ASRM) developed the diagnosis of PCOS requiring only two out of the three features supposed: Anovulation or oligo-anovulation, Clinical or biochemical hyperandrogenism, polycystic ovarian morphology (PCOM) as seen on ultrasound

The Androgen Excess Society (AES) then defined PCOS as hyperandrogenism associated with ovarian dysfunction or polycystic ovaries thus considering androgen excess as a central factor in the development and pathogenesis of PCOS and established that hyperandrogenemia should be present and accompanied by either oligomenorrhea or PCOM or the both features (Azziz *et al*., 2006).

2.4 Symptoms of Pcos

The main cause of PCOS is still unknown but through some signs and symptoms can be used to detect PCOS. Example of these symptoms include hyperandrogenism (hirsutism, acne, Acanthosis nigricans, male pattern balding), obesity, oligo-ovulation, hypertension and dyslipidemia.

2.4.1 Oligo-ovulation

Can be described as irregular or infrequent ovulation. Anovulation is the total absence of ovulation. It can also be accompanied by irregular and heavy periods (Soni *et al*, 2018).

2.4.2 Obesity

A medical condition in which excess fat has accumulated in the body to the point it has a negative effect on the health. A person is normally termed as obese when their Body Mass Index (BMI) is above 30kg/m^2 while being overweight is within the 25-30kg/m^2 range. Being obese can increase the probability of having other diseases such as cardiovascular diseases, type 2 diabetes, and osteoarthritis amongst others (Soni *et al*, 2018).

2.4.3 Dyslipidemia

A condition in which lipid levels are either excessive or deficient. They may be expressed as increased LDL cholesterol, increased triglycerides level, increased cholesterol levels or decreased HDL cholesterol levels (Kannel, 1996)

2.4.4 Hyperandrogenism

A condition also known as androgen excess. It is a disorder characterized by excess production of male sex hormones especially testosterone in the female body coupled with the effects of elevated androgen levels (Soni *et al*, 2018).

2.4.5 Hirsutism

Can be referred to as hair growth in unwanted areas. It entails hair growth in unwanted areas usually in a male pattern usually on the face, chest, back, etc. A PCOS patient may also experience problems sleeping, headaches as well as trouble getting pregnant (Soni *et al*, 2018).

2.5 Etiologies of Pcos

The main cause of PCOS remains unknown till now but due to some research, it has been considered to be a multifactorial condition with genetic components.

Women with PCOS have abnormalities in androgen and estrogen metabolism; secretion and over-production of androgens. This high androgen levels can interfere with brain signals that normally ensure ovulation, thereby resulting in oligo-anovulation, causing the follicles within the ovary to enlarge forming cysts, produce other symptoms like acne, hirsutism, etc.

They also face metabolic disorders such as hyperinsulinemia and insulin resistance with obesity amplifying both abnormalities. Hyperinsulinemia promotes androgen production (Ehrmann, 2005) resulting in symptoms of PCOS, it also increases appetite leading to weight gain and dyslipidemia. It has also been linked to a skin condition known as acanthosis nigricans that affect skin pigmentation leading to dark patches of skin.

2.6 Management Options

PCOS management targets the symptoms that are usually present in patients; anovulation, infertility, acne and hirsutism as the most common symptoms, antagonizes the action of androgens on target tissues, reduces insulin resistance and corrects anovulation. It usually requires the joint effort of an interdisciplinary team and it focuses on two major guidelines which are The American Task Force (Legro *et al*., 2013) and The PCOS Australian Alliance Guidelines (Misso *et al*., 2014). Therapy options can be categorized into:

a) **Symptomatic control:** includes the use of oral contraceptive pills (OCPs), insulin sensitizing drugs, drugs used in treatment of hirsutism
b) **Fertility management:** ovulation induction; through the use of several nonpharmacologic (exercise, diet modifications and weight loss) and pharmacologic (aromatase inhibitors, insulin sensitizers, gonadotropins) approaches. It can be used alone or in combination to achieve ovulation.
c) **Long-term complications of PCOS:** lifestyle interventions, management of the metabolic syndrome are the best management options (Ajayi and Ogunmokun; 2006).

2.6.1 Lifestyle Changes

Lifestyle improvements are a very effective line of treatment and medication (Legro *et al*., 2013; Misso *et al*., 2014). Guidelines recommend exercise and diet should be a crucial part of management mostly in obese women with PCOS (Misso *et al*., 2014).

2.6.2 Medical Treatment

If lifestyle changes are not enough to resolve the symptoms, medical treatment is added to improve the management system.

2.6.2.1 Drugs used for PCOS treatment

Some of the drugs used for PCOS treatment include: metformin, rosiglitazone, pioglitazone, clomiphene citrate, ethinyl estradiol, desogestrel, letrozole, anastrozole, dexamethasone and exemestane.

a) **Insulin sensitizing drugs**: the safety profile of pioglitazone and rosiglitazone is still unestablished, therefore, metformin and clomiphene citrate are choice insulin sensitizing drugs.

b**) Oral contraceptive pills (OCP):** OCPs are the most commonly used medications for treatment of PCOS and have been recommended by the task force and the endocrine society (Legro *et al*., 2013), the Australian alliance (Misso *et al*., 2014), and the PCOS consensus group (Fauser *et al*., 2012) as first line treatment for hyperandrogenism and irregularities in menstrual cycle in PCOS patients. OCP acts by suppressing the hypothalamo-pituitary-ovarian axis (HPO axis), decreasing LH secretion, increasing sex hormone binding globulins and decreasing free testosterone levels (Costello *et al*., 2007). This tackles the hyperandrogenism-related symptoms; acne, hirsutism and menstrual irregularities (Costello *et al*., 2007). A number of clinical trials have raised concerns about the use of OCP and the increased risk of insulin resistance (Baillargeon *et al*., 2003; Legro *et al*., 2013) and the negative effects it has on the cardiovascular profile of PCOS patients (Baillargeon *et al*., 2005; Lidegaard *et al*., 2012). Nevertheless, results of various clinical trials have shown OCPs to be very effective and safe in the treatment of patients with PCOS (Mendoza *et al*., 2014) with their benefits outweighing the risks (Yildiz, 2008).

c) **Metformin:** an oral anti-diabetic drug that acts by hindering glucose production and increasing insulin sensitivity (Bailey and Turner, 1996; Morin-Papunen *et al*., 1998). Velazquez *et al*, carried out a studies in 1994 using metformin on PCOS patients and the results showed 35% reduction in the insulin area and 31% decrease in insulin area to glucose ratio (Velazquez *et al*., 1994). Some research data also revealed that metformin doesn't improve insulin resistance itself but improves glucose effectiveness, that is, the ability of glucose to repress endogenous

glucose production and stimulate glucose uptake (Pau *et al*., 2014). Metformin treatment of adolescents with PCOS was beneficial in improving glucose tolerance and insulin sensitivity, reducing insulinemia and androgen levels (Arslanian *et al*., 2002). A study by Tang *et al*, in contrast showed no significant effect in insulin sensitivity in PCOS patients receiving metformin treatment. This could be ascribed to the high level of obesity and limited weight loss the patients could attain (Tang *et al*., 2006). Other studies also showed metformin to have no effect on insulin resistance in PCOS patients (Ehrmann *et al*., 1997; Açbay and Gündoğdu, 1996). Even though studies have shown contradictory results on metformin effect, it is still suggested as a first line treatment for cutaneous manifestations and pregnancy complications in women with PCOS. It is also used in combination with clomiphene citrate to improve fertility results in clomiphene citrate resistant patients (Legro *et al*., 2013; Misso *et al*., 2014).

d) **Clomiphene citrate**: Clomiphene citrate is a non-setroidal fertility medicine. It causes the pituitary gland to release hormones needed to stimulate ovulation. It is used in treating medical conditions such as PCOS. It increases secretion of LH as well as FSH (Wheeler, 2019) suggesting that they work by stimulating a surge of gonadotropins and follicular rupture (ovulation).

2.7 Medicinal Plants Used In PCOS Treatment

2.7.1 *Asparagus racemosus*

Traditionally used in Indian medicine, it is useful in promoting the development of ovarian follicles, regulating menstrual cycle, and revitalization of the female reproductive system due to the presence of its phytoestrogen. It has also been helpful against hyperinsulinemia.

2.7.2 *Tinospara cordifolia*

A plant known for its hypoglycemic effect, it has also been useful in anti-inflammatory treatments. It helps lower insulin resistance and boosts the general metabolism processes in the body (Chandrasekaran *et al*, 2012).

2.7.3 *Foeniculum vulgare*

The seeds of this plant is used as a good supplement for PCOS management as they are a good source for phytoestrogens. These phytoestrogens helps reduce insulin resistance and

inflammation in PCOS (Jungbauer *et al*, 2015). It has also been helpful in treatment of diabetes, kidney stones, bronchitis nausea and vomiting (Wesam *et al*, 2015)

2.7.4 *Ocimum tenuiflorum*

Ocimum tenuiflorum is a traditional herb that is potentially effective against PCOS as it possesses excellent anti-androgenic properties in reduction of androgen production. It is also a promising mode of treatment against other ailments as well as management of obesity (Swayamjeet *et al*, 2016).

2.7.5 *Actaea racemosa*

Actaea racemosa is useful in treatment of disorders of the female reproductive system such as anovulation, hormonal imbalance and infertility also important issues in PCOS. It has the ability to induce ovulation in PCOS patients (Bency *et al*, 2016). It has also been effective in treating amenorrhea, dysmenorrhea and other uterine conditions (Shari, 2009).

2.7.6 *Lepidium meyenii*

From the brassicaceae family, *Lepidium meyenii* is an herb that is used in relieving menopausal symptoms, helps in stimulating the endocrine system and acts as a hormonal balancer with no side effects (Gonzales *et al*, 2002).

2.7.7 *Taraxacum officinale*

Taraxacum officinale also known as dandelion root is effective as a bile flow stimulant and liver detoxifier. It is used as a cleanser for the liver and helps in getting rid of hormones build-up. This clean up can stimulate SHGB production reducing the level of free testosterone in the blood. It also helps in removal of toxins from the body.

2.7.8 *Ocimum gratissimum*

Ocimum gratissimum, is an herbaceous plant belonging to the family *Lamiaceae* (Calixto, 2000). It is widely distributed and found in warm, temperate regions and tropical regions of Africa and Asia (Okigbo *et al*, 2006) and is the most abundant species of the genus *Ocimum*. In Africa, it is commonly known as scent leaf, clove basil, African basil (Okigbo *et al*, 2006). In Nigeria, it is called *Effinrin* by the Yoruba tribe, *Nchanwu* by the Igbo tribe, and *Daidoya* by the Hausa tribe. It is native to Africa, Madagascar, Southern Asia, Hawaii, Mexico and Brazil. It is also considered invasive in the pacific and Caribbean islands (Calixto, 2000).

2.7.8.1 Morphology of *Ocimum gratissimum*

Ocimum gratissimum is a perennial plant that s woody at the base, they grow to about 1-3 m in height with stemmed branches. The stems are brown in color with leaves from top to bottom. The leaves are mostly green colored and are narrow and ovate-lanceolate in shape measuring about 5-13cm in length and 3-9 cm in width. Petioles of the plant are up to 6cm long and racemes are about 18cm long. The plant stalk is densely pubescent. Flowers are pale yellow and give off a sweet scent (Wagner, 1993).

2.7.8.2 Taxonomy of *Ocimum gratissimum*

Kingdom- Plantae

Domain- Eukaryota

Kingdom- Plantae

Subkingdom- Tracheobionta

Phylum- Spermatophyta

Subphylum- Angiospermae

Superdivision- Spermatophyta

Division- Magnoliophyta

Class- Magnoliopsida

Subclass- Asteridae

Order- Lamiales

Family- *Lamiaceae*

Genus- *Ocimum L.*

Species- gratissimum L.

2.7.8.3 Traditional Uses

Ocimum gratissimum has been used as an herbal plant in many countries. In the north east of Brazil, it is used for medicinal and culinary purposes. Because of its rich oil content, it is used in

preparation of teas and infusion (Rabelo *et al*., 2003). In the coastal regions of Nigeria, it is used in treating epilepsy, high fever and diarrhea (Effraim *et al*., 2003), in the savannah regions, a decoction of scent leaf is useful in treating diarrhea, gonorrhea. In West Africa, it is used as an antipyretic, antimalarial and anticonvulsant. It is also used by the Igbos of Nigeria, to manage babies' cord and keep the wound surfaces sterile. It is useful in treating fungal infections, fever, cold and catarrh (Ijeh *et al*., 2005). Inhabitants of the Brazilian tropical forest use the decoction of *Ocimum gratissimum* roots as a sedative for children (Cristiana *et al*., 2006). People of sub-Saharan communities also use it as a treatment for blocked nostrils by rubbing the leaves between the palms and sniffing it, it is used as treatment for abdominal pains, barrenness, fever, cough, sore eye, convulsions, menstrual irregularity and ear infections (Matasyoh *et al*., 2007).

2.7.8.4 Pharmacological Activity

Many studies have shown *Ocimum gratissimum* to have potential as an antifungal and antibacterial agent (Pandey, 2017; Iqbal, 2015). The plant oil through its etiologic properties have shown a potentiality in inhibiting the virulent strains of shigella isolates, the organism responsible for diarrhea. The pharmacological effect of the aqueous extract of the plants were tested and showed inhibition of the jejunum spontaneous pendular movement in rabbit, non-toxic analgesic effects in mice and non-competitive stomach strip blocking in rats (Aziwa, *et al*, 1999). The plant oil also helped reduce extracellular protease activity, olipopolysaccharide rhamnose content and incidence of kerato conjunctivitis in guinea pigs (Iwalokun, *et al*, 2003). The methanol leaf extracts of the plants can be a therapeutic agent against nicotine toxicity by decreasing lipid-protein damage, generation of free radicals and antioxidant status of male mice murine peritoneal macrophages in vitro (Mahapatra *et al*, 2009). The hexane fraction showed the highest the highest antimicrobial activity against *Vibrio cholerae* and *Klebsiella pneumoniae* and was found to possess the highest grain protectant activity (Ekwenchi *et al*, 2014). The ethanol leaf extracts displayed antimicrobial activity against *S. typhi, N. gonorrhoeae, K. pneumoniae, P. aeruginosa, and V. cholera* (Mann Abdullahi, 2012) and when used synergistically with ampicillin exhibits antibacterial properties against candida albicans isolates (Nweze *et al* 2009).

The aqueous leaf extract showed hypoglycemic effect reducing Triacylglycerol and LDL-Cholesterol as well as lipid level malondialdehyde. It also reduces the plasma glucose level of streptozotocin impelled diabetic rats (Egesie *et al*, 2006) and inhibits liver injuries from CCl4-

impelled rats (Chun-Ching *et al*, 2012) this showing a potentiality in tackling diseases in man. It also inhibits chemotaxis, proliferation and it also reduces tumor sizes in breast cancer cells (Nangia-Marker *et al* 2007). The dichloromethane leaf extract can inhibits myeloid leukemia (Iweala *et al*, 2015) in vitro, showing a potentiality in combatting cancer in man.

2.8 Gas Chromatography Mass Spectrometry (GC-MS)

Gas chromatography coupled to mass spectrometry is a powerful and preferred technique analysis of small volatile molecules. It is also very useful in analysis of unknown compounds and multi component quantitation. Gas chromatography is a technique that vaporizes a sample mixture into gaseous compounds and then separates them according to their boiling points and differential adsorption on a porous or liquid support. It is commonly used for the analysis of volatile and low molecular weight compounds. Mass spectrometry is a highly sensitive detection technique that forms, separates and detects ions in the gaseous phase. When it is used in combination with GC, it ionizes the gaseous eluted compounds, separates the ions in vacuum based on their mass-to charge ratios and measures their individual intensity the output produced is the mass chromatogram. GCMS separates and quantifies multi-component samples, complex matrices and identifies unknown samples. Data obtained from GCMS such as retention time, molecular weight and mass spectra can be used for spectral research. With the aid of an additional software, it can also calculate accurate mass and determine molecular composition. This technique is extremely useful in qualitative analysis, that is, the identification of molecules.

