Selected Toxicity Study on Ethanolic Extract of *Ocimum gratissimum* (Linn.) Leaves in Letrozole–induced Polycystic Ovarian Wistar Rats

BY

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A DISSERTATION TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, BIOCHEMISTRY, MOUNTAIN TOP UNIVERSITY, OGUN STATE, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE IN BIOCHEMISTRY OF THE MOUNTAIN TOP UNIVERISTY.

SEPTEMBER 2021

CERTIFICATION

It is hereby certified that this work was carried out by Ugorji, Zion Kelechukwu with the matriculation number of 17010102012 of the Biochemistry unit, Department of Biological science, of Mountain Top University, Ogun State, Nigeria under the supervision of Dr. F.J. Femi-Olabisi.

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APPROVAL PAGE

This project has been read and approved as having met the requirement of the Department of Biological Science, College of Basic And Applied Science, Mountain Top University, Ibafo, Ogun State, Nigeria, for the award for Bachelor of Science (B.Sc.) degree in biochemistry.

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DECLARATION

I, Ugorji, Zion Kelechukwu hereby declare that this project report entitled "**Selected Toxicity Study on Ethanolic Extract of *Ocimum gratissimum* (Linn.) Leaves in Letrozole–induced Polycystic Ovarian Wistar Rats"** is a record of my research work. The project work was done by me.

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Ugorji Zion Kelechukwu Date

DEDICATION

I dedicate this report to GOD ALMIGHTY for His protection, guidance, provision and also for seeing me through this in good state of health and also to my loving parents, MR & MRS UGORJI for their continuous support and encouragement throughout. May the ALMIGHTY GOD continue to bless you.

ACKNOWLEDGEMENTS

ABSTRACT

Polycystic Ovarian Syndrome (PCOS) or Stein- Leventhal syndrome is a metabolic endocrine disorder that is very common in women of reproductive age affecting approximately 2-10% of them. Although several synthetic drugs such as metformin are available for the treatment of PCOS, the side effects of the use of metformin such as lactic acidosis have continued to limit their acceptability. Thus, an alternative option of plants like *Ocimum gratissimum* leaves should be investigated on. The biochemical and toxicological effects of ethanolic extract of *Ocimum gratissimum* leaves (EEOGL) at the doses of 50 and 100mg/kg body weight on letrozole-induced in Polycystic Ovarian Syndrome (PCOS) was investigated in female Wistar rats. Twenty female Wistar rats with an average weight of (170.81±5.25g) were assigned into 5 groups (A-E) of four each: animals in group A were the control group which received rat pellets and water for 30days while the letrozole-induced rats in groups B, C, D and E also received distilled water, 7.14mg/kg body weight of metformin and 2mg/kg body weight of clomiphene citrate (reference drugs) and the same volume of the extract corresponding to 50 and 100mg/kg body weight of EEOGL respectively after which levels of some biochemical and toxicological indices were determined after each rats were sacrificed by assessing liver function indices, kidney function indices, AST, ALP, ALT data were subjected to analysis of variance and Duncan multiple Range test with statistical significance set at $p < 0.05$. The result reveals: Nine different secondary metabolites were present in the EEOGL, the highest Phyto-components present in the EEOGL is Ethanone, 1-(6,6-dimethylbicyclo [3.1.0] hex-2-en-2-yl)- with the retention time of 11.592 and the lowest is Benzene, 4-ethenyl-1,2-dimethyl- with the retention time of 0.01, a significant increase ($p < 0.05$) and significant decrease ($p < 0.05$) at dose of 50mg/kg and 100mg/kg of EEOGL on kidney function indices as well as liver function indices was noticed. Therefore, the administration of EEOGL at 50 and 100 mg/kg B.W. to letrozole-induced PCOS rats shows mild alteration in the assayed biochemical indices.

Keywords: Biochemical Assessments, Letrozole, Polycystic Ovarian Syndrome, Toxicity

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ABBREVIATIONS

ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ASRM	American Society of Reproductive Medicine
AST	Aspartate Aminotransferase
ADB	Absorbance of Direct Bilirubin
ATB	Absorbance of Total Bilirubin
BUN	Blood Urea Nitrogen
CC	Clomiphene Citrate
CHF	Congestive Heart Failure
DHEA	Dehydroepiandrosterone
EEOGL	Ethanolic extract of *Ocimum gratissimum* leaves
ESHRE	European Society of Human Reproductive and Embryology
FSH	Follicle Stimulating Hormone
GC-MS	Gas Chromatography Mass Spectrometry
GFR	Glomerular Filtration Rate
IR	Insulin Resistance
LH	Luteinizing Hormone
MET	Metformin
NC-CAH	Non-Classical Congenital Adrenal Hyperplasia
NICHD	National Institute of Child Health and Human Disease
NIH	National Institute of Health
NAFLD	Non-Alcoholic Fatty Liver Disease
PCOS	Polycystic Ovarian Syndrome
SHBG	Sex Hormone Binding Globulin
UV	Ultra-Violent

CHAPTER ONE

1.0 Introduction

Polycystic Ovarian Syndrome (PCOS) or Stein- Leventhal syndrome is a metabolic endocrine disorder that is very common in women of reproductive age affecting approximately 2-10% of them (Moran *et al.,* 2010). It has several consequences in female health including alarming rate of infertility (Rojas *et al.*, 2014). This metabolic disorder is caused by several hormonal imbalances, detected through clinical manifestations, of which hyperandrogenism and chronic anovulation are dominant which can cause either a long term or short-term effect on the female health care (Teede *et al*., 2010). Hormonal disturbances associated with PCOS includes insulin resistance and hyperinsulinemia.

In 1935, Stein and Leventhal found a relationship between the polycystic ovaries and amenorrhea (absence of menstruation), hirsutism (male pattern hair growth) and obesity (Marcondes *et al.,* 2011). The clinical manifestation or features of PCOS are not restricted, limited, or confined to the gynecological region (Lane *et al.,* 2006). Women suffering from this disease tend to have an increased prevalence of several other metabolic diseases of which include type 2 diabetes mellitus, dyslipidemia, metabolic syndrome, and obesity (Legro *et al*., 2012).

Amongst all the comorbidities mentioned, obesity have been investigated to be at an epidemic climax with a worldwide rife of 35% in female (Herrero *et al.,* 2013). Due to series of studies and research, it has been investigated that insulin resistance (IR) has a fundamental link associated with these conditions (Reaven, 2011). Although insulin resistance may be present in PCOS patients free of obesity (Toprak *et al.,* 2011).

Plants have been inherited as a source of medication and are an important part of the health care system. Plants contain valuable information in the form of medicinal knowledge that has been preserved for the sake of modern health care system (Pandey, 2017). Plants that are known to possess therapeutic properties due to several investigations, studies and research are commonly known as "Medicinal plants". Plants used in traditional medicine provide a wide variety of drug

and can be used for the treatment of both chronic and infectious diseases (Duraipandiyan *et al*., 2006). Therefore, special focus should be given to the genetic makeup, physiology, morphology of the species to extract its potential as therapeutic value. A good example of this medicinal plant is *Ocimum gratissimum.*

Ocimum gratissimum is also known as clove basil or lemon basil but popularly known as "scent leaf" has been reported to be a culinary herb with wide therapeutic applications (Pandey, 2017). *Ocimum gratissimum* is a medicinal plant that is mainly distributed in tropical regions and native of South Asia, Africa, and various regions of South America. It has antimicrobial, antidiabetic, antidiarrheal, insecticidal, and anti-cancerous potential (Pandey, 2017).

Gas chromatography mass spectrometry also known as GC-MS has been widely recognized as a key mechanical stage for profiling of secondary metabolites in both plant and non-plant species (Keller *et al.,* 2005). The drug discovery processes have been changed with the assistance of powerful computers and information technology to expedite drug discovery, lead optimization, drug development and design (Ekins *et al*., 2007).

1.1 Statement of the Problem

The study was carried out to investigate the safe dose of the ethanolic extract of *Ocimum gratissimum* leaves used in the treatment of letrozole-induced polycystic ovarian syndrome in wistar rats.

1.2 Justification of the Study

Ethnobotanical survey has laid claim to the therapeutic potential of *Ocimum gratissimum* leaves in the treatment of PCOS, therefore it is imperative to scientifically evaluate its safety.

1.3 Aim of the Study

The aim of this study is to evaluate the phytoconstituent of *Ocimum gratissimum* leaves and toxicity of the ethanolic extract in letrozole-induced polycystic ovarian syndrome female rats.

1.4 Specific Objective of the Study

The specific objective of the study includes:

1. To induce polycystic ovarian syndrome in female wistar rats,
2. To determine the phytoconstituents of ethanolic extract of *Ocimum gratissimum* leaves via phytochemical screening, UV-spectroscopy, and GC-MS analysis
3. To evaluate the toxicological effect of the ethanolic extract of *Ocimum gratissimum* leaves on the selected rat tissue as it relates to:
   i. Liver function indices
   ii. Kidney function indices
   iii. Enzyme assay
   iv. Organ-body weight ratio

CHAPTER TWO

2.0 Literature Review

2.1 Historical Perspective of PCOS

Polycystic ovarian syndrome also referred to as PCOS was first regarded to be investigated by Stein and Leventhal in 1935 (Marcondes *et al.,* 2011). In 1721, however, an Italian scientist described a married, infertile woman with shiny ovaries with white surface and the size of pigeon eggs (Vallisneri,1990). It was in the early 1990s that the National Institute of Health also known as 'NIH' sponsored a conference concerning the issue of polycystic ovarian syndrome that formal diagnostic criteria were proposed and eventually largely utilized (Szydlarska *et al.*, 2010). The pathophysiology of PCOS is very difficult to explain as many scientists has tried thus several studies were made. It is now accepted that PCOS is partly genetic through the postulating of many candidate (women) genes (Szydlarska *et al.,* 2010). The Rotterdam criteria incorporated the size and morphology as determined by an ultrasound of the ovary into the diagnostic criteria (Szydlarska *et al.,* 2010). It was observed that insulin resistance was common among women with PCOS especially those with hyperandrogenism (Szydlarska *et al.,* 2010).

2.2 Polycystic Ovarian Syndrome

Polycystic ovarian syndrome also referred to as PCOS as aforementioned can simply be defined as a common, heterogeneous endocrine metabolic disorder affecting of reproductive age affecting approximately 2-10% of them (Moran *et al.,* 2010). It is a condition of which small cysts are multiplied on one or both ovaries and or the ovarian volume exceeds 10ml (the ovary becomes enlarged) (Hamdar *et al.,* 2016). It is characterized by hypothalamic-pituitary-ovary axis dysfunction (excess ovarian activity) and chronic anovulation. It includes androgen excess in serum level of gonadotropins and estrogens (Azizia *et al.,* 2014). Hyperandrogenism is a key feature in PCOS because it is very evident in the genesis of the disorder (Gonzalez, 2011). PCOS was formerly considered to be related to adult women but, due to series of studies, it was discovered that PCOS is a lifelong disorder manifesting since prenatal age (Azizia *et al.,* 2014).

PCOS has been associated with several metabolic features or syndrome which include insulin resistance, hyperandrogenemia, hyperinsulinemia as well as cardiovascular factors (Oliverraj *et*

al., 2017). Insulin resistance and hyperinsulinemia has been pinpointed as the fundamental link associating these conditions of which are said to be a significant cause of hyperandrogenism of women with PCOS (Oliverraj *et al.,* 2017). Women with PCOS tend to have several comorbidities through clinical manifestations which are: obesity, dyslipidemia, hypertension, type 2 diabetes mellitus, metabolic syndrome (Legro *et al*., 2012). Amongst the aforementioned manifestations or complications, obesity turns out to be at a worldwide epidemic proportions averaging 35% of females (Herrero *et al.,* 2013). Obesity aggravates these hormonal imbalances. Hirsutism and menstrual irregularities are observed to be of higher incidence in obese women with PCOS than non-obese with PCOS (Oliverraj et al., 2017).

Due to series of studies of the pathophysiology of PCOS, PCOS is believed to be an inherent metabolic disorder. Anovulation results to increased level of androgen production or release from the ovaries (Callahan *et al.,* 2009). Androgens are stored in the adipose tissue where they are converted to estrogens leading to the increased production of Sex Hormone Binding Globulin (SHBG). The cause of excess production of androgens has been linked to surplus Luteinizing Hormone (LH) stimulation resulting in the presence of cystic changes in the ovaries (Callahan *et al.,* 2009).