2.8.1 Principle of GC-MS

GCMS is comprised of two major parts; the gas chromatograph and the mass spectrometer and they are connected to each other through an interface. The GC separates compounds in a column and is dependent on the nature of the compound, the column's packed material and the column's dimensions; thickness, length and diameter (Shimadzu corp. 2020). In the separation of molecules, the difference in the physio-chemical properties of each compound in the sample mixture is a key factor in the separation of each molecule as they interact with the stationary phase in the column (Shimadzu corp. 2020). The amount of time for each sample to be eluted from the gas chromatograph varies allowing the mass spectrometer to capture, ionize, accelerate, deflect and detect the ionized molecules separately (Shimadzu corp. 2020). The mass

spectrometer detects the ionized molecules by using their charge to mass ratio (Shimadzu corp. 2020).

2.8.2. Applications of GC-MS

a) Environmental monitoring and cleanup: GC-MS is becoming a choice tool for tracking organic pollutants. The cost for GC-MS has decreased and the reliability, hence the increased adoption of the tool in environmental studies.
b) Criminal forensics: GC-MS can analyze particles from a human body in order to help link a criminal to a crime. GC-MS is of essential use here as accuracy of results is needed.
c) Law enforcement: GC-MS is increasingly used in detection of illegal narcotics and forensic technology to find drugs and poisons in biological specimens of suspects or victims (Gohike, 1959).
d) Food beverage and perfume analysis: Food and beverages contain various aromatic compounds. GC-MS is extensively used for the analysis of these compounds such esters, fatty acids, alcohols, aldehydes and terpenes. It is also useful in detection and measurement of contaminants from spoilage which may be harmful.
e) Medicine: in combination with isotopic labeling of metabolic, the GC-MS tool is also used for determining metabolic activity.

2.9 Oestrous Cycle

The oestrous cycle is the reproductive cycle of female species of non-primate vertebrates, like, rats, mice, horses, pigs. This cyclic process consists of a series of events both physiological and behavioral. There are also different forms of the cycle in animals: Polyoestrous animals: their oestrous cycles run throughout the year (e.g. cattle, pigs, mice, and rat), seasonally polyoestrous animals: animals that have multiple oestrous cycles during certain periods of the year (Examples are horses, sheep, goats, deer, and cats), Monooestrous animals: they have only one oestrous cycle per year. (Animals such as dogs, wolves, foxes, bear).

2.9.1 Stages of the oestrous cycle

a) Proestrus: this is the first stage in the cycle which involves the development of the endometrium and ovarian follicles. It is the period between the regression of the corpus luteum of the previous cycle and estrus (Fig.2).

b) Estrus: the second stage in the cycle before the metestrus stage often referred to as the heat period. During this period, pheromones such as estrogen is produced in high quantities. This high estrogen level is responsible for the behavioral signs displayed by the animals such as receptivity to a male and mating example is in the mounting of cows, the willingness to be mounted by another cow (Fig.2).
c) Metestrus: the estrus stage is followed by the metestrus stage that usually lasts for about 3-4 days. It is characterized by sexual inactivity. The corpus luteum is developed under the influence of luteinizing hormone (LH) and begins to produce progesterone (Fig 2).
d) Diestrus: this is the period between the metestrus and the regression of the corpus luteum. It is the last stage in the cycle characterized by a functional corpus luteum and increase in progesterone level in the blood (Fig 2). (Hill, 2021).

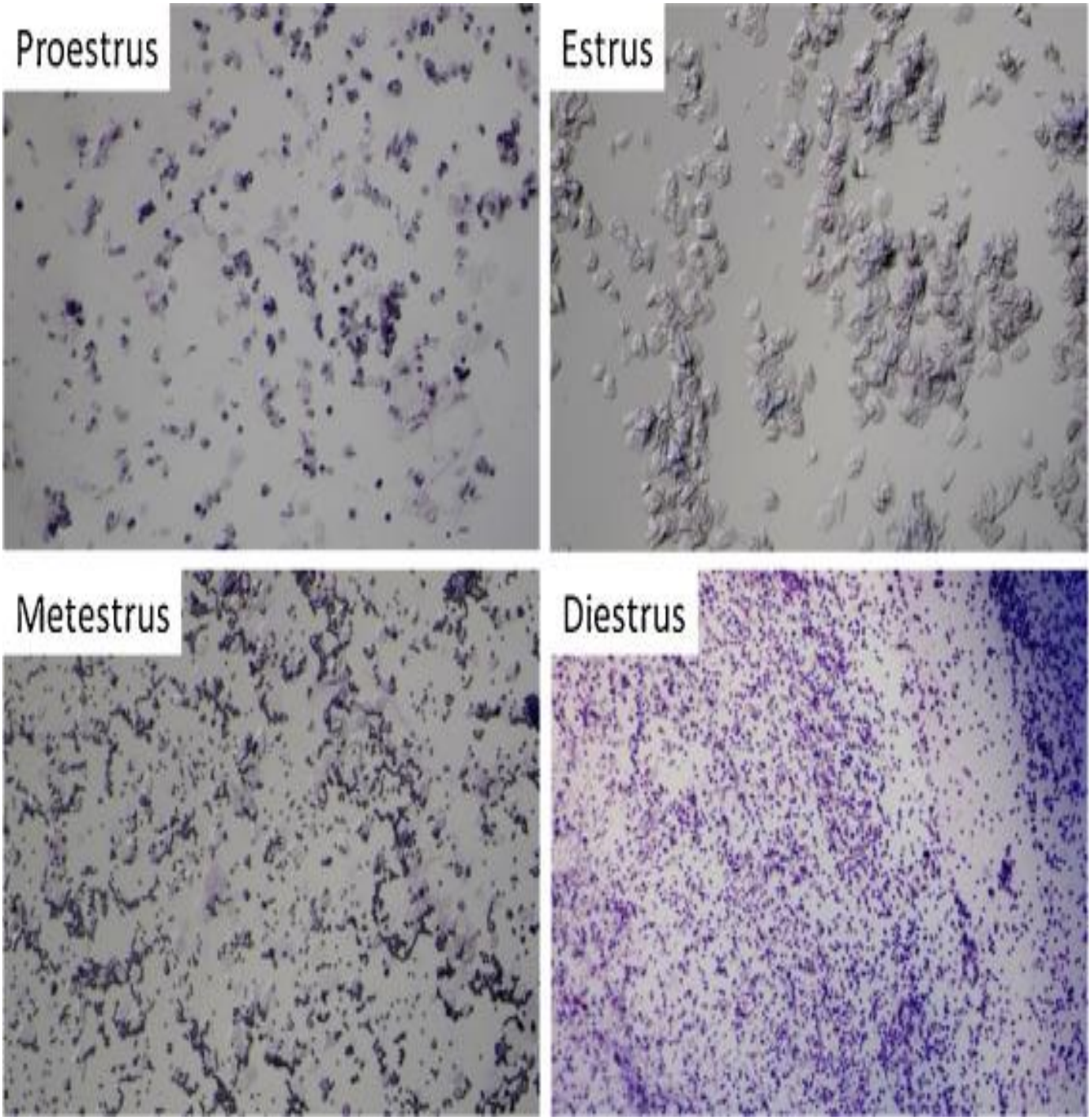


Figure 2: Stages of the oestrous cycle

Source: Inyawilert *et al*., 2016

2.10 Class of Compound: Aromatase Inhibitors

Aromatase inhibitors are compounds that lower estrogen levels by inhibiting an enzyme called aromatase preventing it from converting other hormones into estrogens. Estrogens are believed to fuel the growth of breast cancer cells (Yager and Davidson, 2006). These drugs do not totally stop the production of estrogen but rather they reduce the levels of estrogens especially in women whose ovaries are not producing estrogen. This means less estrogen is available to stimulate growth of hormone-receptor-positive breast cancer cells (Yager and Davidson, 2006).
There are three aromatase inhibitors; Arimidex (chemical name: Anastrozole), Aromasin (chemical name: Exemestane), Femara (chemical name: Letrozole) (Miller, 1999)

Aromatase inhibitors do not stop the ovaries from producing estrogen so they are majorly used to treat postmenopausal women.

2.10.1 Letrozole

Letrozole also known as femara is a non-steroidal aromatase inhibitor. It is an oral anti-estrogen drug used to treat postmenopausal women with breast cancer. Estrogens have been discovered to promote growth of breast cancer. The adrenal glands is where these estrogens are synthesized, Letrozole inhibits the enzyme in the adrenal glands responsible for the production of these estrogens (Nabholtz, 2008).

2.10.1.1 Mechanism of Action of Letrozole

Letrozole is a third generation type II aromatase inhibitor. It acts by competitively inhibiting aromatase (cytochrome P-450 [CYP] 19), preventing it from catalyzing the rate-limiting step in estrogen biosynthesis, that is, the conversion of steroidal C-19 androgens to C-18 estrogens (Nabholtz, 2008). This process leads to uterine weight reduction and increase in concentration of luteinizing hormone. In postmenopausal women, aromatase is responsible for the most estrogen production, therefore inhibiting this enzyme will reduce the availability of estrogen to estrogen-dependent tumors hence preventing their growth (Nabholtz, 2008; Bhatnagar, 2007)

2.11 Phytochemical Constituents and Pcos

Phytochemicals are a wide variety of compounds or secondary metabolites made by plants that possess medicinal properties that are useful in maintaining human health (Grusak, 2002). Some of these secondary metabolites include alkaloids, phenols, glycosides, saponins, tannins, flavonoids, and phlobatannins.

Flavonoids are the most common group of polyphenolic compounds in the human diet and are found in most plants (Ververridis *et al*., 2007). Tannins may promote glucose transport while inhibiting adipocyte development in insulin-resistant type 2 diabetes β-cells (Riedl and Hangerman, 2001). Insulin resistance along hyperinsulinemia are hallmarks of PCOS posing a higher risk of T2DM and impaired glucose tolerance on women with this condition (Ehrmann *et al*., 1999). Saponins have been reported to enhance the synthesis of progesterone (Yang *et al*., 2003). Alkaloids have also been reported to decrease blood glucose and insulin levels (Baldoen *et al*., 2012).

2.12 Hormone Study

2.12.1 Estradiol

Estradiol or oestradiol is one of the three estrogens produced in the human body. It is present in both men and women and plays different roles but women have a higher level of the hormone than men. In men, estradiol helps in bone maintenance, nitric oxide production and brain production. In the female body, its main function is maturation and maintenance of the reproductive system. During the menstrual cycle, increase in estradiol levels cause the egg to mature and be released, it also ensures the thickening of the uterus lining in preparation for the implantation of a fertilized egg. This hormone is made primarily in the ovaries so there is a decline in concentration as women age (Abramowitz, 2020).

High estradiol levels have been linked to acne, constipation, loss of sex drive and depression as well as increasing the risk of uterine and breast cancer and cardiovascular diseases. There may also be weight gain and menstrual problems. In men, it can lead to development of female characteristics and loss of sexual function and muscle tone (Abramowitz, 2020).

In cases of insufficient levels of estradiol, bone growth and development is affected and can result into osteoporosis. Girls may also experience delayed puberty (Abramowitz, 2020).

2.12.2 Progesterone

Progesterone is a steroid hormone secreted by the corpus luteum, a temporary gland formed after ovulation. It prepares the endometrium for possible pregnancy after ovulation by triggering the lining to thicken to accept a fertilized egg. It prevent muscle contractions of the uterus that will cause the body to reject the egg (Fish, 2019).

If pregnancy does not occur, the corpus luteum breaks down and progesterone levels decline. This results in the shedding of the uterine lining known as menstruation, if pregnancy occurs, progesterone stimulates the body to blood vessels that will feed the growing fetus. When the placenta is formed, it also begins to secrete progesterone allowing elevated levels of the hormone throughout pregnancy (Fish, 2019). Women with PCOS are anovulatory in general, and their serum progesterone levels are low and non-ovulatory as a result.

2.12.3 Testosterone

Testosterone is the major sex hormone found in males and this hormone is produced in the testes (testicles). It is responsible for male physical features and also for all the physical changes observed in boys transitioning to men; a stage known as puberty (Puneet Arora, 2020). Some of these changes include growth of the penis and testes, deepening of the voice, building up of the muscles and strong bones. It contributes to sex drive, bone density and muscle strength. Men also need this hormone to produce sperm for the purpose of reproduction (Puneet Arora, 2020). This hormone is also present in women but in lower levels. If testosterone level in women is high, it can lead to development of male pattern baldness, a deep voice and menstrual irregularities. It can also lead to acne, infertility, facial hair growth and changes in body shape (Markus, 2019)

2.12.4 Follicle Stimulating Hormone

Follicle stimulating hormone is a gonadotrophic hormone. It is an essential hormone important to pubertal development also with the proper functioning of ovaries in women and testes in men. In women, FSH stimulates the maturation of the ovarian follicle before ovulation takes place. In men, FSH acts on sertoli cells to stimulate spermatogenesis (Angela and Evelina. 2011). Alongside Luteinizing hormone, it promotes ovulation in women. In women with PCOS, LH levels tend to be higher than FSH levels. This low levels of FSH can lead to poor development at puberty and also poor ovarian function or ovarian failure. The ovarian follicles do not develop

fully and then ovulation does not occur and this can then lead to infertility (Angela and Evelina. 2011).

2.12.5 Luteinizing Hormone

Amongst the hormones that control the human reproductive system is the luteinizing hormone. It plays different roles in ensuring a healthy reproductive system in both men and women. Luteinizing hormone is produced and released in the anterior pituitary gland and is considered a gonadotrophic hormone because of its role in regulating the functions of the gonads in both males and females (Angela and Evelina. 2011). In women, LH stimulates the production of estradiol by the ovaries. An increase in LH levels during a woman's cycle allows the release of a matured egg, a stage known as ovulation and if fertilization of the egg occurs, it stimulates the corpus luteum to produce progesterone to sustain the pregnancy. In men, luteinizing hormone stimulates the leydig cells in the testes to produce testosterone which then stimulates the production of sperm cells and accentuates male features during puberty (Angela and Evelina. 2011).

High levels of luteinizing hormone can lead to infertility and in most cases, it is connected to polycystic ovarian syndrome as it creates excessive levels of testosterone. Low levels of this hormone can also result to infertility as it may limit the ovulation process (Angela and Evelina. 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Collection of Plant Material

Fresh leaves of *Ocimum gratissimum* was collected at Magboro, Ogun state, Nigeria. The plant was identified at the botany department of The University of Lagos, Lagos, Nigeria, by Dr. Nodza George, where a voucher specimen (LUH 8752) was deposited.

3.1.2 Experimental Animals

The experimental animals that were used for the study were obtained from animal house of Mountain top university, Ogun state. The animals were acclimatized for two weeks and were maintained under good environmental conditions with free access to food and water.

3.1.3 Drugs, Assay Kits and Chemicals

Letrozole (2.5mg), Metformin (500mg), Clomiphene Citrate (50mg), Total cholesterol, triglycerides, HDL-Cholesterol assay kits used were products of Randox laboratory, Co-Atrim, United Kingdom. Progesterone, insulin, testosterone, follicle stimulating hormone, luteinizing hormone assay kits were manufactured by Diagnostics Laboratories.

3.2 Methodology

3.2.1 Preparation of Extract

The identified sample was thoroughly washed with clean water to remove contaminants. It was then air-dried and oven dried at 50℃ till it attained a constant weight (340g). The dried sample was then pulverized with the aid of an electric blender and afterwards weighed 338g. 1400ml of ethanol was added to the sample into a container with a lid and kept in a cupboard for 48hours. It was then sieved using a jute bag and was transferred into another container. The sample was further filtered using wathman's filter paper and funnels separating the filtrate from the residue. The residue was collected in beakers and the plant extract was separated from the ethanol using a rotary evaporator. The extracted sample was dried in an oven at 50℃ to concentrate which yielded 31.21g (9.23% yield). The concentrates were refrigerated at -4℃.

3.2.2 Phytochemical Screening

3.2.2.1 Qualitative Phytochemical Analysis

The plant extract was then tested for bioactive compounds using the method described by Roghini and Vijayalakshmi (2018).

a. Test for carbohydrates

1 ml of the molisch reagent was added to 2ml of the extract and the mixture was shaken properly. Few drops of concentrated sulphuric acid was added to the mixture. The presence of carbohydrates was suggested by the appearance of a reddish or purple color (Roghini and Vijayalakshmi, 2018).

b. Test for tannins

To 1ml of the extract, 2ml of 5% ferric chloride was added and shaken properly. The presence of tannin was determined by the appearance of a dark blue or greenish black color (Roghini and Vijayalakshmi, 2018).

c. Test for saponins

In 2ml of distilled water, 2ml of the extract was added and the moisture was shook for 15 minutes in a test tube. Saponin was inferred present due to foam formation (Roghini and Vijayalakshmi, 2018).

d. Test for flavonoids

To 2ml of the extract, 1ml of 2N sodium hydroxide was added. The presence of flavonoids was indicated by the appearance of a yellow color (Roghini and Vijayalakshmi, 2018).

e. Test for alkaloids

In 2ml of the extract, 2ml of conc. HCl was added with few drops of Mayer's reagent. Alkaloids was indicated to be present after a green color or white precipitate appeared (Roghini and Vijayalakshmi, 2018).

f. Test for glycosides

3ml of chloroform and 10% ammonia solution was added to 2ml of the extract. The pink color formed indicated the presence of glycosides (Roghini and Vijayalakshmi, 2018).

g. Test for quinones

1ml of conc. H_2SO_4 was added to 1 ml of the plant extract. Appearance of a red color indicated the presence of quinones (Roghini and Vijayalakshmi, 2018)

h. Test for phenols

2ml of distilled water and few drops of 10% of FeCl was added to 1ml of the plant extract. Presence of phenols was indicated from the appearance of blue or green color (Roghini and Vijayalakshmi, 2018)

i. Test for terpenoids

To 0.5ml of the extract, 2ml of chloroform and conc. H2so4 was added. Appearance of a red or brown color indicated the presence of terpenoids (Roghini and Vijayalakshmi, 2018)

j. Test for cardiac glycosides

To 0.5ml of the extract, 2ml of glacial acetic acid and few drops of ferric chloride were added. The mixture was under layered with 1ml conc. H2so4. Presence of cardiac glycosides was indicated by the presence of a brown ring at the interface (Roghini and Vijayalakshmi, 2018).

k. Ninhydrin test

To 2 ml of the extract, few drops of 0.2% ninhydrin reagent was added and the mixture was heated for 5 minutes. Presence of amino acids was indicated by the presence of a blue color after heating (Roghini and Vijayalakshmi, 2018).

l. Test for coumarins

1ml of 10% NaOH was added to 1ml of the extract. Presence of coumarins was indicated by the appearance of yellow color in the solution (Roghini and Vijayalakshmi, 2018)

m. Test for anthraquinones

To 1 ml of the extract, few drops of 10% ammonia was added and the appearance of a pink colored precipitate indicated the presence of anthraquinones (Roghini and Vijayalakshmi, 2018)

n. Test for steroids

1ml of chloroform and few drops of conc. H2so4 was added to 1ml of the plant extract. Formation of a brown ring indicated the presence of steroids (Roghini and Vijayalakshmi, 2018).

o. Test for phlobatannins

Few drops of 2%HCl was added to 1ml of the extract. Presence of phlobatannins was indicated by the appearance of a red coloration in the solution (Roghini and Vijayalakshmi, 2018).

3.2.3 Animal Grouping and Induction

Sixteen female Wistar rats were acclimatized for two weeks and afterwards PCOS was induced by administering 1mg/kg of letrozole to each rat for 21 days. Group A (control) received 1ml of CMC. The letrozole was dissolved in 0.5% CMC (the vehicle) before administration. Administration was done once daily for 21 days through subcutaneous route.

3.2.3.1 Confirmation of PCOS

The oestrous cycle of the rat was monitored for the 21 days of induction by viewing pap smears under a light microscope for the predominant cell type

3.2.3.2 Vaginal Cytology

The vaginal cytology of the rat was achieved by taking pap smears from the vagina using cotton tipped swabs dipped in distilled water. The swabs were rotated and after 2-3 revolutions, the sample were then smeared on a glass slide and viewed under the light microscope.

3.2.3.3 Determination of Fasting Blood Glucose

The Fasting blood glucose of each rat was determined 24hrs after the last dose of letrozole was administered and after fasting the rats for 12hrs. The fasting blood glucose was determined using Accucheck glucometer kit. The tails of the rats were pierced using a lancet and the blood was dropped on the accucheck strip. The units for the fasting blood glucose was measured in mg/dl

3.2.3 Preparation of Serum and Tissue Supernatants

After sacrificing the rats the organ of choice were isolated in sucrose solution in universal bottles. To prepare the serum, the blood sample collected was centrifuged. The serum was then decanted into well labelled sample bottles and diluted with 0.25% sucrose solution using a dilution factor of x5 for the biochemical assays to be run. The stock solution of the serum was preserved for hormonal assays. In preparing the tissue supernatants, each organ was weighed and homogenized in the presence of sucrose solution using a mortar and pestle. The samples were centrifuged and decanted into sample bottles for each organ. The decanted solutions were diluted with sucrose solution with respect to each organ type.

Table 1: dilution factor for assays

Serum parameter	Serum
Total cholesterol	X5
Triglycerides	X5
High density lipoprotein cholesterol	X5
Low density lipoprotein cholesterol	X5

3.2.4 Animal Grouping and Extract Administration

PCOS was induced in 16 female wistar rats for a period of 21 days. After the last dose of letrozole was administered and the fasting blood glucose was determined, the extract administration was carried out as follows:

a. group A (non-PCOS induced control) received 1ml of distilled water
b. group B (PCOS induced control) received 1ml of distilled water
c. group C (PCOS induced) received 7.14mg/kg of metformin and 2mg/kg body weight of clomiphene citrate (reference drug)
d. group D (PCOS induced) received 50mg/kg of the extract
e. group E (PCOS induced) received 100mg/kg of the extract

At the end of the experimental period. The animals were anesthetized with cotton wad soaked in diethyl ether and were sacrificed by jugular puncture. Thereafter the ovaries, liver and kidneys were isolated.

3.2.5 Determination of Serum Concentration of Hormones

3.2.5.1 Progesterone

The serum progesterone concentration was determined using competitive Accu-Bind kits.

Principle

The principle is based on a one step immunoassay to determine the presence of progesterone using competitive microplate enzyme immunoassay. Progesterone present in the sample competes with enzyme progesterone conjugates for binding with anti-progesterone coated microplate to form an antigen-antibody complex. The activity of the enzyme in the antibody bound fraction is inversely proportional to the native progesterone concentration (Eastham, 1985).

Procedure

To 0.025 ml of each calibrator, control and serum samples in the microplate wells, 0.10 ml of the conjugate was dispensed into each well. The microplate was swirled gently for 30 seconds to mix

and was incubated for 60 minutes at 25°C. The content of the microplate was decanted and 0.30 ml of the washing solution was added repeatedly four times. TMB substrate (0.10 ml) was added and incubated at room temperature for 25 minutes in the dark after which 0.15ml of stopping reagent was pipetted into each well. The absorbance was read on microplate reader at 450nm within 20 minutes after the addition of the stopping reagent.

Calculation

The serum progesterone concentration of the test samples was extrapolated from the calibration curve (figure 10) plotting the absorbance of the standard sample at 450nm against its corresponding concentration.

3.2.6.2 Testosterone

The serum testosterone concentration was quantitatively determined using the direct human testosterone Accu-bind kit.

Principle

The testosterone EIA is based on the principle of competitive binding between testosterone HRP conjugate for a constant amount of rabbit anti-testosterone, Goat anti-rabbit IgG-coated wells and rabbit anti-testosterone reacted with the test sample. HRP-labeled testosterone competes with endogenous testosterone peroxidase conjugate immunologically bound to the well and progressively decrease as the concentration of testosterone in the specimen increases. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled testosterone in the sample (Eastham, 1985).

Procedure

Exactly 0.01ml of standards, control and test sample, 0.1 ml of testosterone HRP conjugate reagent and 0.05ml of rabbit anti-testosterone reagent were dispensed into each well, mixed thoroughly for 30 seconds and incubated at 37°C for 90 minutes. The microwells were rinsed and flicked for 5 minutes with distilled water. A known volume (0.1ml) of 3,3, 5,5-tetramethylbenzidine (TMB) reagent was dispensed into each well, mixed gently for 5 seconds and incubated at room temperature for 20 minutes. The blue color completely turned yellow and the absorbance was read at 450nm with a microtitre well reader within 15 minutes of the standard solutions against its corresponding concentrations. The reaction was stopped with the addition of 1N HCl, afterwards the absorbance was read at 450nm.

Calculation

The serum testosterone concentration was extrapolated from the calibration curve (figure 11). Plotting the absorbance of the standard sample at 450nm against its corresponding concentration.

3.2.6.3 Follicle Stimulating Hormone (FSH)

The serum FSH was quantitatively determined using the direct human serum follicle stimulating enzyme immunoassay kits.

Principle

This is based on the principle of sandwich method. The enzyme assay system utilizes a high affinity and specificity monoclonal antibody (enzyme conjugate and immobilized) directed against a distinct antigenic determinant on the intact FSH molecule (Eastham, 1985).

Procedure

Microplate wells of each reference, control and serum samples to be assayed were in duplicate. A known weight (0.025ml) of each calibrators, control and serum samples were pipetted into appropriate wells. Exactly 0.1ml of the conjugate was dispensed into each well, except for the blank (which contained distilled water) and incubated on the thermo shaker for 30 minutes at 37^{o} C. Each well were washed five times with 0.3ml of working washing solution and tapped firmly against absorbing paper to ensure that it dried. Exactly 0.1ml of 3,3, 5,5-tetramethylbenzidine (TMB) substrate was added to each well, and incubated at 25℃ in the dark for 30 minutes. A known volume of 0.15 ml of stopping reagent was placed into each well and the mixed gently for 10 seconds; the plate was read on microplate reader at 450 nm within 20 minutes after the addition of the stopping reagent. A calibration curve of absorbance standards was plotted against the concentration and this was used in the determination of concentrations for the tests samples.

Calibration

The serum FSH concentration was extrapolated from the calibration curve (figure 12). Plotting the absorbance of the standard sample at 450nm against its corresponding concentration

3.2.6.4 Luteinizing Hormone (LH)

The serum LH was quantitatively determined using the direct human serum follicle stimulating enzyme immunoassay kits.

Principle

This is based on the principle of sandwich method. The enzyme assay system utilizes a high affinity and specificity monoclonal antibody (enzyme conjugate and immobilized) directed against a distinct antigenic determinant on the intact LH molecule (Eastham, 1985).

Procedure

A known volume (0.025 ml) of the standard solutions, control, serum samples were placed in appropriate wells. Exactly 0.1ml of the conjugate was dispensed into each well except blank and incubated on a thermo shaker for 30 minutes at 37°C. each well was washed five times with 0.3 ml of working washing solution and tapped firmly against absorbing paper to ensure its dryness. Exactly 0.1ml of 3,3, 5,5-tetramethylbenzidine (TMB) reagent was dispensed into each well and incubated at 25° C for 30 minutes in a dark place. Thereafter, 0.15 ml of stop reagent was pipetted into each well and mixed gently for 10 seconds. The absorbance of the samples were read on the microplate reader at 450nm within 20 minutes after the addition of stop reagent. Calibration curve of the standard was plotted to get the concentrations of each test sample.

Calculation

The serum LH concentration was extrapolated from the calibration curve (figure 13). Plotting the absorbance of the standard sample at 450nm against its corresponding concentration.

3.2.6.5 Insulin

The serum insulin was quantitatively determined using microplate immunoenzymometric assay kit as described in the manufacturer's protocol version.

PRINCIPLE

The principle is based on immobilization that takes on a surface of a microplate well through the interaction of streptavidin coated on the wells and exogenously added biotinylated monoclonal insulin antibody. The enzyme labelled antibody and a serum containing the native antigen reactive results between the native antigens and antibodies without competition or steric

hindrance to form a soluble sandwich complex. The activity of the enzyme in the antibody-bound fraction is directly proportional to the native antigen concentration (Eastham, 1985).

Calculation

The serum insulin concentration was extrapolated from the calibration curve (figure 14). Plotting the absorbance of the standard sample at 450nm against its corresponding concentration

3.2.7. Lipid Profile Determination

3.2.7.1 Serum Total Cholesterol Concentration

The total cholesterol concentration in the serum of the rats was carried out using the CHOD-PAP reaction (Tietz 1995).

Principle

It is based on the following reactions:

$$\text{Cholesterol} + H_2O \longrightarrow \text{Cholesterol} + \text{Fatty acids}$$

$$+$$

$$H_2O + O_2 \longrightarrow \text{Cholesterol} + H_2O_2$$

$$H_2O_2 + \text{4-Amino antipyrine} + \text{P-Hydrobenzoic acid} \longrightarrow \text{Colored Quinonic derivatives} + 4\ H_2O$$

Procedure

A known volume (2.0ml) of the working reagent was placed into test tubes containing 0.02 ml of the appropriately diluted serum samples. The blank and standard were constituted by substituting the serum with 0.01 ml of distilled water and standard working reagent respectively. The reaction constituent were thoroughly mixed and incubated at 37^{o} C for 5 minutes. Absorbance was spectrophotometrically read at 546nm against the blank.

Calculation

Concentration of cholesterol = $\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard}$ x 5.40mM/L

3.2.7.2 Serum Triglyceride Concentration

The concentration of serum triglyceride, (TG) was determined calorimetrically.

Principle

The triglyceride concentration is determined after enzyme activity hydrolysis with lipases, the indicator is quinonimine formed from hydrogen peroxide, 4- aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase (Tietz 1995).

$$\text{Triglyceride} + H_2O \xrightarrow{\text{Lipases}} \text{Glycerol} + \text{Fatty acid}$$

$$H_2O_2 + \text{4-aminophenazone} \xrightarrow{\text{Peroxidase}} \text{Quinonimine} + HCl + 4\,H_2O + \text{Chlorophenol}$$

Procedure

Exactly 1000µl of the working reagent was added to well clean labelled test tubes consisting of blank, standard and sample. Thereafter 10µl of the distilled water, standard solution (200mg/dL) and serum samples were added respectively. The resulting solution was mixed and incubated for 5 minutes at 37℃. The change in absorbance of standard and sample against the reagent blank was read at 500nm.

Calculation

Concentration of Triglyceride (TG) = $\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard}$ x 2.17mM/L

3.2.7.3 Serum High Density Lipoprotein-Cholesterol Concentration

The determination of serum High Density Lipoprotein Cholesterol (HDL) concentration.

Principle

The low-density lipoprotein (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphortungstic acid in the presence of magnesium. After centrifugation, the cholesterol concentration in the HDL-Cholesterol fraction, which remains in the supernatant is determined (Tietz 1995).

Procedure

Exactly 200µl of the serum samples and standard were dispensed into separate test tubes and 500µl of the reagent was added. The resulting solution was mixed and left for 10 minutes at room temperature. Thereafter, it was centrifuged at 4000rpm for 10 minutes and the clear supernatant was separated for determination of cholesterol content. The concentration of cholesterol was determined using enzymatic saponification procedure as described by Tietz (1995).

Calculation

$$\text{HDL-C} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times 5.25\text{mM/L}$$

3.2.7.4 Serum Low Density Lipoprotein -Cholesterol Concentration

The serum Low Density Lipoprotein (LDL) cholesterol concentration can be expressed as:

LDL Cholesterol = Total Cholesterol – (HDL + TG) (Tietz 1995).

3.2.8 Data Analysis

Data were analyzed by Statistical Product and Service Solution (SPSS) version 26. The statistical differences between groups were determined by one-way of variance (ANOVA) followed by Duncan multiple range test for multiple correlation. Results were expressed as mean ± standard error of mean (SEM). Values were considered statistically significant at $p<0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Chemical Constituents of Ethanolic Extract of *O. gratissimum*

4.1.1 Secondary Metabolites

Qualitative analysis of *O.gratissimum* leaves revealed the presence of tannins, saponins, flavonoids, alkaloids, quinones, phenols, terpenoids, cardiac glycosides and coumarins, while carbohydrates, glycosides, amino acids, anthraquinones, steroids, phlobatannins and anthracyannines were not detected (Table 2).