PCOS has a serious effect including increased risk of development of endometrial hyperplasia and neoplasia due to estrogen production by the ovaries and subcutaneous fat without progesterone opposition which also produces menstrual irregularities (Satler *et al.,* 2015).
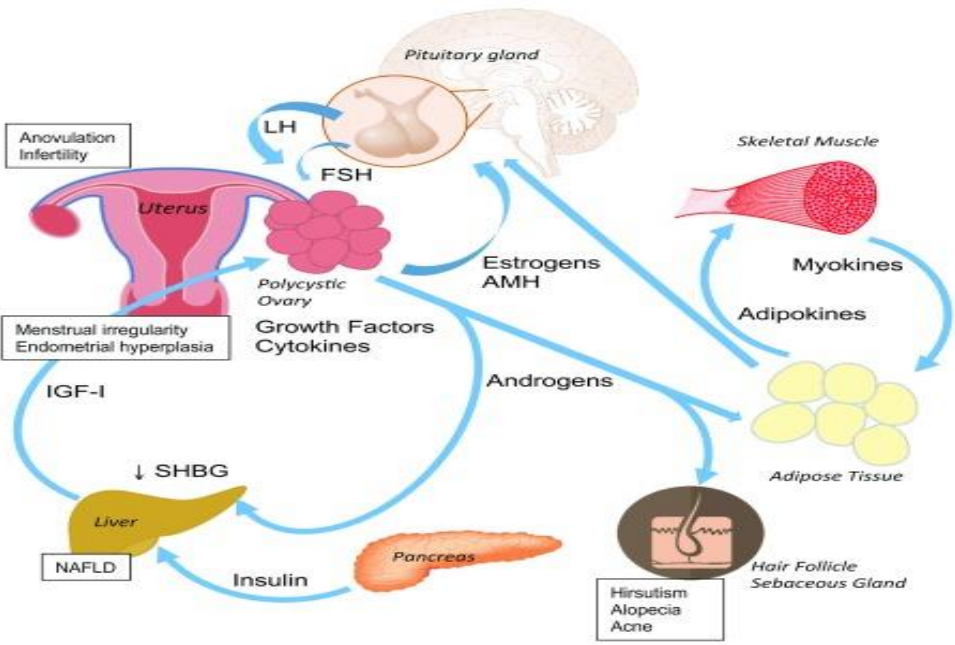
Figure 1: Schematic representation of pathophysiological mechanisms of PCOS (Leo *et al.,* 2016)

2.2.1 Ovarian Dysfunction

Ovarian dysfunction can be best explained as a condition in which the ovaries cease to function, and menstrual periods stops before the age of 40. Ovarian dysfunction or failure is of two types: primary and secondary (Horowitz *et al.,* 2021). Primary ovarian dysfunction simply means the ovaries does not function properly due to surgery or it been removed. It can also be caused by some cancer treatment and certain genetic conditions or diseases. Secondary ovarian dysfunction means the ovaries are in normal condition but there is a problem in getting hormone signals to them from the brain (Horowitz *et al.,* 2021). This occurs when there are diseases of the pituitary gland and the hypothalamus.

Some women with ovarian dysfunction often have menstrual periods and may be able to have children. Ovarian dysfunction is called early menopause, premature menopause, ovarian insufficiency (Horowitz *et al.,* 2021). Ovulatory dysfunction is an abnormal, irregular, or absent ovulation. Irregular menses is with less or equal to 9 menses per year. Diagnosis is often possible by measurement of hormone levels or serial pelvic ultrasonography (Rebar, 2020). Clinical manifestation of ovarian dysfunction includes Oligomenorrhea, amenorrhea, ultrasound polycystic ovaries (Baldani *et al.,* 2012).

2.2.1.1 Infertility

The major key cause of infertility in women with PCOS is ovulatory dysfunction or chronic anovulation (Bassim, 2018). Women with PCOS ovulate irregularly. PCOS patients tend to have reduced fertility based on the endocrine and gynecological metabolic disorders that impact the ovarian cycle (Hart *et al.*, 2015). Statistically, infertility has showed to be 10 times more in PCOS patients. In women with PCOS, conception takes longer, and the rate of miscarriage is higher (Winter *et al.,* 2002). PCOS patients give birth earlier than they planned which can lead to pregnancy complications such as pregnancy related hypertension, gestational diabetes, preeclampsia (Brunyeel *et al.,* 2014; Katulski *et al.,* 2015). Offspring of PCOS patients have a high rate of mortality and morbidity (Katulski *et al.*, 2015). A subset of women with PCOS is infertile. Subfertility is likely to related to plasma luteinizing hormone levels in the follicular phase of the ovarian cycle, leading to a second meiotic division of the oocyte and the release of premature ovocytes (Bassim, 2018).

2.2.2 Metabolic Disturbances of PCOS

Metabolic disturbance or syndrome is a cluster of metabolic abnormalities associated with polycystic ovarian syndrome. These metabolic disturbances primarily include insulin resistance, obesity, dyslipidemia, inflammation, hyperinsulinemia (Panidis *et al.,* 2012). Several studies have been done understanding the metabolic disturbances in PCOS and it was discovered that metabolic syndrome is associated with increased risk in type 2 diabetes mellitus and clinical and subclinical cardiovascular diseases (Panidis *et al.,* 2012). Metabolic syndrome shares several similarities with PCOS. The conjunction of endocrine-metabolic disorders paves ways for the progressive production of several additional comorbidities, be it metabolic or cardiovascular disease which further complicates the management of these PCOS patients (Rojas *et al.,* 2014).

2.2.2.1 Insulin Resistance

Insulin resistance can be defined as metabolic state characterized by the decrease in cellular ability to respond to insulin signaling (Reaven, 2011). Insulin resistance have been discovered to be the major link associating all the metabolic disturbances. Insulin disrupts all the component of the hypothalamus-hypophysis-ovary-axis and ovarian tissue (Rojas *et al.,* 2014). Insulin resistance results in impaired metabolic signaling, favoring hyperandrogenemia which is the main cause of the clinical manifestation of PCOS (Rojas *et al.,* 2014). The prevalence of insulin resistance in PCOS patients ranges from 44 to 77%. This range is due to several factors such as the heterogeneity of PCOS (Dunaif *et al.,* 2012). Insulin resistance as well as hyperinsulinemia plays an important role in androgenic hypersecretion in the development of PCOS.

Decrease in fasting insulin levels in PCOS patients appears to lower androgenemia and improve ovarian functionalism (Legro *et al.,* 2011). Insulin resistance and hyperandrogenemia may create a vicious cycle; stimulating each other in a reciprocal manner (Rojas *et al.,* 2014). Insulin also reinforces adrenal glands as an alternate androgen source parallel to ovaries, by potentiating the hypothalamus-hypophysis-adrenal axis activity at several key sites. Insulin appears to augment adrenal cortex sensitivity to Adrenocorticotropic hormone stimulation with increased androgen secretion (Rojas *et al.,* 2014). Insulin plays a part in the development of Gonadotropin-releasing hormone and Luteinizing hormone pulse secretion seen in PCOS (Rojas *et al.,* 2014).

2.2.2.2 Obesity

Obesity, compared to other associated conditions, have been investigated to be at an epidemic climax with a worldwide rife of 35% in female (Herrero *et al.,* 2013). Obesity is a key magnifying factor of polycystic ovarian syndrome (PCOS). Nearly half of the women with PCOS are clinically obese. Anovulation and menstrual irregularities appear to be more concurrent in obese woman with PCOS than non-obese women with PCOS (Pasquali *et al.,* 2006). Difficulty in conception on a long term is greater in obese PCOS patients (Kahn *et al.,* 2006). Dietary patterns of obese PCOS patients and hyperandrogenemia is somewhat linked.

High-lipid, low fiber diets, both long and short term are related with hyperandrogenemia, through intake-induced hyperinsulinemia which lowers Sex Hormone Binding Globulin (SHBG) which turn increases androgen production or availability (Mukherjee and Maitra, 2010). Diets rich in carbohydrates, may induce oxidative stress in circulating mononuclear blood thus leading to chronic inflammation. Diets rich in lipids have been investigated to increase testosterone synthesis and downregulate SHBG synthesis, resulting in hyperandrogenemia.

These diets also favor endogenous and exogenous Advanced Glycation End product deposition, which results in overall ovarian tissue damage (Rojas *et al.,* 2014). Although obesity is included in the associated conditions of PCOS, it is approximately half of PCOS patients that have obesity. Obese and non-obese women with PCOS have different key features especially in insulin physiology due to the close association between obesity and insulin resistance (Zhao *et al.,* 2010). Whether obesity can lead to PCOS or vice versa can still raise issue for argument or debate (Kamanger *et al.,* 2015).

2.2.2.3 Dyslipidemia

Dyslipidemia plays an important role in the development of PCOS. Dyslipidemia is one of the most common comorbidities found in PCOS patients (Liu *et al.,* 2019). Dyslipidemia is mediated by insulin resistance and excess androgen as well as environmental factors. Dyslipidemia promotes the development of insulin resistance, hyperandrogenism, oxidative stress and anovulation in PCOS patients. (Liu *et al.,* 2019).

Lipid abnormalities often occur in women with PCOS. These abnormalities include reduced high-density lipoprotein cholesterol level, elevated levels of low-density lipoprotein cholesterol, triglycerides levels, total cholesterol, and higher lipoprotein concentration (Tsouma *et al.,* 2014). The most common lipid abnormality found in women with PCOS is reduced high-density lipoprotein cholesterol and elevated level of triglycerides; a common pattern found in insulin resistance (Bargiota and Kandarakis, 2012).

Based on several studies, phenotype specific difference in lipid profiles caused by androgen levels was observed suggesting the importance of androgen in hyperlipidemia (Spalkowska *et al.,* 2018). Changes in genes related to lipid promotes the development of hyperandrogenism. Women with severe hypercholesterolemia, have high body mass index of 29.1kg/m^2 with an average of 25 years (Pergialiotis *et al.,* 2018 and Rocha *et al.,* 2011).

Lipid metabolism is involved in changes in the PCOS oocyte environment. Increased levels of glycerol, lipid region, cholesterol and a slightly higher level of low-density lipoprotein have been observed in PCOS follicular fluids (Zhang *et al.,* 2017). Fatty acids such as palmitoleic acid and linoleic acid are increased in PCOS. These changes suggest that dyslipidemia influences the development of follicles in PCOS, which in turn leads to infertility (Zhang *et al.,* 2017). Abnormal lipid metabolism can promote hyperandrogenism, insulin resistance, oxidative stress, and infertility in PCOS (Zhang *et al.,* 2017).

2.2.2.4 Inflammation

Severe low-grade inflammation has become a major key in the development of polycystic ovarian syndrome. Glucose, a dietary trigger has the ability to incite inflammatory response and oxidative stress from mononuclear cells of PCOS patients. Glucose-stimulated inflammation promotes ovarian androgen production in women with PCOS (Gonzalez *et al.,* 2011). In the fasting state, hyperandrogenism is able to activate mononuclear cells by increasing the mononuclear cell sensitivity to glucose which a mechanism for promoting diet-induced inflammation in PCOS patients (Gonzalez *et al.,* 2011).

Mononuclear cells derived macrophages are the main source of cytokine production in excess adipose tissue and promotes adipocyte cytokine production. Increased abdominal adiposity is

continuous in all weight classes in PCOS patients (Gonzalez *et al*., 2011). Glucose indigestion incites oxidative stress in normal weight PCOS patients even in the absence of increased abdominal adiposity. Markers of inflammation and oxidative stress are highly linked with circulating androgens in PCOS (Gonzalez *et al.,* 2011).

Suppression of ovarian androgen production does not affect inflammation in normal weight PCOS patients. The pro-inflammatory stimuli (cytokine tumor necrosis factor) could upregulate the ovarian theca cell steroidogenic enzyme responsible for androgen production which implies that inflammation directly stimulates the polycystic ovary to produce androgens (Gonzalez *et al.,* 2011). The inflammatory load gotten from adipose tissue in PCOS is in proportion to body mass.

2.2.2.5 Type 2 Diabetes Mellitus

Based on the relationship between insulin resistance and polycystic ovarian syndrome, PCOS is therefore, associated with impaired glucose tolerance which a key risk factor in the development of type 2 diabetes mellitus (Fauser *et al.,* 2012). Due to several studies, PCOS patients have a high risk of developing type 2 diabetes mellitus or impaired glucose tolerance (Lerchbaum *et al*., 2013). Development of diabetes in PCOS patients varies with respect to body mass index (BMI). Increasing insulin resistance helps in the development of impaired glucose tolerance and type 2 diabetes mellitus (Fauser *et al.,* 2012). Family history of diabetes increases the prevalence of type II diabetes mellitus in PCOS patients. However, the prevalence of diabetes in PCOS patients with no family history of diabetes was still much higher than normal women (Hamdar et al., 2016). Family history and obesity are major contributors in the development of diabetes in PCOS patients, diabetes can still occur in lean PCOS patients who have no family history, mainly secondary to insulin resistance (Dunaif, 1999).

2.3 Diagnostic Criteria of Polycystic Ovarian Syndrome

The diagnostic criteria of polycystic ovarian syndrome were proposed by three groups; National Institute of Health/ National Institute of Child Health and Human Diseases (NIH/NICHD) in 1992, European Society of Human Reproduction and Embryology/ American Society of Reproductive Medicine (ESHRE/ASRM) also known as Rotterdam criteria, Androgen Excess and PCOS society

(Zawadski and Duniaf, 1992; Rotterdam, 2003; Azziz *et al.,* 2006). All three groups views PCOS as a diagnosis for exclusion although each group defines PCOS in a different manner.

National Institute of Health (NIH) diagnostic criteria for polycystic ovarian syndrome entails that, women with PCOS should demonstrate these features:

1. Oligo-amenorrhea and or chronic anovulation

2. Clinical and or biochemical signs of hyperandrogenism (Zawadski and Duniaf, 1992).

Later, Rotterdam criteria defines PCOS using polycystic ovarian morphology on ultrasound and added to the criteria of National Institute of Health. The Rotterdam criteria includes:

1. Clinical and or biochemical signs of hyperandrogenism
2. Oligo-amenorrhea and or chronic anovulation
3. Polycystic ovaries (Rotterdam, 2003).