Table 2: secondary metabolites of ethanolic extract of *O.gratissimum* leaves

SECONDARY METABOLITES	LEAVES
Carbohydrates	--
Tannins	++
Saponins	++
Flavonoids	++
Alkaloids	++
Glycosides	--
Quinones	++
Phenols	++
Terpenoids	++
Cardiac glycosides	++
Ninhydrin	--
Coumarins	++
Anthraquinones	--
Steroids	--
Phlobatannins	--
Anthracyanines	--

-- signifies absence of secondary metabolite

++ signifies presence of secondary metabolite

4.1.2 UV-Visible Spectroscopy Analysis

The ultraviolet visible spectroscopy analysis of ethanolic extract of *O.gratissimum* leaves revealed the varying absorbance of the plant extract at varying wavelengths ranging from 200nm-600nm with the highest absorbance of the extract at wavelengths ranging from 320-390nm as shown in figure 3.

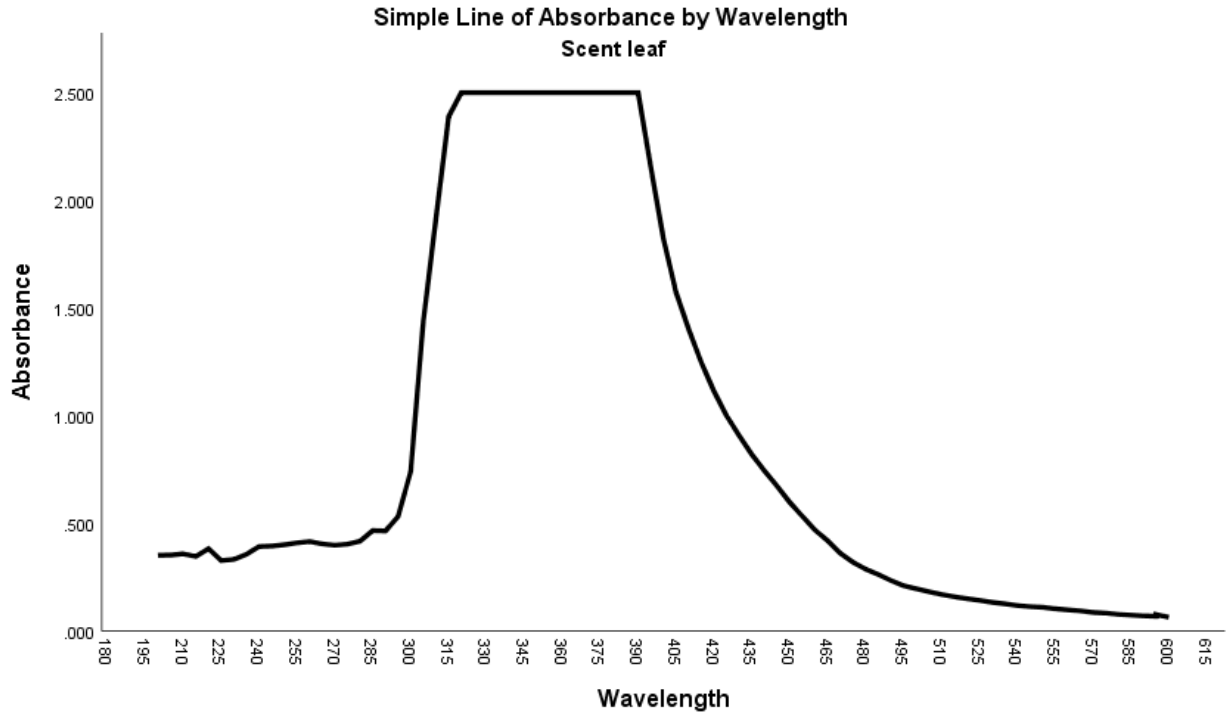


Figure 3: UV-Spectroscopy of *O. gratissimum* leaves

4.1.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

4.1.3.1 Chromatogram of the Ethanolic Extract of *O. gratissimum* Leaves

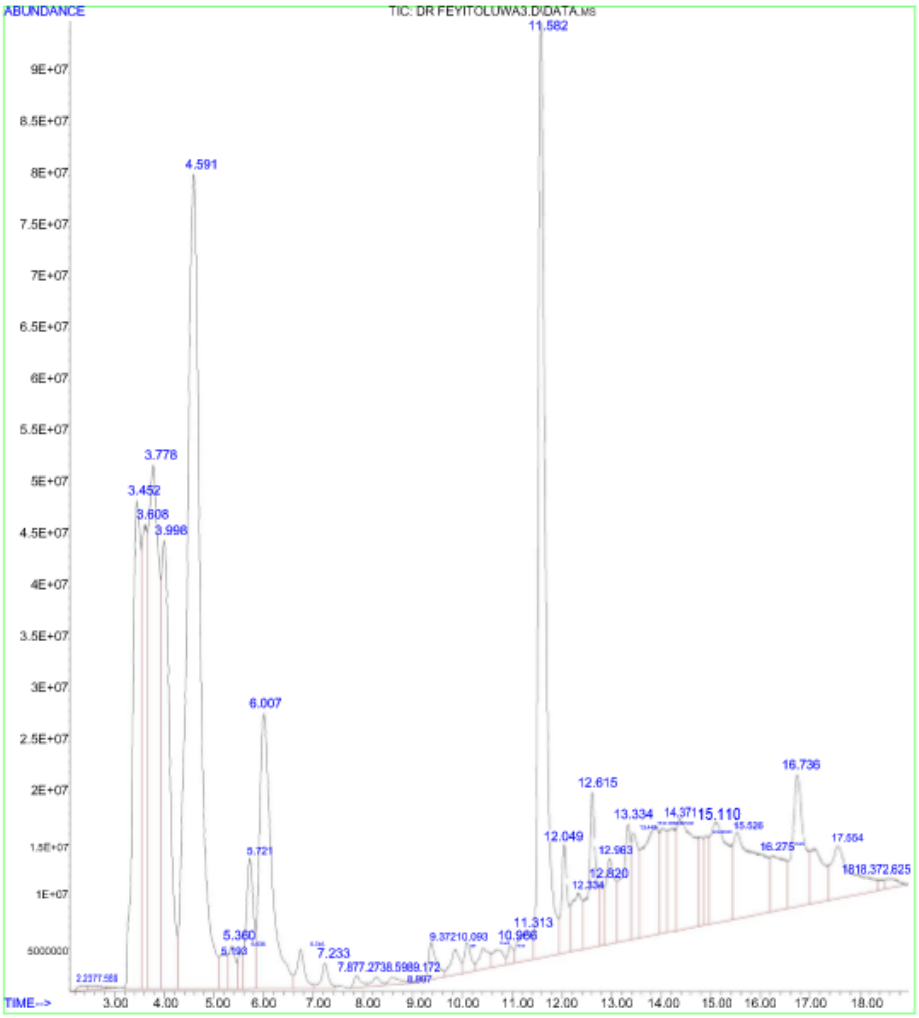


Figure 4: GC-MS Chromatogram of ethanolic extract of *O. gratissimum* leaves

4.1.3.2 Phyto Components of Ethanolic Extract of *O. gratissimum* Leaves.

The phyto components of the ethanolic extract of *O. gratissimum* plant were identified. Table 3 shows the peak, retention time, % total and chemical formula of the plant. From figure 4, the chromatogram shows the highest peak with retention time 11.582 to be the phyto-component Ethanone, 1-(6,6-dimethylbicyclo [3.1.0] hex-2-en-2-yl) and other peaks to be other phyto-components.

Table 3: Phyto components of ethanolic extract of *O.gratissimum* leaves

Serial No	Name of Compound	Retention time	Area %	Chemical formula	%Total
1	Glycerol triethyl ether	2.377	0.07	$C_9H_{20}O_3$	0.071
2	Fumaric acid, 2-ethoxyethyl ethyl ester	2.589	0.09	$C_{10}H_{16}O_5$	0.089
3	D-Fucose	3.452	6.50	$C_6H_{12}O_6$	6.499
4	Cyclopentanol, 3-methyl-	3.608	4.22	$C_6H_{12}O$	4.220
5	2-Hexene, 5-methyl-, (E)	3.778	9.08	C_7H_{14}	9.083
6	Ethane, diazo-	3.998	6.30	$C_2H_4N_2$	6.301
7	Cyclopropane, 1-methyl-2-(3-methyl pentyl)	4.591	18.03	$C_{10}H_{20}$	18.030
8	d-Gulopyranose	5.193	0.45	$C_6H_{12}O_6$	0.453
9	1-Butanol, 3-methyl-, acetate	5.360	0.49	$C_7H_{14}O_2$	0.490
10	1-Butanol, 3-methyl-, acetate	5.536	0.26	$C_7H_{14}O_2$	0.265
11	Peroxide, dimethyl	5.721	1.60	$C_2H_6O_2$	1.600
12	Propanenitrile, 2-hydroxy-	6.007	5.62	C_3H_5NO	5.617
13	Pentane, 1-(1-ethoxyethoxy)-	6.746	0.52	$C_9H_{20}O_2$	0.519
14	Hexanoic acid, ethyl ester	7.233	0.30	$C_8H_{16}O_2$	0.296
15	o-Cymene	7.877	0.14	$C_{10}H_{14}$	0.143
16	.alpha.-Phellandrene	8.273	0.13	$C_{10}H_{16}$	0.134
17	1-Methyl-5-mercaptotetrazole	8.598	0.14	$C_2H_4N_4S$	0.138
18	Benzene, 4-ethenyl-1,2-dimethyl-	8.907	0.01	$C_{10}H_{12}$	0.015
19	2,5-Dimethylcyclohexanol	9.172	0.05	$C_8H_{16}O$	0.046
20	2(1H)-Pyridinone, 6-hydroxy-	9.372	0.50	$C_5H_5NO_2$	0.497
21	1,3-Benzenediamine	9.857	0.38	$C_6H_8N_2$	0.376
22	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	10.093	0.33	$C_{10}H_{18}O$	0.331
23	Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	10.419	0.34	$C_{11}H_{16}O$	0.339

24	(S)-(+)-4-Isopropyl-2-oxazolidinon	10.730	0.30	$C_6H_{11}NO_2$;	0.295
25	L-Proline, 1-acetyl-	10.966	0.16	$C_7H_{11}NO_3$	0.157
26	Furan, 2,3,5-trimethyl-	11.313	0.65	$C_7H_{10}O$	0.648
27	Ethanone, 1-(6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)	11.582	10.97	$C_{10}H_{14}O$	10.967
28	Caryophyllene	12.049	1.07	$C_{15}H_{24}$	1.072
29	Humulene	12.334	0.97	$C_{15}H_{24}$	0.975
30	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]	12.615	1.94	$C_{15}H_{24}$	1.936
31	Phenol, 3,5-dimethyl-	12.820	0.44	$C_8H_{10}O$	0.440
32	Silane, trimethyl(3-methylphenoxy)	12.963	1.33	$C_{10}H_{16}OSi$	1.326
33	Caryophyllene oxide	13.334	1.69	$C_{15}H_{24}O$	1.695
34	2-Pyrrolidineacetic acid	13.449	1.16	$C_6H_{11}NO_2$	1.156
35	Benzene, 4-ethyl-1,2-dimethoxy-	13.894	2.88	$C_{10}H_{14}O_2$	2.883
36	1-Hexanol, 5-methyl-2-(1-methylethyl)-, acetate	14.027	1.16	$C_{12}H_{24}O_2$	1.159
37	1,2-Benzenedicarboxylic acid, decyl octyl ester	14.221	1.24	$C_{26}H_{42}O$	1.239
38	Phthalic acid, 1-adamantylmethyl ethyl ester	14.371	3.28	$C_{21}H_{26}O_4$	3.276
39	2-Nonenoic acid, 9-(dimethylamino) -7-hydroxy-2-methyl-9-oxo-, methyl ester, (E)-	14.800	0.73	$C_{13}H_{23}NO_4$	0.734

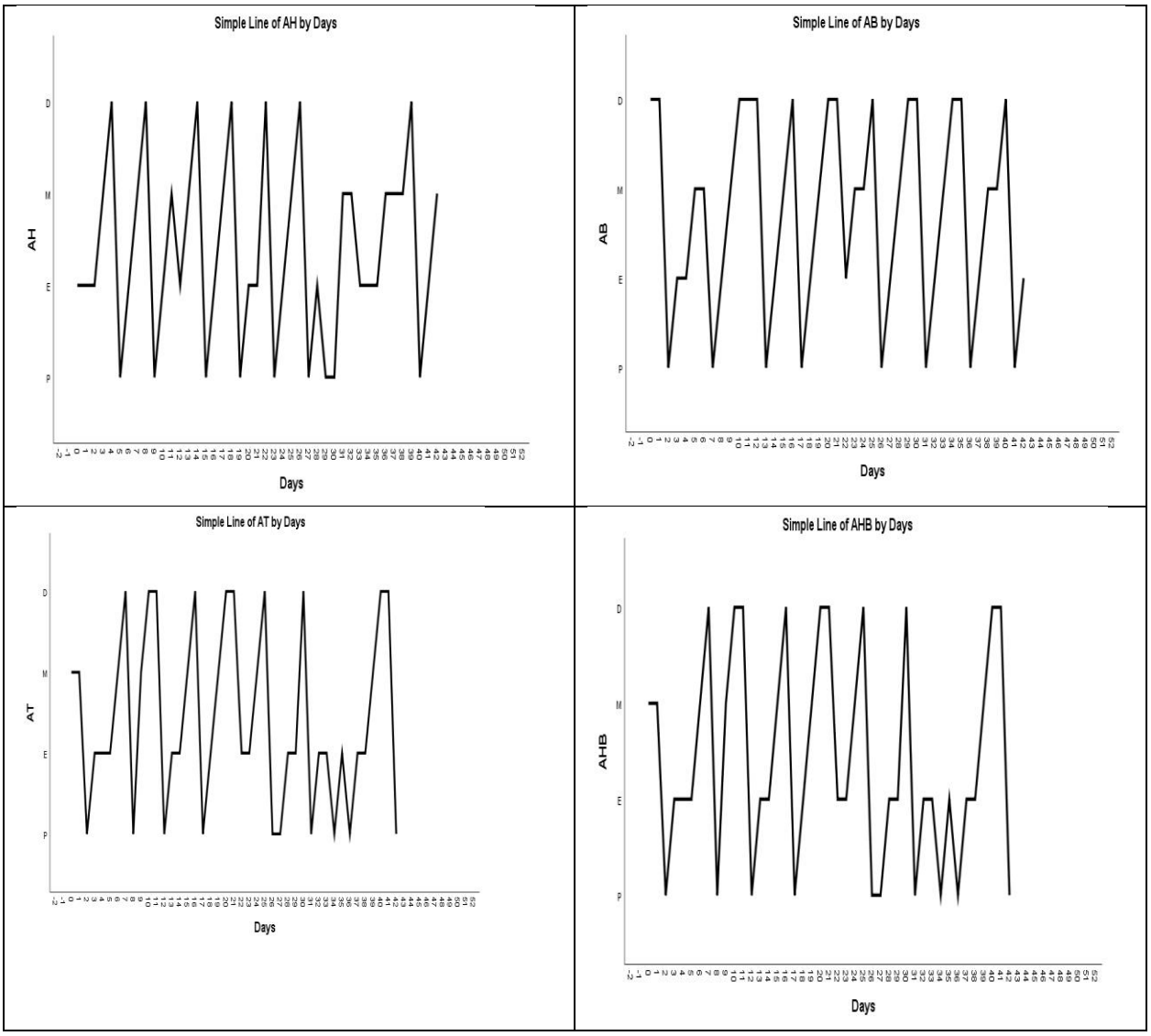
40	Phthalic acid, hex-2-yn-4-yl undecyl ester	14.910	0.63	$C_{25}H_{36}O_4$	0.630
41	Oxazole, 2-hexyl-4,5-dimethyl-	15.110	2.88	$C_{11}H_{19}NO$	2.881
42	Hexadecanoic acid, ethyl ester	15.526	3.64	$C_{18}H_{36}O_2$	3.644
43	3-(Adamantan-1-ylamino)-2-(2,4-dichloro-5-fluorobenzoyl)acrylic acid , ethyl ester	16.275	1.37	$C_{22}H_{24}Cl_2FNO_3$	1.373
44	Phytol	16.736	2.56	$C_{20}H_{40}O$	2.559
45	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	17.079	1.21	$C_{20}H_{34}O_2$	1.209
46	Phytol, acetate	17.554	1.88	$C_{22}H_{42}O_2$	1.883
47	.beta.-Alanine, N-neopentyloxycarbonyl-, neopentyl ester	18.372	0.09	$C_{14}H_{27}NO_4$	0.093
48	Phenol, 2-(4-diethylaminophenyliminomethyl)-6-methoxy-	18.625	0.22	$C_{18}H_{22}N_2O_2$	0.221

4.3 Results of Pharmacological Study

4.3.1 Oestrous Cycle of Letrozole Treated Rats after Administration of *O.gratissimum*

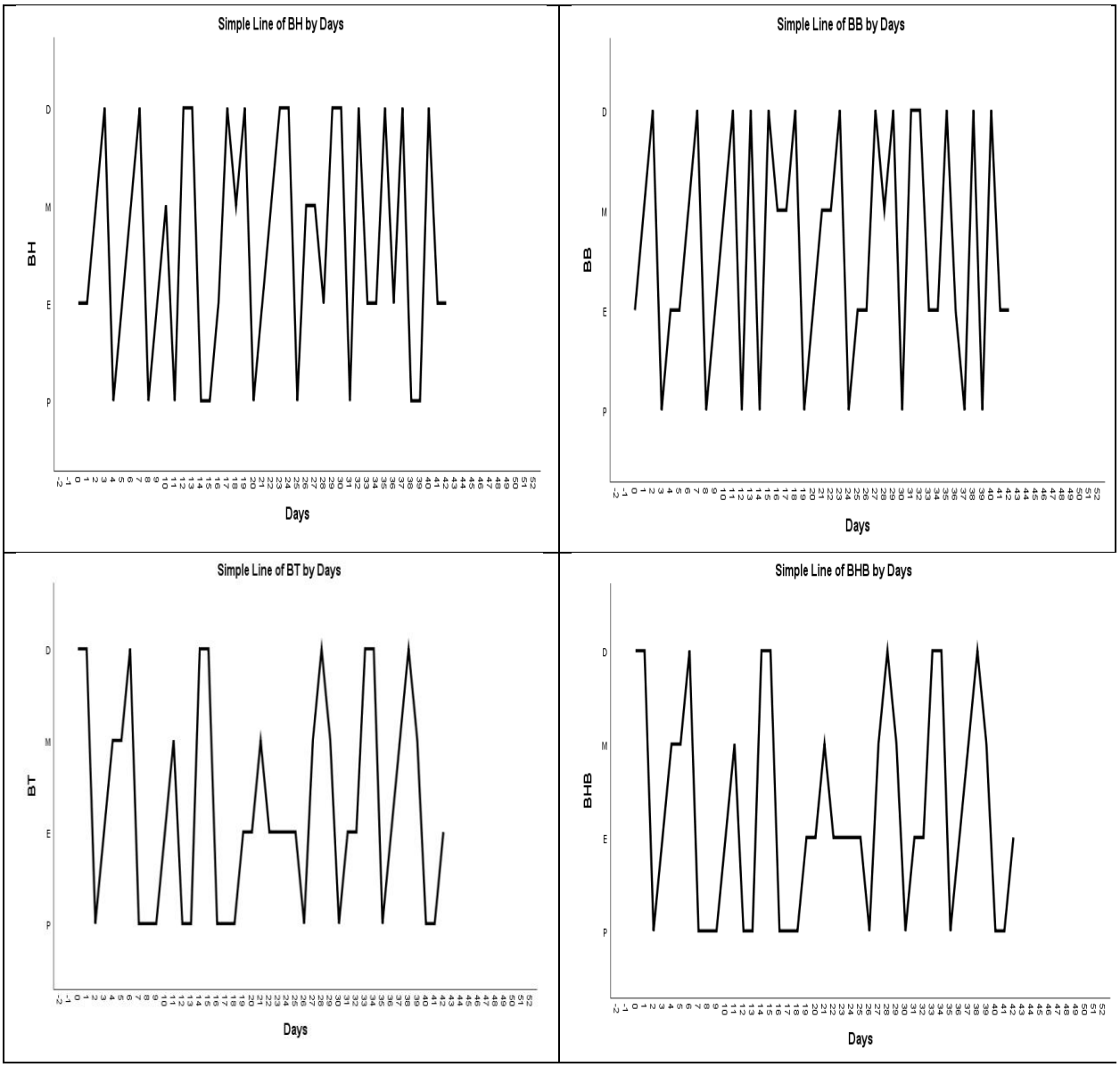
The alterations of the oestrous cyclicity of the rats administered letrozole was not restored after administration of 50mg/kg and 100mg/kg body weight of the ethanolic extract of *Ocimum gratissimum* leaves when compared to the control group.

P-Proestrus, E-Estrus, M-Metestrus, D-Diestrus



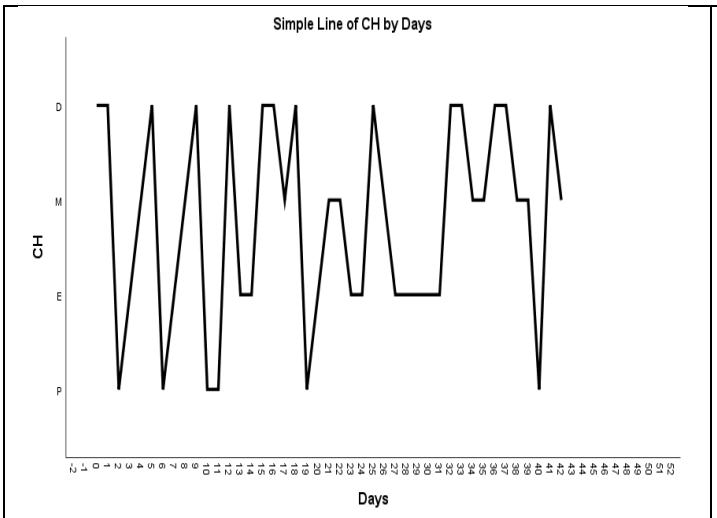
P-Proestrus, E-Estrus, M-Metestrus, D-Diestrus

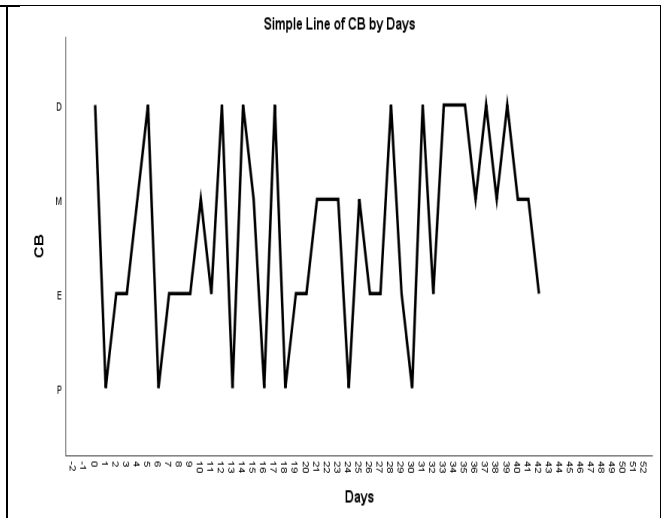
Figure 5: graph showing the oestrous cyclicity of female rats in control group



P-Proestrus, E-Estrus, M-Metestrus, D-Diestrus

Figure 6: graphs showing the oestrous cyclicity of female rats in group B


Simple Line of CH by Days
CH
D
M
E
P
Days


Simple Line of CB by Days
CB
D
M
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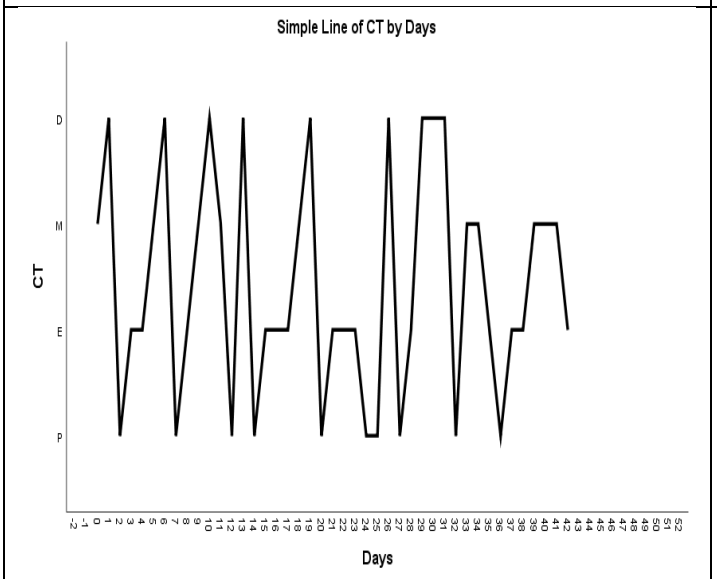
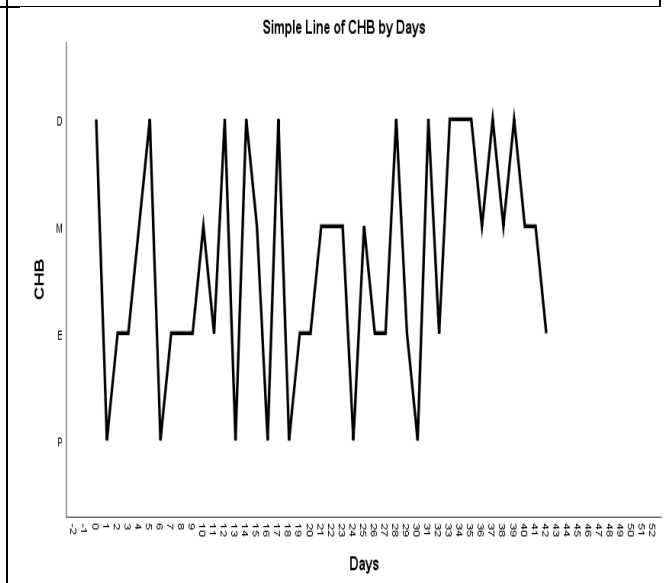
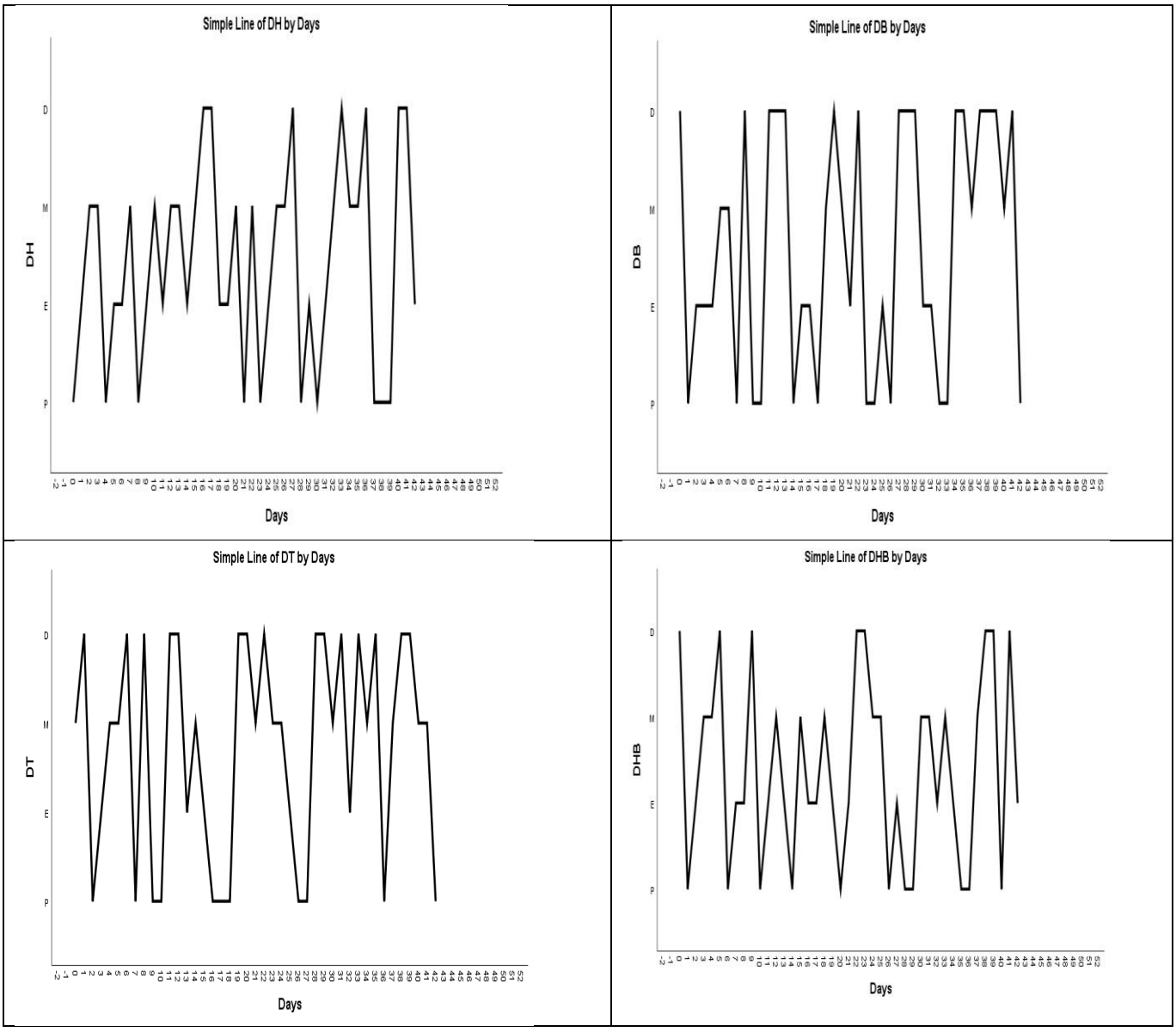
Days


Simple Line of CT by Days
CT
D
M
E
P
Days


Simple Line of CHB by Days
CHB
D
M
E
P
Days

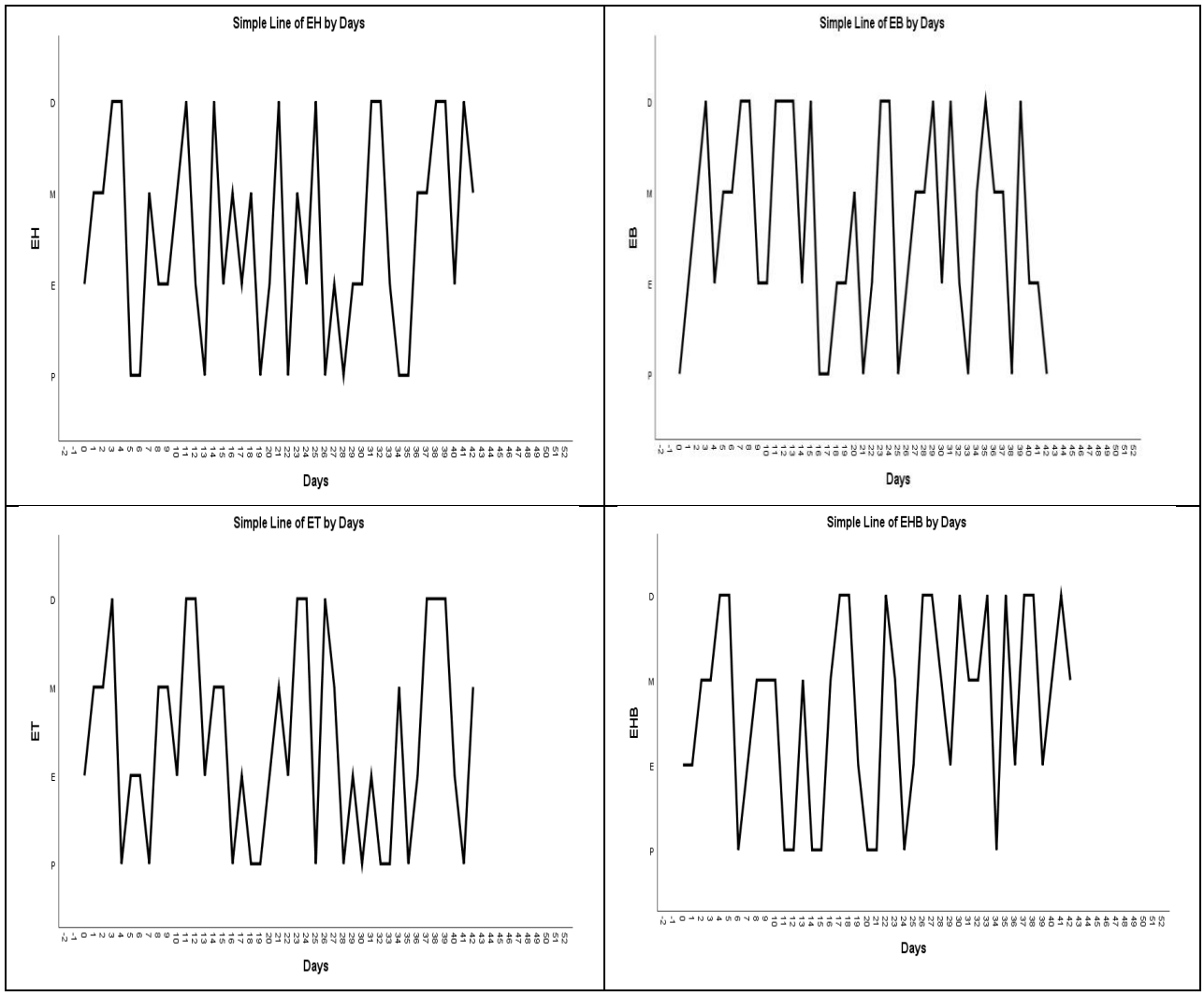


P-Proestrus, E-Estrus, M-Metestrus, D-Diestrus

Figure 7: Graphs showing the oestrous cyclicity of female rats in Group C

P-Proestrus, E-Estrus, M-Metestrus, D-Diestrus

Figure 8: Graphs showing the oestrous cyclicity of female rats in Group D



P-Proestrus, E-Estrus, M-Metestrus, D-Diestrus

Figure 9: graphs showing the oestrous cyclicity of rats in Group E

4.3.2 Fasting Blood Glucose concentration letrozole-induced PCOS rats administered ethanolic extract of *O. gratissimum* leaves