Androgen Excess Society defines PCOS and considered that androgen excess is essential in the development of polycystic ovarian syndrome and establishes that androgen excess should be present in the diagnostic criteria of PCOS. These criteria include that patient should demonstrate.

1. Hyperandrogenemia and or Hirsutism
2. Oligo-anovulation and or polycystic ovaries (Azziz *et al.,* 2006).

Exclusion of other androgen excess disorders should be excluded such as non-classical congenital adrenal hyperplasia (NC-CAH), Cushing's syndrome, androgen-secreting tumors, hyperprolactinemia, thyroid diseases, drug-induced androgen excess, as well as other causes of oligomenorrhea or anovulation (Spritzer, 2014). In 2012, National Health Institute (NIH) sponsored an evidence-based methodology which classified PCOS phenotypes into four classes:

1. Phenotype A: hyperandrogenism, ovulatory dysfunction, polycystic ovary morphology
2. Phenotype B: hyperandrogenism, ovulatory dysfunction
3. Phenotype C: hyperandrogenism, polycystic ovary morphology
4. Phenotype D: ovulatory dysfunction and polycystic ovary morphology (Johnson *et al.*, 2012; Lizneva *et al.*, 2016).

A conclusive diagnosis of PCOS can be done if a patient has at least 2 or all of the following features: hyperandrogenism, menstrual abnormalities and polycystic ovaries (Hailes, 2018).

2.4 Prevalence of PCOS

The prevalence of polycystic ovarian syndrome is said to be between 3% to 10% but differs based on geographical population, race, and ethnicities. (Wolf *et al.,* 2018). Due to the inconsistences and variabilities in the diagnostic criteria of PCOS, it is therefore difficulty to determine the prevalence of PCOS (Wolf *et al.,* 2018). The prevalence of PCOS may be difficult to determine when conducted on a large scale due to the fact a large percentage of individual (reported to be 75%) remains undiagnosed when visiting a doctor (Wolf *et al.,* 2018).

Prevalence rates of PCOS have been recorded to be as low as 1.6% when carried out using the combination of the three diagnostic criteria than when carried out separately (Okoroh *et al.,* 2012). Prevalence of PCOS is preferred to be carried out in a subpopulation and the result varies when done with the different diagnostic criteria (Okoroh *et al.,* 2012).

2.5 Symptoms Of PCOS

Symptoms of polycystic ovarian syndrome varies in women with reference to age. In young women with PCOS, they mainly complain of reproductive and psychological problems while older women experience metabolic syndromes (Teede *et al.,* 2011). For proper diagnosis, a thorough clinical examination, laboratory test and medical history examination must be conducted (Witchel *et al.,* 2015). The major signs and symptoms of PCOS are chronic anovulation, hyperandrogenism and menstrual irregularities (Azziz *et al.,* 2009). Other signs and symptoms associated with polycystic ovarian syndrome include:

1. Hirsutism (male pattern of hair growth)
2. Cystic acne
3. Infertility
4. Diabetes mellitus
5. Alopecia (loss of hair)
6. Oligomenorrhea (menstrual irregularities)
7. Amenorrhea (absence of menstruation)

8. Obstructive sleep apnea (trouble sleeping) (Rotterdam, 2004; Legro *et al*., 2013; Chhabra et al., 2005).

2.6 Etiologies of Polycystic Ovarian Syndrome

The cause of PCOS is yet to known because of its heterogeneity. Although, it is believed to be caused by elevated levels of male hormones such as testosterone, androgen stopping the ovaries from producing hormones and making eggs normally (Watson, 2021). Polycystic ovarian syndrome patients have abnormalities in androgen and estrogen metabolism as well as control of androgen production. The abnormalities associated with PCOS are amplified by insulin resistance, hyperinsulinemia, and obesity (Barber *et al*., 2006). Because the level of follicle stimulating hormone is reduced in respect to luteinizing hormone, the ovarian granulosa cells cannot convert androgen to estrogen causing a reduction in estrogen level (Dunaif *et al.,* 2001).

2.7 Management Options of PCOS

In order to manage polycystic ovarian syndrome, the symptoms are usually targeted. Symptoms of polycystic ovarian syndrome varies in adolescent girls and women (Ehrmann *et al.,* 1999). The first treatment for both adolescent girls and women is considered to be lifestyle changes such as diets and exercise (Ehrmann *et al.,* 1999). Diets and exercise are not good enough for the treatment of symptoms associated with polycystic ovarian syndrome because for proper treatment of PCOS effective, it has to be interdisciplinary (Legro *et al.,* 2013). No specific diet or exercise has been reported to have any effect in the management of PCOS (Hayek *et al.,* 2016).

Medical treatment is considered to be better for the management of PCOS. Medical management of polycystic ovarian syndrome is targeted at the treatment of metabolic syndrome (Callahan *et al.*, 2009). The use of insulin-sensitizing drugs aids in improving insulin sensitivity with a reduction in circulating androgen levels, as well as improvement in both the ovulation rate and glucose tolerance (Callahan *et al.,* 2009). First-line medical therapy usually consists of an oral contraceptive to induce regular menses. Contraceptive inhibits ovarian androgen production as well as increases sex hormone-binding globulin (SHBG) production (Callahan *et al.,* 2009).

Suggested medications for polycystic ovarian syndrome are metformin, oral contraceptives, clomiphene citrate, spironolactone, leuprolide, prednisone (Baldauff and Arslanian, 2015). Each medication is responsible for managing one or more metabolic disturbances or syndrome.

2.7.1 Metformin

Metformin or Glucophage is an oral antidiabetic drug that reduces insulin resistances and reduces hyperinsulinemia in women with PCOS. It acts by hindering the production of glucose by the lungs and elevating the peripheral insulin sensitivity (insulin sensitizer) (Lord *et al.,* 2003). Around a treatment period of 36 months, metformin helps in improving the metabolic profile of PCOS patients especially by improving circulation of high-density lipoprotein cholesterol, body mass index, diastolic blood (Cheang *et al.,* 2009).

Metformin has advantageous effect although small but can cause a responsible reduction in androgen levels about 11% (Lord *et al.,* 2003). Although there are contrary results on metformin effect, it is suggested to be the first line of treatment in pregnancy complication in women with PCOS. When combines with clomiphene citrate, it improves the fertility outcomes of PCOS patients who are resistant to clomiphene citrate (Legro et al., 2013; Misso et al., 2014).

2.7.2 Oral Contraceptives

Oral contraceptives are suggested to be first line of treatment in PCOS women with hyperandrogenism and menstrual irregularities recommended by PCOS consensus group, Endocrine society and Taskforce, Australian Alliance (Fauser *et al.,* 2012; Legro *et al.,* 2013; Misso et al., 2014). Oral contraceptive pills decrease luteinizing hormone secretion, increase sex hormone binding globulin, and decrease free testosterone levels by suppressing the hypothalamo-pituitary ovarian axis (Costello *et al.,* 2007). All oral contraceptive has the capability to reduce ovarian androgen production via the inhibition of gonadotropin secretion and also decrease the production of adrenal androgens (Legro *et al.,* 2013).

Although all oral contraceptives reduce levels of free testosterone by estimation of 50%, preparation of contraceptives which differ also have different effect on ovarian production and sex hormone binding globulin (Costello *et al.,* 2007). Oral contraceptive pills tend to have a negative effect on women with PCOS having cardiovascular profiling; a statement of which is still

debatable (Lidegaard *et al.,* 2012). The most appropriate way to administer oral contraceptive to PCOS patients is in low doses which contains anti-androgenic or neutral progestins because high doses of contraceptive progestins inhibits 5-alpha reductase (Yildiz, 2015).

2.7.3 Clomiphene Citrate

Clomiphene citrate (Serophene or Clomid) is a selective estrogen receptor modulator that binds to estrogen receptors thereby inducing ovulation by increasing the output of pituitary gonadotropins. Clomiphene citrate acts directly by producing a surge of luteinizing hormone which could ovulation within a couple of days (Lucidi, 2019). In a case of ovulation induction when fertility is longed-for, clomiphene citrate is suggested to be the first line of treatment (Vause *et al.,* 2010). Some women with patients after being administered with clomiphene citrate turns out to be resistant. Therefore, second line of management can be considered. (Spritzer *et al.,* 2015). Laparoscopic ovarian drilling, exogenous gonadotropins and *in vitro* fertilization have been considered to be the second line of management of PCOS when clomiphene citrate with or without metformin fails to achieve fertility (Spritzer *et al.,* 2015).

2.8 Medicinal Plants used in PCOS

Plants have been inherited as a source of medication and are an important part of the health care system. Plants contain valuable information in the form of medicinal knowledge that has been preserved for the sake of modern health care system (Pandey, 2017). Plants that are known to possess therapeutic properties due to several investigations, studies and research are commonly known as "Medicinal plants". Plants used in traditional medicine provide a wide variety of drugs and can be used for the treatment of both chronic and infectious diseases (Duraipandiyan *et al*., 2006). Therefore, special focus should be given to the genetic makeup, physiology, morphology of the species to extract its potential as therapeutic value (Pandey, 2017). Examples of medicinal plants used in treating certain sometimes associated with PCOS include.

1. *Asparagus racemosus* (**Shatavari**): It is recognized in traditional Indian medicine for promoting the development of ovarian follicles, combating hyperinsulinemia, regulating menstrual cycle, and revisiting the reproductive system of a female due to its phytoestrogen (Ayurvedic, 2014).

2. ***Tinospora cordifolia* (Guduchi):** It is a medicinal plant that has hypoglycemic effects and anti- inflammatory properties (Chandrasekaran *et al.,* 2012). It helps in reducing insulin resistance, naturally boosting metabolic processes, revitalizing body tissues because inflammation in tissues can lead to ovarian cysts and insulin imbalance (Chandrasekaran *et al.,* 2012).

3. ***Ocimum tenuiflorum* (Holy Basil):** It is a traditional medicinal plant known to be effective in treating PCOS due anti-androgenic properties to reduce androgen production (Hyperandrogenism) (Baby *et al.,* 2016). It is used in management of obesity and its metabolic disturbances (Satapathy *et al.,* 2016).

4. ***Actaea racemose* (Black Cohosh):** It is used in several disorder especially those associated with the female reproductive system such as PCOS and its comorbidities. *Actaea racemose* has the potential to induce ovulation in women with PCOS (Baby *et al.,* 2016). It is recognized to be very essential in childbearing and menstrual cycles as well as in treating amenorrhea, dysmenorrhea, leucorrhea, and other uterine conditions (Lieberman, 2009).

5. ***Grifola frondosa* (Maitake Mushroom):** It is a fungus used because of its hypoglycemic effect and is helpful in the management of diabetes (Pachiappan *et al.,* 2017). Its extract has the ability to induce ovulation in PCOS patients (Hudson, 2011). It helps in regulating blood glucose levels and increasing insulin sensitivity (Hudson, 2011).

2.8.1 *Ocimum gratissimum*

Ocimum gratissimum leaf is a medicinal plant. *Ocimum gratissimum* has several names all depending on the geographical location (Effraim *et al.,* 2003). In India, *Ocimum gratissimum* is referred to as 'Vriddhutulsi' in Sanskrit speaking tribe, 'Ram tulsi' in Hindi speaking tribe, 'Nimma tulasi' in Kannada speaking tribe (Effraim *et al.,* 2003). In Nigeria mainly the southern part, it is called 'Daidoya' by the Hausa speaking tribe, 'Nchonwu' by the Igbo speaking tribe and Yoruba speaking tribe called 'effinrin' (Effraim *et al.,* 2003). In French (menthe gabonaise), Indonesian (ruku-ruku rimba). *Ocimum gratissimum* is also known as clove basil or lemon basil as its English name but commonly known as "scent leaf" (Pandey, 2017). *Ocimum gratissimum* is a distributed to tropical regions and a native of South Asia, West Africa and some parts of South America, Polynesia, Bismarck, Archipelago, West Indies (Singh, 2012).

Ocimum gratissimum is a polymorphic branched, aromatic shrub nearly 0.5 to 3m tall with erect stem, woody at its base with the epidermis peeling in strips (Singh, 2012). The leaves measure up to 10 x 5cm. The leaves show the presence of covering and glandular trichomes. Ordinary trichomes are few, the long ones (6-celled) are mostly found in the margin while the short ones (2-celled) are found in the lamina (Prabhu *et al.,* 2009). The petioles are about 6cm long and the racemes are up to 18cm long. *Ocimum gratissimum* has densely pubescent peduncles (Prabhu *et al.,* 2009). The calyx is 5mm long and the campanulate is 5 to 7mm long. *Ocimum gratissimum* is dotted on both sides, coarsely blunt or round downwards (Prabhu *et al.,* 2009). It prefers open environment, disturbed lands, wet and fertile conditions and can as well tolerate drought (Orwa *et al.,* 2009). In some regions in Asia especially South-East, it grows up to 300m long and can grow as a hedge plant (Orwa *et al.,* 2009).

It is cultivated in South Sea Islands, Nepal, Bengal, Deccan, Ceylon and also with Chittagong. The shrubs, leaves, seeds as well as the whole plant has great relevance as well as ethno-pharmacological significance.

Figure 2: Picture of *Ocimum gratissimum*

Source: http//: henriettesherbal.com

2.8.1.1 Taxonomy of *Ocimum gratissimum*

Kingdom	Plantae - Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	*Asteridae*
Order	*Lamiales*
Family	*Lamiaceae* – Mint family
Genus	*Ocimum* L. – basil
Species	*gratissimum* L. – African basil

2.8.1.2 Traditional uses of *Ocimum gratissimum*

In traditional medicine, *Ocimum gratissimum* has been used widely in so many countries such as the Northeast of Brazil, Coastal areas of Nigeria, India (Rabelo et al., 2003; Effraim et al., 2003; Akinmoladun et al., 2007). In Northeast of Brazil, *Ocimum gratissimum* is not only used for medicinal purposes but also culinary purposes because of the richness of essential oil in the flowers and leaves enabling it to be used in preparing of tea (Rabelo *et al.,* 2003). In the Coastal areas of Nigeria, it is used in the treatment of diarrhea, high fever, and epilepsy (Effrim *et al.,* 2003). The Savannah areas of Nigeria use it to treat mental illness (Akinmoladun et al., 2007).

In the Southeast of Nigeria, it is used in the keeping wound surfaces sterile, treatment of fungal infections, cold and catarrh and in managing of a baby's cord (Ijeh *et al.,* 2005). Certain tribes in Nigeria uses the extract of the leave to treat diarrhea and the cold leave can be used to relief stomach pain and hemorrhoids (Kabir et al., 2005). In India, sunstroke, headache, and influenza

has been treated using the whole plant of *Ocimum gratissimum* because of it anti-inflammatory, diaphoretic, and antipyretic properties (Tania *et al.,* 2006).

In Kenya, it is used in treating sore eyes, cough, barreness, fever, abdominal pains, convulsions as well as tooth gargle, regulation of menstruation (Matasyoh *et al.,* 2007). It can be used as sedative for children in the Brazilian tropical forests (Cristiana *et al.*, 2006).

2.8.1.3 Pharmacological Activities of *Ocimum gratissimum*

Ocimum gratissimum is a popularly used medicinal plant with a wide range of therapeutic effect. The extracts gotten from *Ocimum gratissimum* has several pharmacological applications and ethno-medicinal properties (Pandey, 2017). *Ocimum gratissimum* has antimicrobial, antibiotic, antidiabetic, antidiarrheal, antiurolithiatic, antioxidant, insecticidal, antimutagenic, antitumor, anticancerous properties (Ekunwe *et al.,* 2010; Orwa *et al.,* 2009; Nwanjo *et al.,* 2007; Odukoya *et al.,* 1993; Agarwal *et al.,* 2014; Pandey, 2017). Ethanolic leaf extracts of *Ocimum gratissimum* tends to have hepatoprotective effect (Surana and Jain, 2010; Arhoghro *et al.,* 2009).

Ocimum gratissimum is recognized to have insecticidal value as well as the potential to be used in treating miscarriage (Oparaocha *et al* 2010; Ogbe *et al* 2009). Ethanolic leaf extracts of *Ocimum gratissimum* shows antimicrobial activity against certain bacteria such as *N. gonorrhea, K. pneumonia, S. typhi, P. aeruginosa and V. cholera* (Mann, 2012). *Ocimum gratissimum* combined with ampicillin exhibits antibacterial properties against *E. coli, Proteus mirabilis* and *S. aureus* (Macdonald *et al.,* 2010; Nweze and Eze, 2009). Several leaf extracts be it ethanol, methanol, aqueous or dichloromethane has its own pharmacological activity when used in different case studies.

Methanol leaf extracts of *Ocimum gratissimum* reduces damage of lipid-protein, generation of free radical as well as possess antioxidant properties (Mahapatra *et al.,* 2009). Dichloromethane leaf extracts inhibits myeloid leukemia *in vitro* with the potential ability to cancer in man (Iweala *et al.,* 2015). Aqueous leaf extracts (fresh) reduce the size of tumors in breast cancer cells (Nangia-Makker *et al.,* 2007).

2.9 Class of Compound of Letrozole

Letrozole (Femara) belongs to the class of compound knowns as "aromatase inhibitors" of which it is the third-generation type which are used to treat breast cancer (Yager, 2006). The function of aromatase inhibitors is the stop the production of estrogen by blocking the aromatase enzyme which converts androgen into estrogen in minute quantities (Khanna, 2020). Aromatase inhibitors are of three classes: Aromasin (exemestane), Arimidex (anastrozole), Femara (letrozole).

2.10 Mode of Action of Letrozole

Letrozole also known as Femara is a generic oral drug that stops the production of estrogen (anti-estrogen drug). Adrenal glands are the major source for circulating estrogens because it contains the enzyme aromatase which produces estrogen, estradiol and estrone (Ogbru, 2020). Letrozole blocks the active site of aromatase as well as the electron transfer chain of cytochrome- P450 19A1 (Nabholtz, 2008). This leads to high levels of luteinizing hormone, FSH and uterine weight loss. Reduction in the availability of estrogens due to the induction of Letrozole, makes estrogen-dependent tumor regress (Nabholtz, 2008).
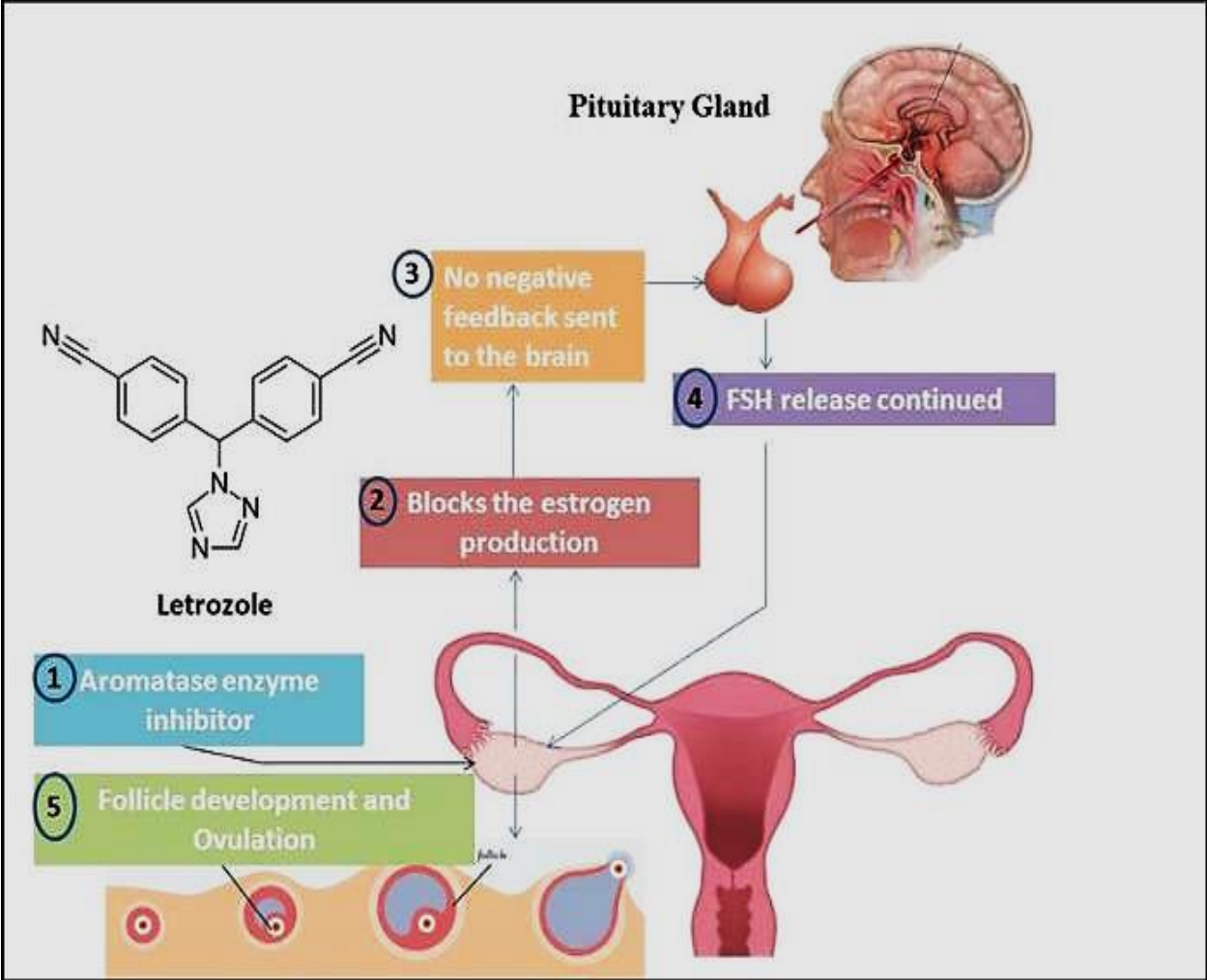
Figure 3: Diagram of mode of action of letrozole in the body (Nabholtz, 2008).

2.11 Phytochemical Constituents and PCOS

According to (Chetia *et al.*,2014), the phytochemical constituents of aqueous extract of *Ocimum gratissimum* leaves indicates the presence of several bioactive compounds such as steroids, tannins, flavonoids, saponins, terpenoids, alkaloids, phenolic compounds, glycosides, phlobatannins, reducing sugars and so on. The phytochemical screening of the ethanol extract carried out on the aerial part of *Ocimum gratissimum* indicates the presence of several secondary metabolites such as phenolic compounds, carbohydrates, terpenoids, anthraquinones, sterols (Mann *et al.,* 2012).

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, phlobatannins.

Flavonoids are the most common group of polyphenolic compounds in the human diet and are found in most plants (Ververidis *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).

Examples of plants with phytochemical constituents used to treat PCOS are as follows:

1. ***Foeniculum vulgare* (Shatapushpa)**: The seeds of the plant are regarded as good in the management of PCOS because of it is a rich source of phytoestrogens (Jungbauer *et al.,* 2014). Phytoestrogens in the *Foeniculum vulgare* helps in reducing inflammation in PCOS as well as reducing insulin resistance. It also helps in reducing hormonal imbalances that leads to metabolic disturbances in PCOS (Jungbauer *et al.,* 2014). Different parts of *Foeniculum vulgare* can be used in treating diseases related to the digestive system such as diabetes, kidney stones, bronchitis (Kooti *et al.,* 2015).

2. ***Galega officinalisi* (Goats Rue):** *Galega officinalisi* is believed to have a beneficial effect in PCOS patients, although further studies are needed to be done (Pachippan *et al.,* 2017). In the Middle Ages, it has been recognized for reliving the symptoms of diabetes mellitus. It turned out to be guanidine which is a substance that decreases insulin resistance by reducing blood sugar (Pachippan *et al.,* 2017).

   Galega officinalisi is a natural source of guanidine which is an anti-diabetic drug belonging to biguanides class as well as metformin which a drug used in the management of PCOS belonging to the same class (Pachippan *et al.,* 2017). This link enables *Galega officinalisi* to be considered as a possible second option for treating PCOS (Pachippan *et al.,* 2017).

3. ***Lepidium meyenii* (Maca):** It is a traditional plant used in stimulating the endocrine system and relieving menopausal symptoms. It also acts as a natural hormonal balancer by encouraging a health menstrual cycle with the help of estrogen and progesterone (Gonzales *et al.,* 2002). It is also a good fertility food and an adaptogen (Gonzales *et al.,* 2002).

2.11.1. Gas Chromatography Mass Spectrometry

Gas Chromatography-Mass spectrometry also known as GC-MS is a technique used to ensure proper and good separation with high selectivity and low detection limits of analytes (Hubschmann, 2015). GC-MS is a technique that uses both the features of gas-liquid chromatography and mass spectrometry to identify different substances in a sample (Hubschmann, 2015). It can detect trace elements in materials and identify substances in luggage or in human.

GC-MS consists of the separation power of gas chromatography and identification characteristics of mass spectrometry (Hubschmann, 2015). It is used in metabolic profiling and identifying metabolic disorders, monitoring of conventional and emerging organic pollutants, analysis of drugs and metabolites in blood, urine and so on (Hubschmann, 2015).

2.11.1.1 Principle of GC-MS

Gas Chromatography- Mass spectrometry is connected through an interface which acts as a transfer line to carry the pressurized gas chromatography output into the evacuated ion source of the mass spectrometer (Hubschmann, 2015). The gas chromatography section takes place in the column and is determined based on the nature of compound, column packed material and column

dimension (Hubschmann, 2015). The difference in the physio-chemical properties between different molecules in a mixture depends on interactions with the stationary phase in the column (Hubschmann, 2015).

Each molecule has a different retention time to elute from the column and allows the downstream mass spectrometer to capture, ionize, accelerate, deflect, and detect the ionized molecules separately by breaking each molecule into ionized fragments (Hubschmann, 2015). The mass spectrometer detects the fragments via their mass to charge ratio (Hubschmann, 2015).

2.11.1.2 Applications Of GC-MS

GC-MS has a wide range of application because of its versatility. It is used in various fields such as health industry, food and beverage industry, biological sciences, environmental safety, security, criminal forensics and so on (Shanmugam *et al.,* 2010). Applications of GC-MS are:

1. Analysis of aromatic compounds such as fatty acids
2. Screening of food contaminants
3. Monitoring persistent organic pollutants
4. Analysis of blood, urine for the presence of drug metabolites
5. Profiling of bioactive compounds in plants
6. Analysis of fire debris (Shanmugam *et al.,* 2010).