The fasting blood glucose of letrozole treated rats administered distilled water was significantly ($P < 0.05$) reduced in comparison to the control group. Administration of the 50 mg/kg body weight of the extract to letrozole treated rats significantly ($P < 0.05$) increased in comparison to the animals that received the reference drugs, metformin and clomiphene citrate in contrast to the administration of the 100mg/kg body weight of the extract to letrozole treated rats which significantly ($P < 0.05$) decreased the fasting blood glucose concentration of the animals in a similar manner to the animals that received the reference drugs, metformin and clomiphene citrate (table 4).

Table 4: Fasting blood glucose of female rats administered extract of *O.gratissimum* leaves

GROUPS	Fasting blood glucose before induction	Fasting blood glucose after induction	Fasting blood glucose after treatment
Control	74.00 ± 0.58[a]	87.50 ± 0.96 [a]	114.50 ± 0.29 [a]
PCOS+Distil. H_2O	75.00 ± 0.58 [a]	78.50 ± 0.96 [e]	100.00 ± 0.00 [d]
PCOS+MET+CC	63.50 ± 2.89 [c]	64.00 ± 1.15 [c]	79.00 ± 0.58 [b]
PCOS+ 50 mg/kg b.w. of EEOGL	75.00 ± 0.58 [a]	104.50 ± 0.29 [b]	91.50 ± 0.87 [c]
PCOS+ 50 mg/kg b.w. of EEOGL	78.00 ± 0.58[b]	71.50 ± 0.87 [d]	79.50 ± 0.87 [b]

Data are means of four determinations ± SEM. Values with different superscripts in each column are significantly different (P<0.05);

4.3.3 Weight of Letrozole Treated Rats Administered *O.gratissimum*

The body weight of letrozole-treated rats administered 50mg/kg of the plant extract increased significantly ($P < 0.05$) in contrast to the control group. This significant increase ($P < 0.05$) was also revealed in the animals treated with the reference drugs. Administration of 100mg/kg of the plant extract also increased the body weight of the rats significantly ($P < 0.05$) in contrast to the control group as shown in table 5.

Table 5: Body weights of female rats administered extract of *O. gratissimum* leaves

Groups	Weight before induction	Weight after induction	Weight after treatment
Control	138.49 ± 1.03 [a]	150.20 ± 1.41 [a]	162.52 ± 0.66 [a]
PCOS+Distil. H_2O	155.22 ± 0.11 [c]	189.45 ± 0.71 [d]	182.12 ± 0.81[c]
PCOS+MET+CC	192.83 ± 0.76 [d]	219.66 ± 0.01 [e]	211.44 ± 7.06 [d]
PCOS+ 50 mg/kg b.w. of EEOGL	135.13 ± 0.28[e]	173.71 ± 0.62 [b]	176.14 ± 0.80 [c]
PCOS+ 100 mg/kg b.w. of EEOGL	140.96 ± 0.44 [b]	184.02 ± 2.24 [c]	168.69 ± 1.71 [b]

Data are means of four determinations ± SEM. Values with different superscripts in each column are significantly different (P<0.05)

4.3.4 Effect of Ethanolic Extract of *O. gratissimum* Leaves on the Concentration of Serum Hormones of PCOS-induced Female Rats.

The administration of 100mg/kg body weight of the extract significantly increased ($P < 0.05$) the serum insulin concentration of the PCOS-induced groups as in contrast to the control group (Fig. 10).

The administration of distilled water to the PCOS-induced rats did not significantly alter the serum testosterone concentration of the animals in contrast to the control. However, the animals that were administered the plant extract ad also the reference drugs had their serum testosterone concentration significantly increased ($P < 0.05$) as in comparison to the control group (Fig. 11).

The serum progesterone concentration of the rats administered the reference drugs compared favourably ($P > 0.05$) with the control group. However, the serum progesterone of the animals administered 50mg/kg and 100mg/kg of the plant extract was significantly decreased when compared to the control group. (Fig. 12).

The serum LH concentration of the animals that receive 100mg/kg body weight of the extract, the reference drug and distilled water were significantly increased ($P < 0.05$) in comparison to the control group. The animals that were administered 50mg/kg of the extract had their serum LH concentration significantly decreased ($P < 0.05$). (Fig. 13).

There was a significant increase of serum FSH concentration in all the animals that received distilled water, reference drugs and 50mg/kg body weight of the extract, however, the group that was administered 100mg/kg body weight of the extract produced a significant decrease ($P < 0.05$) in their serum FSH concentration (Fig. 14).

The rats that were administered the reference drugs had their estradiol levels significantly ($P < 0.05$) increased in comparison to the control group. However administration of both doses that is, 50mg/kg and 100mg/kg body weight of the plant extract significantly decreased ($P < 0.05$) the serum estradiol concentration in contrast to the control group (Fig. 15).

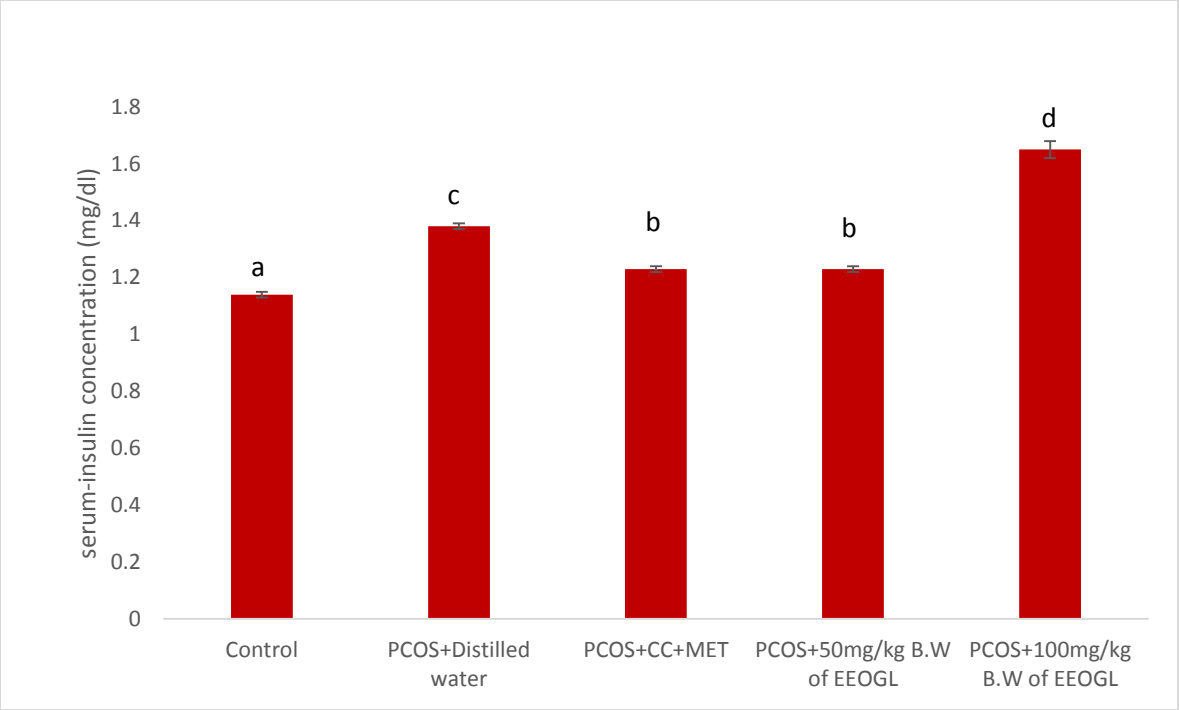


Figure 10: effects of ethanolic extract of *Ocimum gratissimum* leaves on serum insulin concentration of letrozole-treated female rats

Data are means of four determinations ± SEM. Values with different superscripts in each column are significantly different (P<0.05);

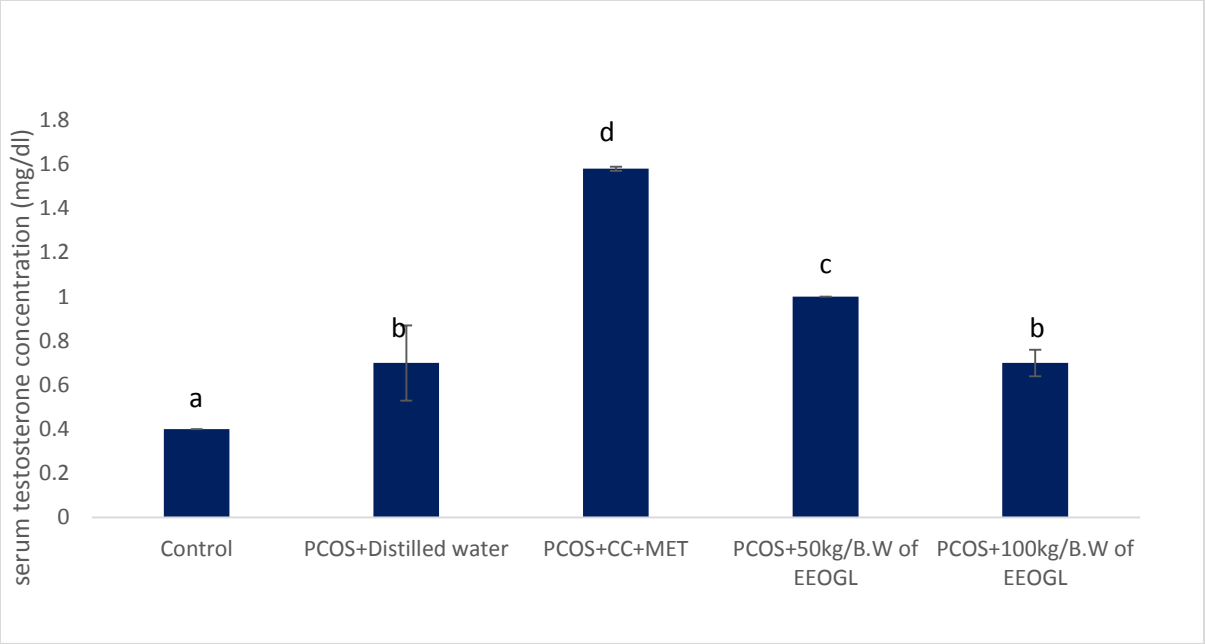


Figure 11: effects of ethanolic extract of *Ocimum gratissimum* leaves on serum testosterone concentration of letrozole treated female rats

Data are means of four determinations ± SEM. Values with different superscripts in each column are significantly different (P<0.05);

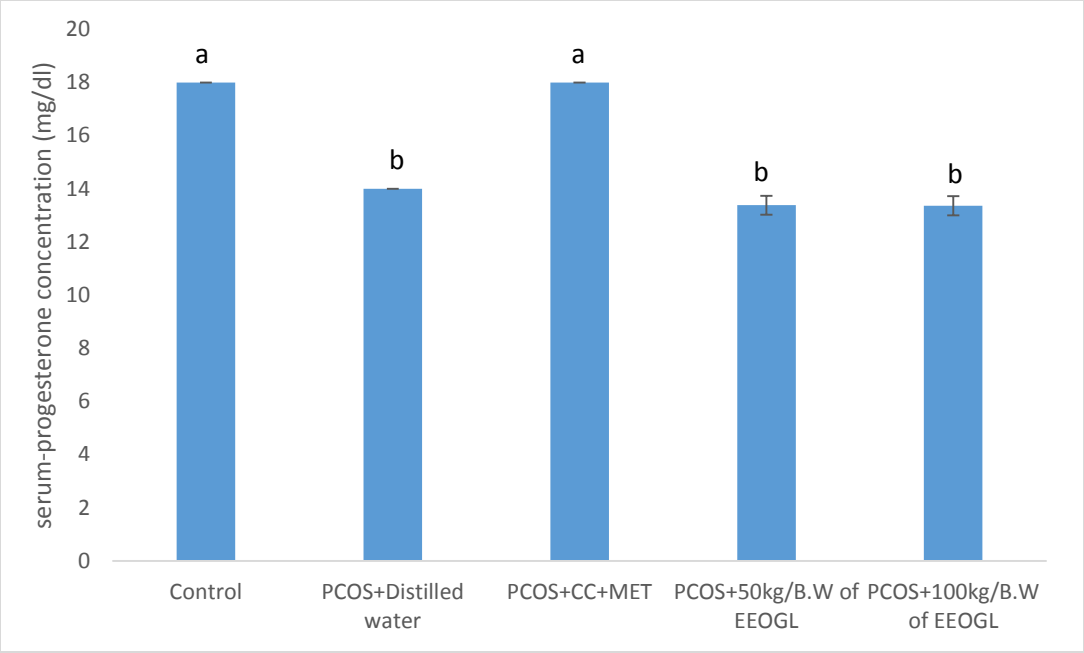


Figure 12: effects of ethanolic extract of *Ocimum gratissimum* on serum progesterone concentration of letrozole treated female rats

Data are means of four determinations ± SEM. Values with different superscripts in each column are significantly different (P<0.05);