2.11.2 UV-Visible Spectrometry

2.11.2.1 Principle

Ultraviolet and visible absorption spectroscopy (often abbreviated to UV-Vis) is a method of spectroscopy that involves calculating the attenuation (strength/intensity weakening) of a light beam after it passes through a sample or reflects from a sample surface. The UV-Visible Spectroscopy principle is based on chemical compounds absorption of ultraviolet or visible light, which results in the formation of distinct spectra. The interaction between light and matter is the basis of spectroscopy. Excitation and de-excitation occur as matter absorbs light, resulting in the formation of a spectrum. In the field of analytical chemistry, UV-Visible spectroscopy is commonly used, especially during the quantitative analysis of a specific analyte.

2.11.2.2 Applications

UV-Vis is a technique that is easy and cheap which allows sample recovery and good discrimination between clear compounds without any need for derivative (Niamh, 2019). Applications of UV-Vis are:

1. Food and Agriculture
2. Pharmaceutical research
3. Life science
4. Quality control
5. Petrochemistry
6. Traditional chemistry
7. Cosmetic industry

2.12 Organ Study

In the study of polycystic ovarian syndrome, there are certain major organ that must be studied to have a proper understanding about polycystic ovarian syndrome. These organs control the basis of which polycystic ovarian syndrome functions. These organs are ovary, uterus, liver, kidney. These organs play a role in the metabolic endocrine disorder common in adolescent and reproductive age women.

2.12.1 Ovary

The ovary is the primary reproductive gonads in a woman. It consists of germ cells and somatic cells whose relationship leads to the formation of oocyte-containing follicles, development of both germ cells and somatic cells (Richards and Pangas, 2010). A mature ovary is mainly controlled by two hormones namely, luteinizing hormone and follicle-stimulating hormone secreted from the anterior pituitary gland by the influence of gonadotropin-releasing hormone from the hypothalamus (Richards and Pangas, 2010).

Ovarian follicle development is regulated by hypothalamic-pituitary-ovarian axis in which gonadotropin- releasing hormone controls the release of the gonadotropic hormones, follicle-stimulating hormone, and luteinizing hormone. On the secretion of gonadotropin-releasing

hormone, ovarian steroids exert positive and negative regulatory effects (Richards and Pangas, 2010). Both high and low frequency of gonadotropin-releasing hormone pulses has an effect in a woman's menstrual cycle.

High frequency leads to a rise in luteinizing hormone levels which leads to formation of corpus luteum and ovulation while low frequency enhances follicle growth and stimulates a slight increase in follicle stimulating hormone levels (Richards and Pangas, 2010). The ovary ensures the release of oocytes that can be fertilized are on time and the maintenance of luteal cell function which is necessary for pregnancy through feedback mechanism to the hypothalamus and pituitary (Richards and Pangas, 2010).

When a woman is diagnosed of PCOS, the ovaries begin to develop several small collections of fluids known as 'follicle' and fail to regularly release eggs (Mayo, 2020).
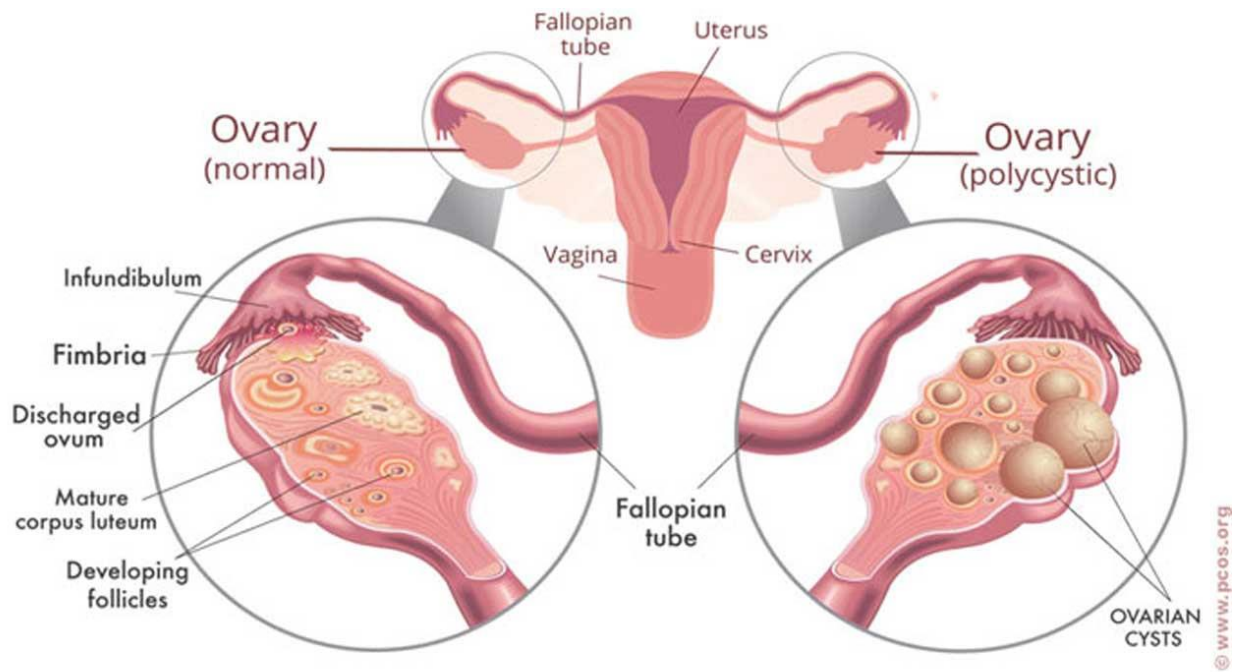
Figure 4: Diagram of a polycystic ovary

Source: Adunwoke, 2019

2.12.2 Liver

Liver is the largest organ and gland in the body. The liver is of two different gland types. The secretory gland and the endocrine gland. The secretory gland is responsible for the secretion of bile into bile ducts while the liver as an endocrine gland is responsible for secretion of chemicals into the blood that has an effect on the other body organs (Stoppler, 2019). When the reproductive system of a women is affected with PCOS, there is an increased rate for liver fibrosis and steatosis as well as nonalcoholic fatty liver disease (NAFLD) with insulin resistance and hyperandrogenism as main factors in liver damage for PCOS patients (Estes, 2021).

Androgens such as testosterone, dehydroepiandrosterone (DHEA) and dihydrotestosterone have been identified as pro-apoptotic agents (induce apoptosis by targeting specific survival pathways) that act on hepatocytes (Baranova *et al.*, 2013). Overproduction of these androgens leads to an androgen- dependent pro-apoptotic environment that may aid in progression of liver diseases (Baranova *et al.,* 2011). Features of PCOS that increases the risk of NAFLD is still unknown especially if NAFLD is higher in all PCOS patients (Kumarendran *et al.,* 2018).

2.12.3 Kidney

All vertebrates have a pair of kidneys, which are bean-shaped organs. They remove waste from the body, keep electrolyte levels regulated, and keep blood pressure in check (Kaygusuz *et al*., 2013). The kidneys are located toward the rear of the abdomen, one on each side of the spine. To make room for the liver, the right kidney is slightly smaller and lower than the left. Male kidneys weigh 125–170 g, while female kidneys weigh 115–155 g (Nath *et al.,* 2013).

The kidneys' primary function is to maintain homeostasis. This means they keep track of fluid levels, electrolyte balance, and other factors that keep the body's internal environment stable and comfortable. Other functions of the kidney include (Knoedler *et al.,* 2015): Excretion of waste, nutrient re-absorption, keeping the right pH, regulation of osmolality, keeping blood pressure in check, active compound secretion. The kidneys can be affected by a variety of disorders. Kidney disease can be caused by environmental or medical conditions, which can create functional and structural difficulties in some persons from birth.

2.12.4 Percentage Organ Body Weight Ratio

Changes in organ weight acts as an indicator to identify organ damage that has been chemically induced, although it can be difficult to analyze because a change in organ weight can reflect chemically induced changes in overall body weight (Lazic *et al.,* 2020). Interpreting the organ weight data can be difficult because you have to identify whether a chemically induced compound affects the organ directly through changes in the body weight (Lazic *et al.,* 2020). To calculate the ratio (relative organ weight), the organ of the animal is dividing by the body weight of the animal the multiplied by hundred. Unfortunately, with this method, the ratio fails to adjust the differences in the body weight between groups properly, but this is mostly ignored (Michael *et al.,* 2007). The percentage organ- body weight ratio was obtained using the following expression:

$$\text{Percentage organ-body weight ratio} = \frac{\text{Organ weight}}{\text{Total body weight of the animals}} \times 100$$

2.13 Biochemical Assessment

2.13.1 Kidney Function Indices and PCOS

Biochemical kidney function indices can be determined by running biochemical assays such as creatinine, uric acid, urea, and blood urea nitrogen (BUN) creatinine ratio (Edmud *et al.,* 2006). The body produces creatinine, which is the breakdown product of creatine phosphate in muscles, based on muscle size. When evaluating the kidney's function, creatinine is a regularly utilized measurement. Serum creatinine concentration be used to calculate glomerular filtration rate (Miller *et al.,* 2005). Testing for creatinine clearance serves as a way to track the course of kidney disease. As a general rule, renal failure is suspected when serum creatinine levels are higher than what is considered "normal." Creatinine excretion by the glomeruli and tubules is reduced over time in chronic renal failure and uremia (Edmud *et al.,* 2006).

Urea is a significant nitrogenous end product of protein and amino acid degradation. Water is partially reabsorbed by glomeruli in the kidneys after urea has been removed from the circulation. In order to estimate renal function, urea in the serum is the most commonly used clinical indicator.

In the differential diagnosis of acute renal failure and pre-renal conditions where the blood urea nitrogen–creatinine ratio is elevated (Pagana *et al.,* 2002). Since its overproduction rate depends on various nonrenal factors, including food and urea cycle, urea clearance does not provide a good indication of the glomerular filtration rate (GFR) (Pagana *et al.,* 2002).

A rise in blood urea nitrogen (BUN) is noticed in patients with renal illness or failure, kidney stone blockage, congestive heart failure (CHF), dehydration, fever (feverishness), shock, and gastrointestinal bleeding. It's possible to have high BUN levels throughout late pregnancy, or even after eating a lot of protein-rich foods. For example, high levels of BUN indicate renal injury, while low levels of BUN indicate fluid retention (Pagana *et al.,* 2002).

PCOS is thought to be a pro-inflammatory condition. A number of recent studies have shown that women with PCOS, regardless of their body mass index (BMI), have an inflammatory response that is triggered by glucose (Gonzalez et al., 2006). PCOS is associated with elevated levels of circulating proatherogenic inflammatory mediators (Diamanti-Kandarakis *et al.,* 2006).

2.13.2 Liver Function Indices and PCOS

Biochemical liver function indices can be determined by running biochemical assays such as serum total protein, albumin, total and direct (conjugated) bilirubin, globulin and albumin-globulin ratio, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) (Salmela *et al.,* 2004). ALT and AST are indicators of hepatocyte damage because they detect the concentration of intracellular hepatic enzymes that have spilled into the blood (Salmela *et al.,* 2004). ALP and bilirubin act as markers of biliary function and cholestasis while albumin with prothrombin reflects liver synthetic function (Salmela *et al.,* 2004). Elevations of AST and ALT can cause drug and toxin-induced liver injury, viral hepatitis and so on (Pasquali *et al.,* 2011).

According to (Schwimmer et al., 2005), liver diseases are often undiagnosed because they are silent and become discovered when they reach advanced levels. Certain medications can cause toxicity to the liver if NAFLD is present because some physicians are unaware of NAFLD as a morbidity of PCOS. PCOS patients tend to have elevated blood levels of ALT and should therefore avoid alcohol and acetaminophen (Schwimmer *et al.,* 2005).

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials

3.1.1 Plant material and Authentication

Fresh leaves of *Ocimum gratissimum* plant were collected from Magboro market, Ogun state, Nigeria. The plant authentication was carried out at Department of Plant Biology, University of Lagos, Lagos, Nigeria by Mr. Nodza George. A voucher specimen number LUH 8752 was prepared and deposited at the herbarium of the department.

3.1.2 Experimental animals

Twenty female albino rats with an average weight of (170.81 ± 5.25g) was obtained from the Animal Holding unit of the University of Lagos, Lagos, Nigeria. They were kept in a well-ventilated house condition and fed with rat pellets (Vital feeds, grand cereals) and water.

3.1.3 Assay kits and drugs

Albumin, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, uric acid, creatinine, total protein, cholesterol, high density lipoprotein cholesterol and triglyceride assay kits were products of Randox laboratory, Liquizyme, United Kingdom. Progesterone, testosterone, estradiol, insulin, follicle stimulating hormone (FSH) and luteinizing hormone (LH) assay kits were manufactured by Diagnostics laboratories.

3.2 Methods

3.2.1 Preparation of Ethanolic Extract of *O. Gratissimum Leaves*

Reagents/Materials

2.5L absolute (concentrated) ethanol, warring electric blender, Rotatory evaporator, Beakers, Funnel, Whattman's No.1 filter paper, Measuring cylinder, Jute bag, Hot air oven and Plastic container, UV-Visible Spectrophotometer, cuvette, 40 ml distilled water, 0.17g *Ocimum gratissimum,* filter paper, conical flask.

Procedure

A known weight of 1468g of *Ocimum gratissimum* leaves was washed to remove contaminants, which was sun dried to remove water content of the leaves and the oven dried at 50℃ to attain a weight of 340g. When completely dried, was pulverized with an electric blender. The pulverized leaves which weighed 388g was soaked in 1400ml of absolute ethanol for 48hours. The extract was sieved with a jute bag and poured in a plastic container and filtered with Whattman's No. 1 filter paper, funnel, and conical flask. The filtrate was subjected to the rotatory evaporator to get back the absolute ethanol which is the solvent and the concentrate of the plant sample. The concentrate was kept in the oven at 50℃ until completely dry to obtain a yield of 31.21g (9.23%).

3.2.2 Phytochemical Screening of the Extract

3.2.2.1 Qualitative screening of secondary metabolites

Ocimum gratissimum leaves was screened for secondary metabolites present as described by (Arvindganth *et al.,* 2015).

1. **Test for Carbohydrate**

   2ml of plant extract was treated with 1ml Molisch's reagent and 5 drops of concentrated sulphuric acid in a test tube. Formation of purple or reddish colour solution gives the presence of carbohydrate (Arvindganth *et al.,* 2015).

2. **Test for Tannins**

   1ml of plant extract was added with 2ml of 5% ferric chloride in a test tube. Formation of dark blue or greenish black solution indicates the presence of tannins (Arvindganth *et al.,* 2015).

3. **Test for Saponins**

   2ml of plant extract was added with 2ml of distilled water in a test tube and was shaken for 15 minutes lengthwise. Formation of foam indicate the presence of saponins (Arvindganth *et al.,* 2015).

4. **Test for Alkaloids**

   2ml of plant extract was added with 2ml of concentrated hydrochloric acid and 5 drops of Mayer's reagent in a test tube. Formation of green colour solution or white precipitate gives the presence of alkaloids (Arvindganth *et al.,* 2015).

5. **Test for Flavonoids**

   2ml of plant extract was added with 1ml of 2N sodium hydroxide in a test tube. Formation of yellow colour solution gives the presence of flavonoids (Arvindganth *et al.,* 2015).

6. **Test for Glycosides**

   2ml of plant extract was added with 3ml of chloroform and 10% ammonia solution in a test tube. Formation of pink colour solution indicates the presence of glycosides (Arvindganth *et al.,* 2015).

7. **Test for Quinones**

   1ml of plant extract was added with 1ml of concentrated sulphuric acid in a test tube. Formation of red colour solution indicate the presence of quinones (Arvindganth *et al.,* 2015).

8. **Test for Phenols**

   1ml of plant extract was added with 2ml of distilled water and 5 drops of 10% ferric chloride in a test tube. Formation of blue or green colour solution indicates the presence of phenols (Arvindganth *et al.,* 2015).

9. **Test for Terpenoids**

   0.5ml of plant extract was added with 2ml of chloroform and 2ml of concentrated sulphuric acid in a test tube. Formation of a reddish-brown colour solution gives the presence of terpenoids (Arvindganth *et al.,* 2015).

10. **Test for Cardiac Glycosides**

    0.5ml of plant extract was added with 2ml of glacial acetic acid, 5 drops of ferric chloride, and 1ml of concentrated sulphuric acid in a test tube. Formation of brown ring at the interface indicates the presence of cardiac glycosides (Arvindganth *et al.,* 2015).

11. **Test for Ninhydrin**

    2ml of plant extract was added with 5 drops of 0.2% ninhydrin reagent and heated for 5 minutes in a test tube. Formation of blue colour solution indicate the presence of ninhydrin (Arvindganth *et al.,* 2015).

12. Test for Coumarins

1ml of plant extract was added with 1ml of 10% sodium hydroxide in a test tube. Formation of yellow colour solution gives the presence of coumarins (Arvindganth *et al.,* 2015).

13. Test for Anthraquinones

1ml of plant extract was added with 5 drops of 10% ammonia solution in a test tube. Formation of pink colour precipitate indicate the presence of anthraquinones (Arvindganth *et al.,* 2015).

14. Test for Steroids

1ml of plant extract was added with 1ml of chloroform and 5 drops of concentrated sulphuric acid in a test tube. Formation of brown ring gives the presence of steroids (Arvindganth *et al.,* 2015).

15. Test for Phlobatannins

1ml of plant extract was added with 5 drops of 2% hydrochloric acid in a test tube. Formation of red colour solution indicate the presence of Phlobatannins (Arvindganth *et al.,* 2015).

16. Test for Anthracyanin

1ml of plant extract was added with 1ml of 2N sodium hydroxide and heated for 5 minutes at 100 ºC in a test tube. Formation of bluish green colour solution gives the presence of anthracyanin (Arvindganth *et al.,* 2015).

3.3 UV- Visible Spectrophotometry

3.3.1 Principle

Ultraviolet and visible absorption spectroscopy (often abbreviated to UV-Vis) is a method of spectroscopy that involves calculating the attenuation (strength/intensity weakening) of a light beam after it passes through a sample or reflects from a sample surface. The UV-Visible Spectroscopy principle is based on chemical compounds absorption of ultraviolet or visible light, which results in the formation of distinct spectra. The interaction between light and matter is the basis of spectroscopy. Excitation and de-excitation occur as matter absorbs light, resulting in the formation of a spectrum (Niamh, 2019).

In the field of analytical chemistry, UV-Visible spectroscopy is commonly used, especially during the quantitative analysis of a specific analyte.

Procedure

0.17g of *Ocimum gratissimum* was dissolved in 40 ml distilled water in a conical flask, the solution was filtered using a filter paper to give a clear solution. The plant sample solution was placed in a clean cuvette, held at the opaque portion of the cuvette, and placed in the UV-Visible spectrophotometer. The absorbance of the ethanolic plant extract was determined by a wavelength with range between 200 and 600nm. The UV-visible spectra were recorded on a (Shimadzu UVd-1800 PC, Japan) UV-Vis spectrophotometer (Niamh, 2019).

3.3.2 GC-MS Analysis

The GC-MS study was carried out using a Hewlett Packard Gas Chromatograph (Model 6890 series) fitted with a flame ionization detector and a Hewlett Packard 7683 series 250 °C MS transfer line temperature injector. The GC was fitted with an HP-5MS (30 x 0.25 mm) fused silica capillary column, 1.0µm film thickness. The oven temperature was kept at 50 ° C for 5 min of held time and increased at a rate of 2 ° C / min from 50 to 250 ° C, using helium gas (99.999 percent) as a carrier gas at a steady flow rate of 22 cm / s. A 1.0micron extract (1 mg diluted in 1 ml of absolute alcohol) was injected at a 1:30 split ratio. Agilent Science Network Mass Spectrometer (Model 5973 series) coupled with Hewlett Packard Gas Chromatograph (Model 6890 series) with NIST08 Library software database was studied by MS. Mass spectra were taken at 70 eV/200 °C, 1 scan/s scanning rate. Compound recognition was carried out using the NIST08 Library database. The mass spectrum of each unknown compound was compared with the known compounds contained in the library software database (Lian *et al*., 2013)

3.4 Induction of PCOS

3.4.1 Animal grouping and induction of PCOS

Twenty female wistar rats of average weight of (170.81±5.25g) were acclimatized for one week to standard housing conditions and fed with rat pellets and water. The Wistar rats were induced when they in their estrus stage. The animals were grouped based on similarity in body weight into five groups of four animals as follows:

Group 1 (Control group)- received only animal feed and water

Group 2 (Letrozole-induced)- received 1ml of distilled water

Group 3 (Letrozole-induced)- received 0.5ml of 7.14mg/kg body weight of metformin and 0.5ml of 2mg/kg body weight clomiphene citrate (Reference drugs).

Group 4 (Letrozole-induced)- received 0.5ml ethanolic extract of *Ocimum gratissimum* leaves

Group 5 (Letrozole-induced)- received 1ml of ethanolic extract of *Ocimum gratissimum* leaves.

To those who required 0.5 ml and 1ml of the plant extract, 1ml of distilled water and 1ml of metformin + clomiphene citrate which corresponded to their respective doses were administered once daily for twenty-one days using oral administration.

3.4.2 Confirmation of PCOS

The estrous cycle of the rats was monitored by vaginal cytology and using a light microscope to know the cell type present in the vagina at the time to know the stage of the cycle. This was done daily during to know the period of induction (Yakubu *et al.,* 2008).

3.4.3 Vaginal Cytology

Vaginal smears were obtained from the rats using cotton buds and distilled water. The buds were dipped into the distilled water, damped, and inserted into the vagina of the rats. The sample was then smeared onto a microscope slide and viewed under a microscope.

3.5 Preparation of Serum and Tissue Supernatants

The rats were weighed individually and thereafter anaesthetized in a jar containing cotton wool soaked in diethyl ether. The neck area was cleared of fur and skin to expose the jugular veins. The jugular veins were displaced slightly from the neck region and thereafter cut with a sharp sterile blade. The animals were held head downwards, allowed to bleed into clean, dry centrifuge tubes and left at room temperature for 10 minutes to clot. The blood samples were centrifuged at 3000rpm for 10 minutes using Thermo Scientific Centrifuge (Heraeus Megafuge 8).

The sera were thereafter aspirated using Microflux pipette into clean, dry, sample bottles and were then stored frozen (4ºC) overnight. The animals were quickly dissected, and the liver, kidney and ovary were excised, cleaned of fatty layers, weighed, and transferred into ice cold 2M sucrose

solution. Thereafter, each organ was blotted with blotting paper, cut thinly with a sterile blade, and homogenized separately in ice cold 2M sucrose solution (1:4 w/v). The homogenates obtained were centrifuged at 3000rpm for 10 minutes to obtain the supernatants which were then gently collected into sample bottles, stored frozen (4⁰C) overnight before being used for the various biochemical assays (Yakubu *et al.,* 2008).

Table 1: Dilution factor for the various assays

Assays	Serum	Liver	Kidney
ALT	×5	×30	
ALP	×5	×30	×60
AST	×5	×30	
BUN	×5		
Creatinine	×5		
Albumin	×5		
Globulin	×5		
Total Protein	×5		×60
Albumin-Globulin ratio	×5		
Urea	×5		
Uric acid	×5		
Direct Bilirubin	×5		
Total Bilirubin	×5		

3.6 Biochemical Assessments

3.6.1 Determination of Liver Function Indices

3.6.1.1 Serum Total Protein

The method was first described by Knipe (1998) was used to determine serum total protein

Procedure:

20µl of the sample (serum) was added to the samples test tubes, 20µl of water was added to the blank test tube and 20µl of standard was added to the standard test tube. 1ml of Reagent 1 was added to the sample test tubes, blank test tube and standard test tube, which the solution was mixed and incubated in the hot-air oven for 30 minutes at 25ºC. The absorbance of the samples was read against the blank using UV-Visible spectroscopy at 546nm wavelength.

3.6.1.2 Serum Albumin Concentration

The procedure described by Doumas *et al* (1971) was employed for the determination of serum albumin in the animals.

Principle:

The determination of albumin concentration is based on the quantitative binding to the indicator 3, 3', 5, 5'-tetrabromocresol sulphonapthelein. The albumin-BCG complex absorbs maximally at 578nm. The absorbance is directly proportional to the concentration of albumin in the sample.

Procedure:

5µl of enzyme source (serum) was added to the sample test tubes, 5µl of water was added to the blank test tube and 5µl of standard was added to the standard test tube. 1.5ml of Reagent 1 was added to the sample test tubes, blank test tube and standard test tube, which the solution was mixed and kept in the hot-air oven for 10 minutes at 37ºC, which the absorbance of the samples was read against the blank using UV-Visible spectroscopy at 630nm wavelength.

Calculation:

Concentration of albumin= $\dfrac{(\text{Asample} \times \text{concentration of standard})}{\text{Astandard}}$

Asample= Absorbance of sample

Astandard= Absorbance of standard

Concentration of standard= 4.68g/dl

3.6.1.3 Serum Globulin Concentration

The serum globulin level was assayed using the method described by Tietz (1995). The concentration of globulin was expressed in g/dl. The serum globulin level concentration was determined using

Globulin= Total protein – Albumin

3.6.1.4 Serum Total and Direct Bilirubin Concentration

The method described by Evelyn and Malloy (1938) was used to determine serum total and direct bilirubin concentrations.

Principle:

Direct bilirubin in the sample reacts with diazotized sulphanilic acid in alkaline medium to form blue colored complex. Total bilirubin in the sample was determined by the presence of caffeine benzoate and acetate as accelerator to form azobilirubin (albumin bound bilirubin).

Procedure:

For the determination of total bilirubin, a drop of reagent 2 was placed into 0.1ml of reagent 1 after which 0.5ml of reagent 3 was added subsequently. Thereafter, 0.1ml of appropriately diluted serum was added to the mixture. The blank was constituted by replacing the serum with distilled water without reagent 2. For the determination of direct bilirubin, a drop of reagent 2 was added to 0.1ml of reagent 1 followed by the addition of 0.1ml of 0.9% NaCl. 0.1ml of the sample was added to

the mixture. The blank was constituted by replacing serum with distilled water without adding reagent 2.