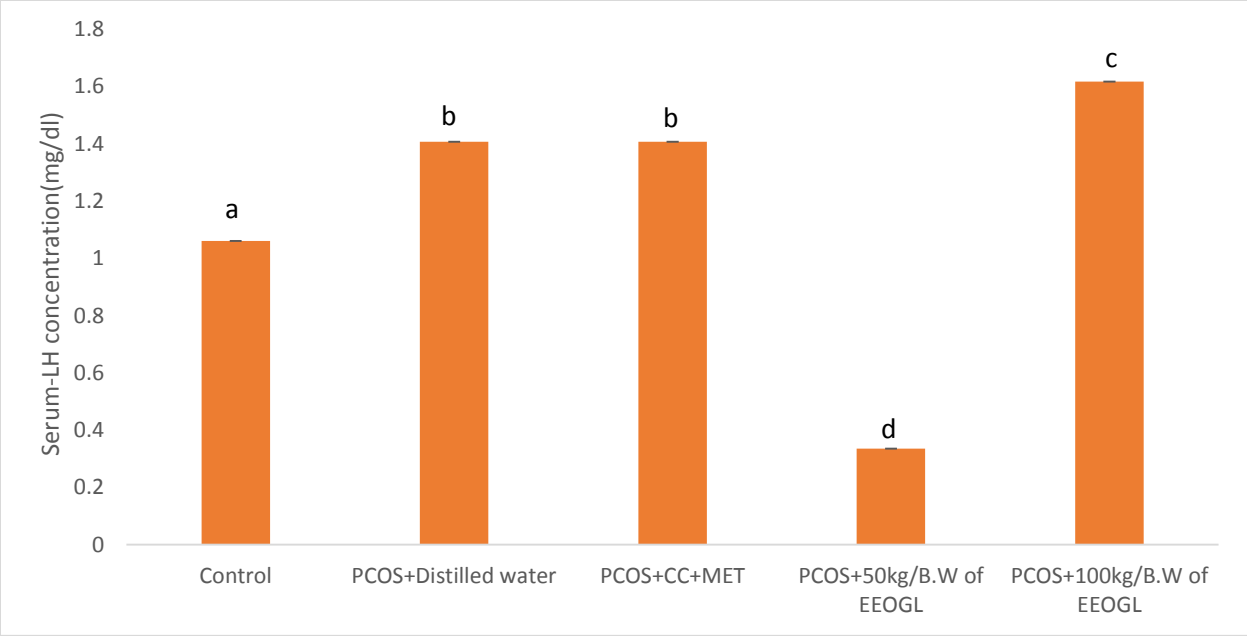


Figure 13: Effects of ethanolic extract of *Ocimum gratissimum* leaves on serum LH concentration of letrozole-treated rats

Data are means of four determinations ± SEM. Values with different superscripts in each column are significantly different (P<0.05);

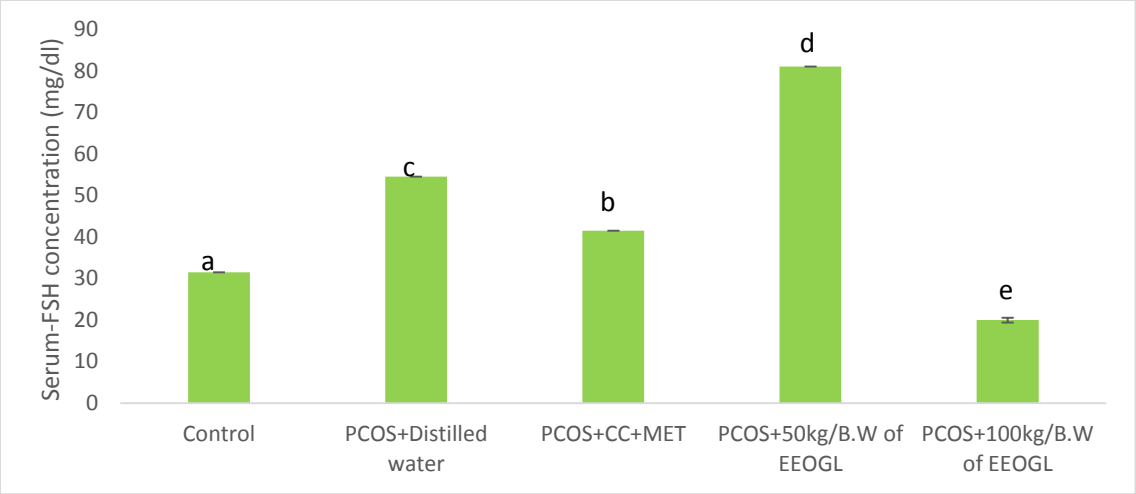


Figure 14: effects of ethanolic extract of *Ocimum gratissimum* leaves on serum FSH concentration of letrozole-treated female rats

Data are means of four determinations ± SEM. Values with different superscripts in each column are significantly different (P<0.05);

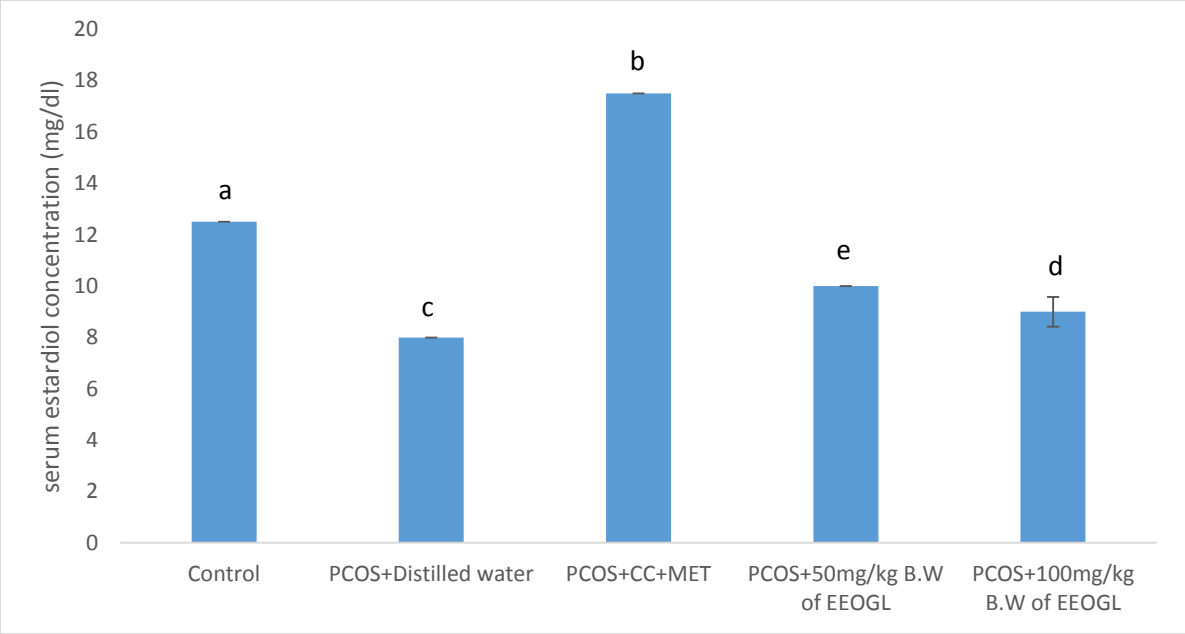


Figure 15: Effects of ethanolic extract of *Ocimum gratissimum* leaves on serum estradiol concentration of letrozole-treated female rats

Data are means of four determinations ± SEM. Values with different superscripts in each column are significantly different (P<0.05);

4.3.5 Serum Lipid Profile of Letrozole-Treated Rats after Administration Of Ethanolic Extract of *O. gratissimum*

The administration of the 50 mg/kg body weight of the plant extract significantly increased ($P < 0.05$) the concentration of the serum cholesterol of letrozole treated rats. However, administration of 100mg/kg of extract significantly decreased ($P < 0.05$) the serum concentration of cholesterol of letrozole treated rats. Furthermore, letrozole treated rats that were administered the reference drugs produced cholesterol concentration that compared favorably with the control group (Table 6).

Administration of 100mg/kg body weight of the extract significantly increased ($P < 0.05$) the serum HDL concentration of letrozole treated rats in comparison to the control groups. Administration of 50mg/kg body weight of the extract, and administration of the reference drug significantly decreased ($P < 0.05$) the concentration of serum HDL concentration in comparison to the control group (Table 6).

Administration of 100mg/kg body weight of the plant extract to letrozole treated rats significantly increased ($P < 0.05$) the serum concentration of LDL when compared to the control group. However, the weight of the animals that received 50mg/kg of the extract, and the reference drugs compared favourably ($P > 0.05$) with the control group (Table 6).

The concentration of serum triglycerides of the animals that were administered 50,100mg/kg body weight of the plant extract and the reference drug were all significantly increased ($P < 0.05$) in comparison to the control (Table 6).

Table 6: Serum lipid profile of letrozole-induced PCOS rats administered ethanolic extract of *O. gratissimum* leaves

SERUM PARAMETER	CONTROL	PCOS+ distil. water	PCOS+ MET+ CC	PCOS+50 of EEOGL	PCOS+100 mg/kg of EEOGL
Total cholesterol (mmol/L)	10.23± 0.00[a]	68.78 ± 0.00[c]	8.03 ± 0.37[a]	27.57 ± 2.38[b]	5.97 ± 0.33[d]
HDL(mmol/L)	4.85 ± 0.2[a]	0.60 ± 0.26[c]	0.38 ± 0.06[c]	0.30 ± 0.11[c]	5.59 ± 0.32[b]
LDL(mmol/L)	9.11 ± 0.57[a]	45.90 ± 0.64[c]	10.70 ± 1.56[a]	8.87 ± 2.73[a]	18.80± 0.49[b]
TRIGLYCERIDES (mmol/L)	14.50± 0.85[a]	22.30 ± 0.38[c]	18.37 ± 1.25[b]	18.41 ± 0.47[b]	19.26± 0.16[b]

Data are means of four determinations ± SEM. Values with different superscripts in each row are significantly different (P<0.05);

CHAPTER FIVE

5.0 Discussion

The detection of some phytochemicals revealed the potential medicinal properties of *O. gratissimum* leaves. Alkaloids and flavonoids have been reported to show a reducing effect on plasma concentrations of LH, estradiol and FSH (Bianco *et al*., 2006; Lauritzen *et al*., 1997; Browning *et al*., 1998). Tannins have been reported to improve stimulation of glucose transport in type 2 diabetic patients due to insulin resistance (Riedl and Hangerman, 2001). Saponins have also been reported to enhance progesterone synthesis (Yang *et al*., 2003). Flavonoids and Alkaloids have also been reported to decrease blood glucose and insulin levels (Waltner-law *et al*., 2002; Baldeon *et al*., 2012).

PCOS has been identified as one of the most common disorder found in women of reproductive age. It has been characterized by elevated levels of testosterone, and LH, elevated levels of insulin and low levels of FSH and progesterone as well as disoriented ovulatory cycles. The aim of this study was to determine the type of effect that *Ocimum gratissimum* would have on the letrozole induced PCOS rats. Hyper androgenemia is seen as one of the hall mark of PCOS, due to the blockage of aromatase activity and excess production of testosterone. Letrozole is an aromatase inhibitor that inhibits aromatase, resulting in the non-conversion of androgens to estrogens. This eventually leads to hyperandrogenemia from excess testosterone levels. As predicted, induction of PCOS in the rats resulted in imbalance in hormonal concentrations and disorganized oestrous cycle in comparison to the oestrous cycle of the control group. As the plant extract was administered to the rats, the both doses, that is, the 50 and 100mg/kg body weight of the extract, did not reverse the effects of the letrozole on the oestrous cycle of the rats. From the study, administration of the plant extract decreased the fasting blood glucose of the rats in a similar manner to the rats treated with metformin and clomiphene citrate. This antihyperglycemic activity could be attributed to the presence of flavonoids, cardiac glycosides, tannins, saponins, in the plant (Mohammed *et al*., 2007).

PCOS has also been known to have effect on the lipid profile of PCOS patients. Patients may experience high LDL cholesterol, triglycerides, and total cholesterol levels, as well as low HDL cholesterol levels (Tsouma *et al*., 2014; Ghaff arzad *et al.,* 2016). Experimental rats exhibited ovarian changes and increase in follicular wall thickness in a high fat diet induced PCOS,

suggesting infertility being associated with dyslipidemia in PCOS (Patel and Shah, 2018). In the study, it was observed that induction of PCOS in the rats caused their body weight to increase compared to the control group. However, administration of the both doses of the ethanolic extract of *Ocimum gratissimum* leaves did not have a significant effect in reducing their body weight.

Insulin resistance and hyperinsulinemia are common hallmarks of PCOS posing a great risk for type 2 diabetes in PCOS patients. From this present study, the administration of the extract to the animals caused an increase in their serum insulin concentration when compared to the control. This effect was also found in the rats that were administered.

The serum testosterone concentrations of testosterone of the rats administered the plant extract were increased in comparison to the control as well as the rats administered the standard drugs. The both doses of the plant extract decreased the serum concentration of progesterone in contrast to the control group. However the standard drugs that were administered to the standard drugs treated rats reversed the effect that the letrozole administration had on the serum progesterone concentration showing the action of clomiphene citrate. Progesterone is the hormone secreted by the corpus luteum after successful ovulation (Fish, 2009). In PCOS patients however, the progesterone levels are usually low as observed also in this study in the letrozole treated rats administered distilled water. Clomiphene citrate reverses the blockage of estrogen synthesis allowing synthesis of the sex hormones. But in this study, the both doses of the extract administered did not reverse the letrozole effect on the rats. In PCOS patients, serum levels of LH are higher than normal but FSH levels are lower or normal. Administration of the 50mg/kg plant extract reduced the serum concentration of LH compared to the control group but in the rats administered 100mg/kg of the extract the concentration of LH was increased. On the other hand, the concentration of FSH in rats administered 50mg/kg was significantly increased past the threshold while the 100mg/kg treated rats had their FSH levels decreased.

Women with PCOS have low estrogen levels. Estradiol concentrations of the animals that were administered both doses of the plant extract were significantly decreased in comparison to the control group and in the rats administered the reference drug, their estradiol levels increased, this result also shows that the plant extract did not ameliorate the effect of letrozole induced PCOS on the reproductive profile of the rats.

5.1 Conclusion

This study shows that letrozole induced PCOS in rats is associated with reproductive and metabolic disorders. The ethanolic extract of *O. gratissimum* leaves did not completely restore the oestrous cyclicity, decreased the insulin levels alter the lipid profile and sex hormones of hormones associated to PCOS rats after 14 days of treatment.

5.2 Recommendations

Further studies should be carried out on the effects of ethanolic extract of *O. gratissimum* leaves at higher doses on the reproductive and metabolic state of PCOS rats.

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Appendix

0.25M Sucrose solution

171.15g of sucrose was dissolved in small quantity of water and made up to 2 litres with distilled water.

Preparation of 7.14mg/kg of Metformin

Each tablet of metformin drug contains 500mg of the active ingredient and is used by humans with approximate body weight of 70kg. The average weight of the experimental animals was 170.81g, that is, 0.17kg. Therefore 7.14mg/kg of metformin was used.

Preparation of 1mg/kg of Letrozole

Each tablet of letrozole contains 2.5mg of the active ingredient and is used by humans with approximately 70kg body weight. The average weight of the experimental animals was 170.81g. Therefore, 1mg/kg body weight of letrozole was used.

Preparation of 2mg/kg of Clomiphene citrate

Each tablet of clomiphene citrate contains 2mg of the active ingredient of the active ingredient and is used by humans with approximately 70kg body weight. The average weight of the experimental animals was 170.81g. Therefore, 2mg/kg of clomiphene citrate was used.