The mixture of both total and direct bilirubin was incubated at 25°C for 10minutes after which 0.5ml of reagent 4 was dispensed into the total bilirubin preparation. The mixtures were further incubated for another 30mintues at 25°C and absorbance was read spectrophotometrically at 578nm.

Calculation:

Total bilirubin (mg/dl) = $10.8 \times A_{TB}$

Direct bilirubin (mg/dl) = $14.4 \times A_{DB}$

A_{TB} = Absorbance of total bilirubin

A_{DB} = Absorbance of direct bilirubin

10.8 = Milligram of total bilirubin per 100ml

14.4 = Milligram of direct bilirubin per 100ml

3.6.1.5 Serum Albumin-Globulin Ratio

The determination of serum albumin-globulin ratio was done using the method described by Melnick *et al* (1940) by dividing the concentration of serum albumin by serum globulin content. It is expressed mathematically as:

$$\text{Albumin-globulin ration} = \frac{\text{Serum albumin (g/dl)}}{\text{Serum globulin (g/dl)}}$$
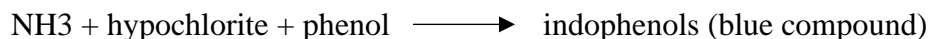
3.6.2 Determination of Kidney Function Indices

3.6.2.1 Serum Urea Concentration

The procedure described by Veniamin and Vakirtzi (1970) was used for the determination of urea in the serum.

Principle:

Urea in the serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then determined photometrically by Berthelot's reaction as derived by the following:

$$Urea + H_2O \longrightarrow 2NH_3 + CO_2$$

$$NH3 + hypochlorite + phenol \longrightarrow indophenols \text{ (blue compound)}$$
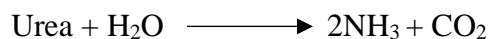
Procedure:

0.05ml of the solution 2 was added to 0.005ml of the serum sample. The standard was constituted by substituting the sample with 0.005ml of standard reagent. The blank was constituted by replacing the serum with 0.005ml distilled water. The mixture was incubated for at 37°C for 5 minutes. The absorbance of the sample and the standard were read spectrophotometrically against the blank at 578nm.

Calculation:

$$Urea \; concentration \; (mg/dl) = \frac{Asample \times Concentration \; of \; standard}{Astandard}$$

Concentration of standard = 50mg/dl

3.6.2.2 Serum Creatinine Concentration

The method described by Bartels and Bohmer (1972) was used for the determination of serum creatinine.

Principle:

Creatinine in alkaline solution reacts with picric acid to form a red colored complex. The amount of the complex formed is directly proportional to the creatinine concentration read spectrophotometrically at 492nm.

Procedure:

100μl of enzyme source (serum) was added to the sample test tubes, 100μl of water was added to the blank test tube and 100μl of standard was added to the standard test tube. 1ml of the Reagent

was added to the sample test tubes, blank test tube and standard test tube, which the solution was mixed, and the absorbance of the samples was read against the blank at 30 seconds (A_1) and 2 minutes (A_2) intervals using UV-Visible spectroscopy at 492nm wavelength.

Calculation:

$A_2 - A_1 = \Delta A\text{sample or } \Delta A\text{standard}$

$$\text{Concentration of creatinine in the sample (mg/dl)} = \frac{\Delta A\text{sample} \times \text{concentration of standard}}{\Delta A\text{standard}}$$

Concentration of the standard = 0.158mg/dl

Asample = Absorbance of the test sample

Astandard = Absorbance of the standard

3.6.2.3 Serum Uric Acid Concentration

Serum uric acid concentration was determined according to the method described by Tietz (1995).

Principle:

Uric acid is converted by uricase to allantoin and hydrogen peroxide which under the catalytic influence of peroxidase, oxidizes 3,5 – Dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to red violet quinonimine compound.

Procedure:

0.5ml of working reagent was added to 0.01ml of the diluted serum sample. The standard was constituted by replacing the serum with 0.01ml of standard reagent. The blank was constituted by replacing the test sample with 0.01ml of distilled water. The resulting mixture was incubated at 37°C for 5minutes and the absorbance of the serum samples and the standard were read spectrophotometrically at wavelength of 520nm against reagent blank within 30 minutes.

Calculation:

Concentration of uric acid (mg/dl) = Absorbance of test × Concentration of standard

Absorbance of standard

Concentration of standard = 6.0mg/dl

3.6.2.4 Serum Blood Urea Nitrogen (Bun) – Creatinine Ratio

The serum BUN- Creatinine ratio was determined using the method described by Tietz (2006). It is mathematically as:

BUN-RATIO (mg/dl) = Serum urea

Serum creatinine

3.6.3 Determination of Enzyme Activity

3.6.3.1 Alkaline Phosphatase Activity

The method described by Wright et al (1972) was used for the determination of alkaline phosphatase activity.

Principle:

The amount of phosphate ester split within a given period is a measure of the phosphatase enzyme. Para- nitrophenyl phosphate was hydrolyzed to para- nitrophenol and phosphoric acid at a pH of 10.1. The para-nitrophenol confers a yellowish colour on reaction.

Procedure:

20µl of enzyme source (serum) was added to the sample test tubes and 20µl of water was added to the blank test tube. 1ml of Reagent 1 was added to the sample test tubes and blank test tube, which the solution was mixed, and the absorbance of the samples was read against the blank at 1minute (A_1), 2 minutes (A_2) and 3 minutes (A_3) intervals using UV-Visible spectroscopy at 405nm wavelength.

Calculation:

To calculate ALP activity:

U/I = 2742 × ΔA 405 nm/min

ΔAbs/min = Change in Absorbance per minute

U/I = activity of enzyme

3.6.3.2 Aspartate Aminotransferase Activity

The method described by Reitman and Frankel (1957) was used for assaying the activity of aspartate aminotransferase

Principle:

The enzyme catalyzes the reversible reaction involving α-ketoglutarate and L-aspartate to form L-glutamate and oxaloacetate. The activity of aspartate aminotransferase was determined by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl hydrazine at 546nm

Procedure:

50µl of the enzyme source (serum/liver) was added to the samples test tubes and 50µl of water was added to the blank test tube. 250µl of Reagent 1 was added to the sample test tubes and blank test tube which the solution was mixed and incubated in the hot-air oven for 30 minutes at 37⁰C. 250µl of Reagent 2 was added to both samples test tubes and blank test tube, which the solution was allowed to stand for exactly 20 minutes at room temperature (25⁰C). 2.5ml of Reagent 3 was also added to both samples test tubes and blank test tubes, which the solution was mixed, and the absorbance of the samples was read against the blank using UV-Visible spectroscopy at 546nm wavelength.

3.6.3.3 Alanine Aminotransferase Activity

The method described by Reitman and Frankel (1957) was used for assaying the activity of alanine aminotransferase.

Principle:

Alanine aminotransferase activity was determined by monitoring the concentration of pyruvate hydrazone formed with 2,4- dinitrophenyl hydrazine.

ȧ-oxoglutarate + L-alanine ——> L-glutamate + pyruvate

Reagents

Reagent 1: Buffer (Phosphate buffer, L-alanine, ȧ-oxoglutarate)

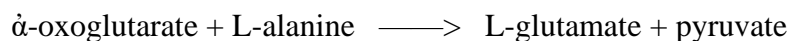Reagent 2: 2,4-dinitrophenylhydrazine (DNP)

Reagent 3: 0.4N Sodium Hydroxide

Procedure:

50μl of the enzyme source (serum/liver) was added to the samples test tubes and 50μl of water was added to the blank test tube. 250μl of Reagent 1 was added to the sample test tubes and blank test tube which the solution was mixed and incubated in the hot-air oven for 30 minutes at 37⁰C. 250μl of Reagent 2 was added to both samples test tubes and blank test tube, which the solution was allowed to stand for exactly 20 minutes at room temperature (25⁰C). 2.5ml of Reagent 3 was also added to both samples test tubes and blank test tubes, which the solution was mixed, and the absorbance of the samples was read against the blank using UV-Visible spectroscopy at 546nm wavelength.

3.7 Determination Of The Percentage Organ-Body Weight Ratio

This method was by (Michael *et al.,* 2007). The percentage organ- body weight ratio was obtained using the following expression:

$$\text{Percentage organ-body weight ratio} = \frac{\text{Organ weight}}{\text{Total body weight of the animals}} \times 100$$

CHAPTER FOUR

4.0 Results

Chemical constituents of ethanolic extract of *O. gratissimum leaves*

4.1 Phytochemical Analysis

The result in Table 2 reveals the qualitative analysis carried out on the ethanolic extract of *Ocimum gratissimum* indicated the presence of tannins, saponins, flavonoids, alkaloids, quinones, phenols, terpenoids, cardiac glycosides, coumarins while carbohydrates, glycosides, ninhydrin, anthraquinones, steroids, Phlobatannins, anthracyanin were not detected (Table 2).

Table 2: Secondary metabolites of ethanolic extract of *Ocimum gratissimum*

SECONDARY METABOLITES	RESULTS
Carbohydrates	--
Tannins	++
Saponins	++
Flavonoids	++
Alkaloids	++
Glycosides	--
Quinones	++
Phenols	++
Terpenoids	++
Cardiac Glycosides	++
Ninhydrin	--
Coumarins	++
Anthraquinones	--
Steroids	--
Phlobatannins	--
Anthracyanine	--

++ indicates present

-- indicates absent

4.2 UV- Spectroscopy Analysis of Ethanolic Extract of *O. gratissimum*

The UV- spectroscopy analysis of the ethanolic extract of *Ocimum gratissimum* leaves revealed varying absorbance of the plant extract at various wavelengths. The EEOGL reached its highest absorbance of 2.500 at a wavelength 245nm and the lowest absorbance of 1.648 at a wavelength of 205nm.
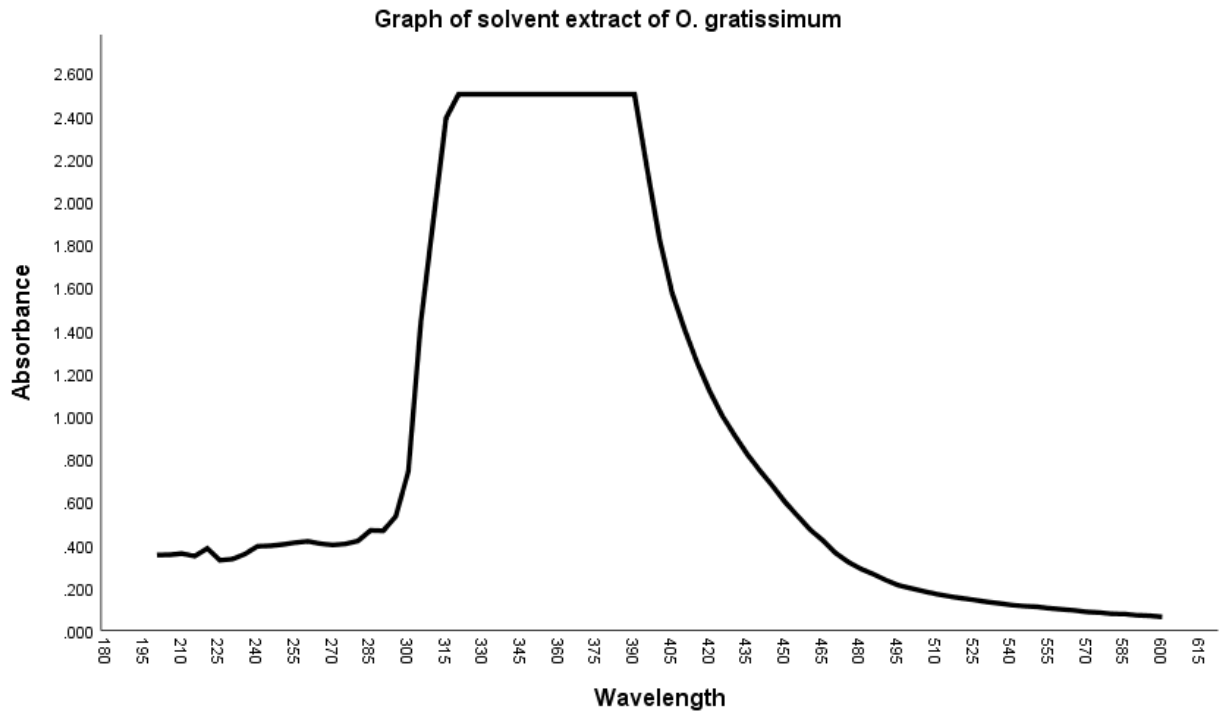
Figure 5: UV- Spectroscopy of ethanolic extract of *O. gratissimum* plant