Calibration Curves

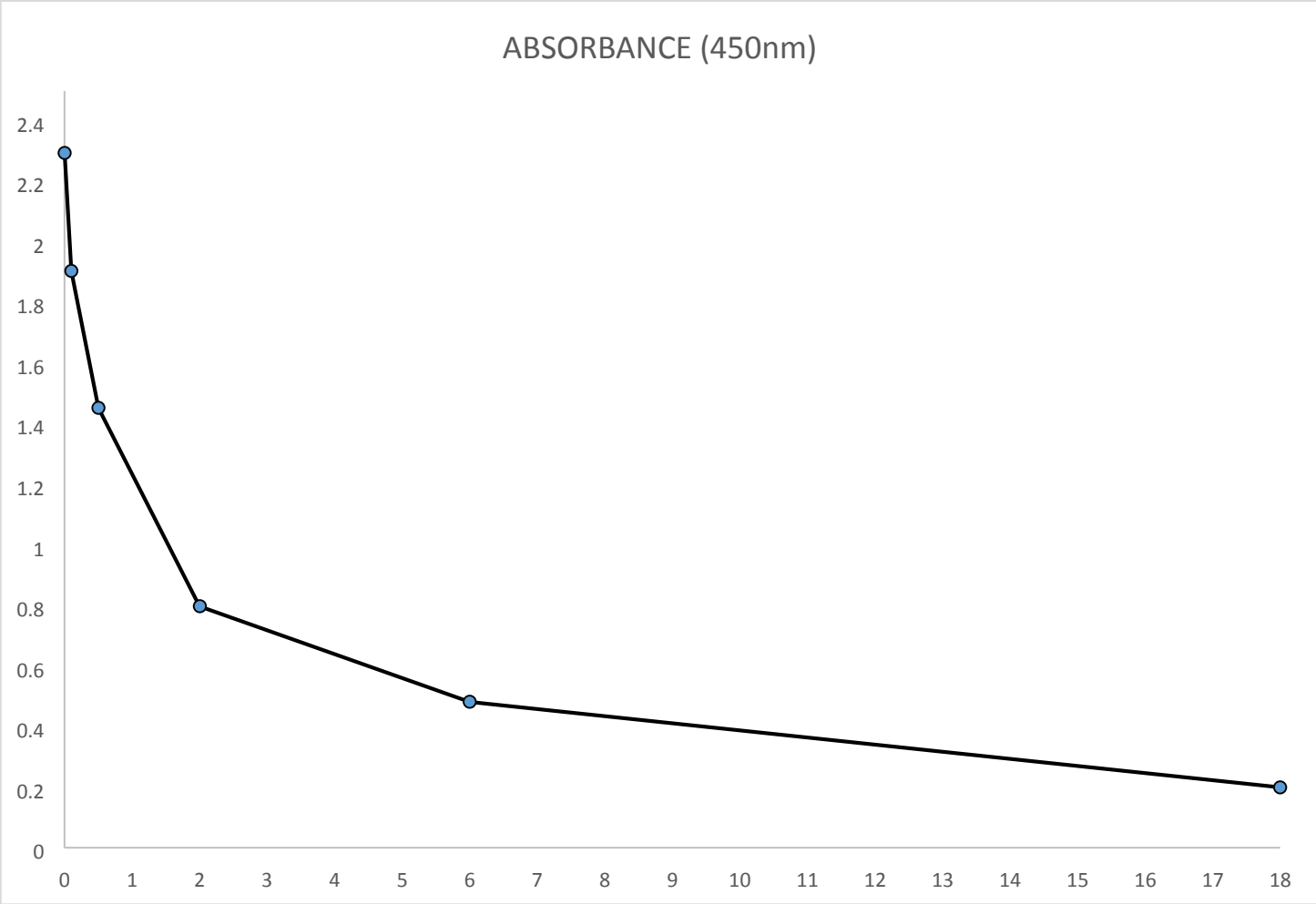

Figure 16: Calibration curve for testosterone

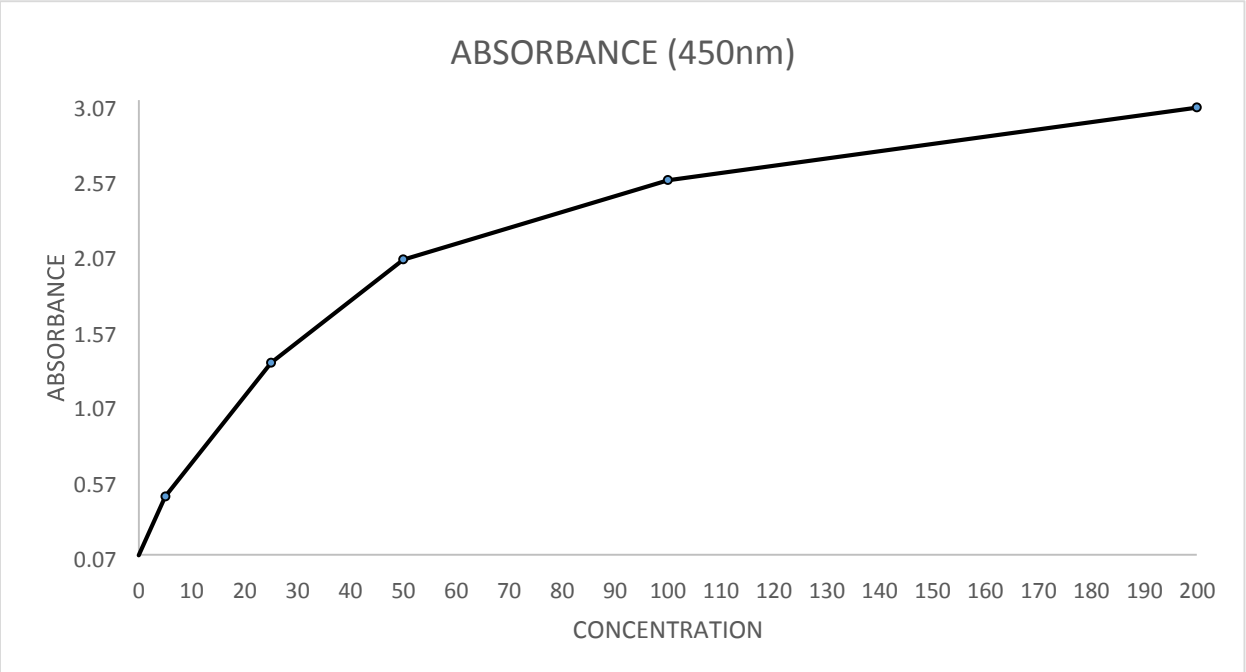

Figure 17: Calibration curve for Insulin

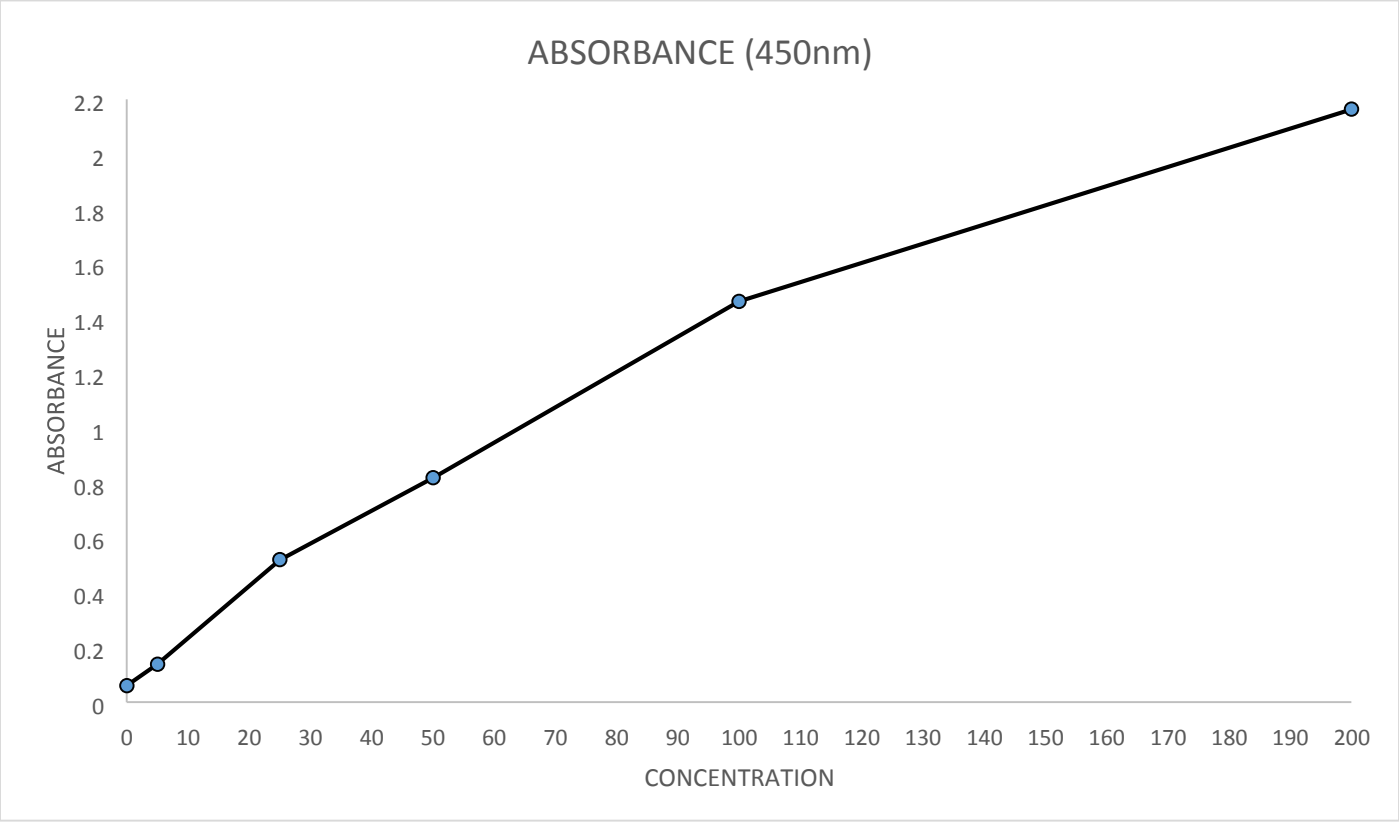

Figure 18: Calibration curve for Luteinising hormone

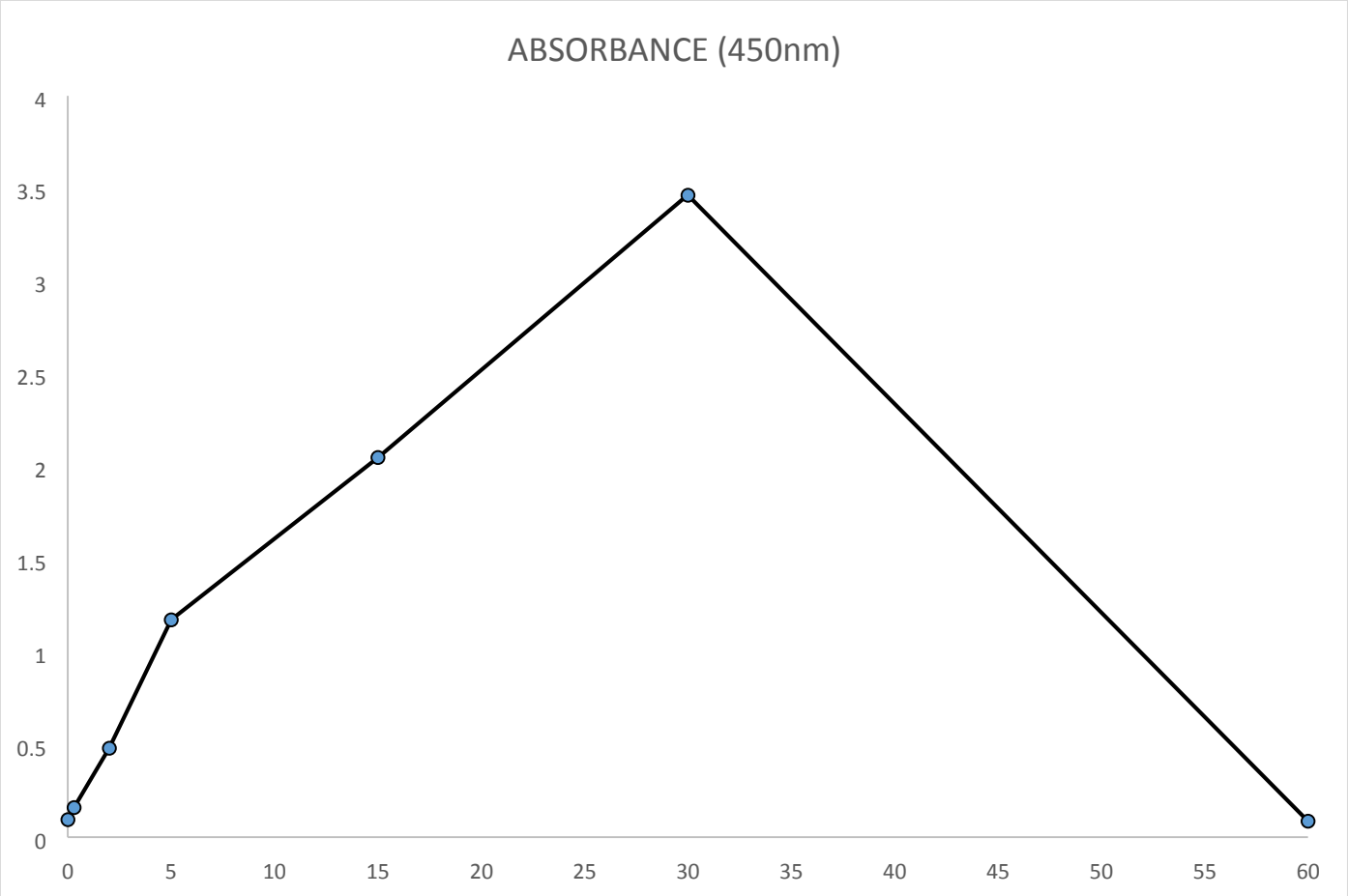

Figure 19: Calibration curve for Progesterone

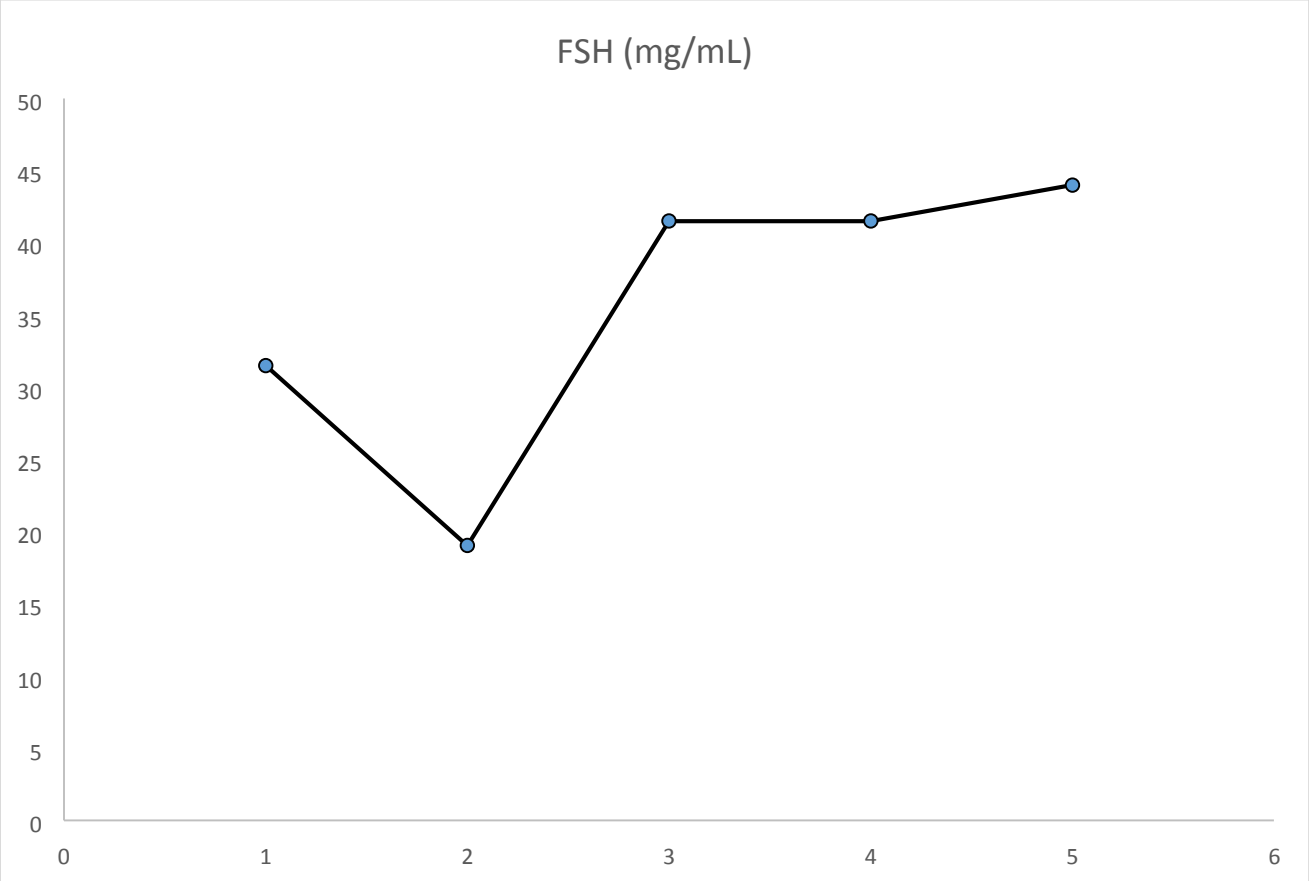

Figure 20: **Calibration cure for Follicle stimulating Hormone**