4.3 Gas Chromatography – Mass Spectrometry (GC-MS) Analysis

4.3.1 Chromatogram of ethanolic extract of *Ocimum gratissimum* leaves

The chromatogram showing below reveals the phtyochemical components present in *O. gratissimum* leaves indicating Ethanone, 1-(6,6-dimethylbicyclo [3.1.0] hex-2-en-2-yl)- as the highest peak with the retention time of 11.582 while the other peaks were of other phyto-components present in the plant.
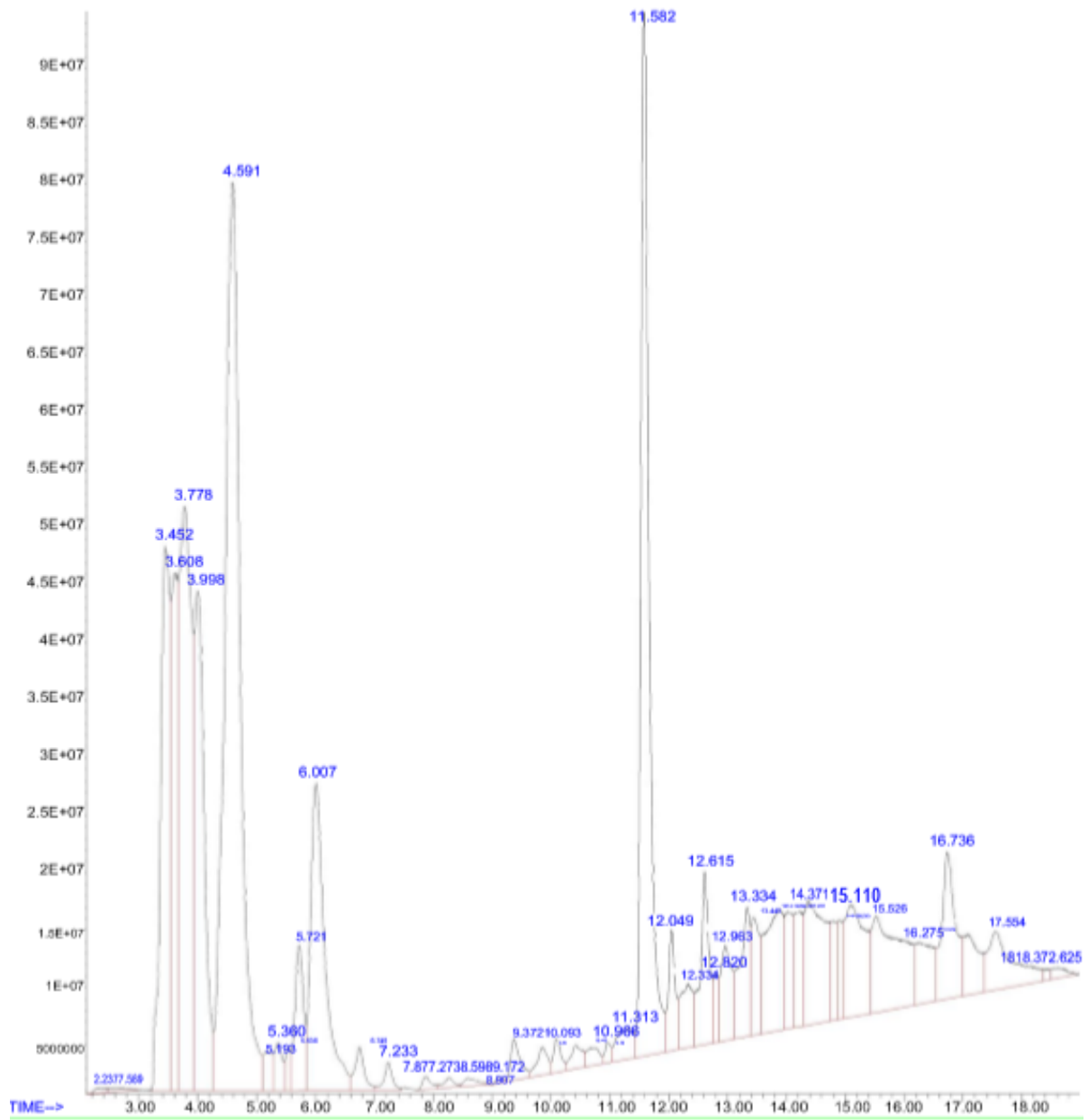
Figure 6: GC-MS chromatogram of ethanolic extract of *Ocimum gratissimum* leaves

Other Phyto-components of ethanolic extract of *O. gratissimum* leaves other than Ethanone, 1-(6,6-dimethylbicyclo [3.1.0] hex-2-en-2-yl)- were identified. The phytochemical components identified in the ethanolic extract of *O. gratissimum* leaves by GC-MS showing their peak, retention time, area %, name of compound, % of the total and the chemical formula is shown in (table 3).

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

S/N	Retention time	Area %	Name of Compound	% Of total	Chemical formular
1	2.2377	0.07	Glycerol triethyl ether	0.071%	$C_9H_{20}O_3$
2	3.452	6.50	D-Fucose	6.499%	$C_6H_{12}O_5$
3	3.608	4.22	Cyclopentanol, 3-methyl-	4.220%	$C_6H_{12}O$
4	3.778	9.08	2-Hexene, 5-methyl-, (E)-	9.083%	C_7H_{14}
5	3.998	6.30	Ethane, diazo-	6.301%	$C_4H_8N_2O_2$
6	4.591	18.03	Cyclopropane, 1-methyl-2-(3-methyl pentyl)-	18.030%	$C_{10}H_{20}$
7	5.193	0.45	D-Gulopyranose	0.453%	$C_6H_{12}O_6$
8	5.360	0.49	1-Butanol, 3-methyl-, acetate	0.490%	$C_7H_{14}O_2$
9	5.721	1.60	Peroxide, dimethyl	1.600%	$C_2H_6O_2$
10	6.007	5.62	Propanenitrile, 2-hydroxy-	5.617%	C_3H_5NO
11	7.233	0.30	Hexanoic acid, ethyl ester	0.296%	$C_8H_{16}O_2$
12	7.877	0.14	O-Cymene	0.143%	$C_{10}H_{14}$
13	8.273	0.13	Alpha, - Phellandrene	0.134%	$C_{10}H_{16}$
14	8.598	0.14	1-methyl-5-mercaptotetrazole	0.138%	$C_2H_4N_4S$
15	8.907	0.01	Benzene, 4-ethenyl-1,2-dimethyl-	0.015%	$C_{10}H_{12}$
16	9.172	0.05	2,5- Dimethylcyclohexanol	0.046%	$C_8H_{16}O$
17	9.372	0.50	2(1H)- Pyridinone, 6-hydroxy	0.497%	$C_6H_7NO_2$

18	10.093	0.33	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	0.331%	$C_{10}H_{18}O$
19	10.966	0.16	L-Proline, 1-acetyl-	0.157%	$C_7H_{11}NO_3$
20	11.313	0.65	Furan, 2,3,5-trimethyl-	0.648%	$C_7H_{10}O$
21	11.582	10.97	Ethanone, 1-(6,6-dimethylbicyclo [3.1.0] hex-2-en-2-yl)-	10.967%	$C_{10}H_{14}O$
22	12.049	1.07	Caryophyllene	1.072%	$C_{15}H_{24}$
23	12.334	0.97	Humulene	0.975%	$C_{15}H_{24}$
24	12.615	1.94	Naphthalene, decahydro-4a-methyl-1-methylene-7- (1-methylethenyl)-, [4aR- (4a. alpha., 7. alpha.,8a. beta.)]	1.936%	$C_{15}H_{24}$
25	12.820	0.44	Phenol, 3,5-dimethyl-	0.440%	$C_8H_{10}O$
26	12.963	1.33	Silane, trimethyl(3-methylphenoxy)	1.326%	$C_{10}H_{16}OSi$
27	13.334	1.69	Caryophyllene oxide	1.695%	$C_{15}H_{24}0$
28	14.371	3.28	Phthalic acid, 1-adamantylmethyl ethyl ester	3.276%	$C_{21}H_{26}O_4$
29	15.110	2.88	Oxazole, 2-hexyl-4,5-dimethyl-	2.881%	$C_{11}H_{19}NO$
30	15.526	3.64	Hexadecanoic acid, ethyl ester	3.644%	$C_{18}H_{36}O_2$
31	16.275	1.37	3-(Adamantan-1-ylamino)-2-(2,4-dichloro-5-fluorobenzoyl) acrylic acid, ethyl ester	1.373%	$C_{22}H_{24}Cl_2NO_3$
32	16.736	2.56	Phytol	2.559%	$C_{20}H_{40}O$
33	17.554	1.88	Phytol, acetate	1.883%	$C_{22}H_{42}O_2$
34	18.372	0.09	. beta. -Alanine, N-neopentyloxycarbonyl-, neopentyl ester	0.093%	$C_{17}H_{32}N_2O_5$
35	18.625	0.22	Phenol, 2- (4-diethylaminophenyliminomethyl) -6-methoxy-	0.221%	$C_{18}H_{22}N_2O_2$

4.4 Percentage relative organ-body weight ratio of Letrozole-induced rats administered ethanolic extract of *O. gratissimum*

The result obtained from the liver-body weight ratio (table 4) of letrozole-induced PCOS in rats orally administered distilled water and 100mg/kg body weight of the extract was decreased significantly ($p<0.05$) compared to that of the control animals while the organ body ratio of liver of letrozole-induced PCOS in female rats orally administered 50mg/kg body weight of the extract, and clomiphene citrate plus metformin (reference drugs) was increased significantly ($p<0.05$) compared to that of the control animals.

The kidney-body weight ratio of letrozole-induced PCOS in female rats orally administered distilled water, clomiphene citrate plus metformin, 50mg/kg body weight of the extract and 100mg/kg body weight of the extract was increased significantly ($p<0.05$) compared to that of the control animals.

The ovaries-body weight ratio of letrozole-induced PCOS in female rats orally administered distilled water, clomiphene citrate plus metformin, 50mg/kg body weight of the extract and 100mg/kg body weight of the extract was decreased significantly ($p<0.05$) compared to that of the control animals.

Table 4: Effect of ethanolic extract of *O. gratissimum* leaves on some organ- body weight ratio of letrozole-induced PCOS in female rats.

	Organ-body weight ratio (%)		
	Liver	**Kidney**	**Ovary**
Control	2.67 ± 0.02^a	0.47 ± 0.02^a	0.50 ± 0.00^a
PCOS + distilled water	2.63 ± 0.06^a	0.50 ± 0.02^b	0.09 ± 0.01^b
PCOS + Met+ CC	3.30 ± 0.09^b	0.57 ± 0.02^b	0.07 ± 0.01^b
PCOS + 50mg/kg b.w. of EEOGL	2.84 ± 0.09^a	0.50 ± 0.02^b	0.05 ± 0.02^a
PCOS + 100mg/kg b.w. of EEOGL	2.64 ± 0.16^a	0.53 ± 0.53^b	0.07 ± 0.00^b

Data are means of four determinations $\pm$ SEM. Values with different superscripts are significantly different (P< 0.05).

4.5 Biochemical Assessments

4.5.1 Liver Function Indices and PCOS

4.5.1.1 Effect of ethanolic extract of *Ocimum gratissimum* leaves on some liver function indices of Letrozole-induced female rats.

The result obtained from albumin concentration (table 5) increased significantly ($p<0.05$) in letrozole-induced PCOS in female rats orally administered distilled water, clomiphene citrate and metformin, 50mg/kg body weight of the extract and 100mg/kg body weight of the extract was increased significantly ($p<0.05$) compared to that of the control animals. The serum albumin levels of Letrozole-induced rats administered distilled water compared favorably ($p>0.05$).

Globulin concentration decreased significantly ($p<0.05$) in Letrozole-induced rats orally administered distilled water while the globulin concentration in Letrozole-induced rats orally administered clomiphene citrate and metformin, 50mg/kg body weight of the extract and 100mg/kg body weight of the extract was increased significantly ($p<0.05$) compared to that of the control animals. The serum globulin levels of Letrozole-induced female rats administered distilled water compared favorably ($p>0.05$).

Total protein concentration decreased significantly ($p<0.05$) in Letrozole-induced rats orally administered clomiphene citrate and metformin while the total protein concentration in Letrozole-induced rats orally administered distilled water, 50mg/kg body weight of the extract and 100mg/kg body weight of the extract was increased significantly ($p<0.05$) compared to that of the control animals.

Direct bilirubin concentration decreased significantly ($p<0.05$) in Letrozole-induced rats orally administered distilled water, 50mg/kg body weight of the extract and 100mg/kg body weight of the extract while the direct bilirubin concentration increased significantly ($p<0.05$) in Letrozole-induced rats orally administered clomiphene citrate and metformin compared to that of the control animals.

Total bilirubin concentration increased significantly ($p<0.05$) in Letrozole-induced rats orally administered distilled water and 100mg/kg body weight of the extract while total bilirubin concentration decreased significantly ($p<0.05$) in Letrozole-induced rats orally administered

clomiphene citrate and metformin and 50mg/kg body weight of the extract compared to that of the control animals.

Albumin-globulin ratio concentration increased significantly ($p < 0.05$) in Letrozole-induced rats orally administered distilled water while albumin-globulin ratio concentration decreased significantly in Letrozole-induced rats orally administered clomiphene citrate and metformin, 50mg/kg body weight of the extract and 100mg/kg body weight of the extract was increased significantly ($p < 0.05$) compared to that of the control animals.

Table 5: Effect of ethanolic extract of *O. gratissimum* leaves on liver function indices in Letrozole- induced female rats

Groups	Albumin (mg/dL)	Globulin (mg/dL)	Total Protein (mg/dL)	Direct Bilirubin (mg/dL)	Total Bilirubin (mg/dL)	Albumin - Globulin ratio (mg/dL)
Control	6.81 ± 0.69^a	5.60 ± 1.01^a	1.22 ± 0.33^a	5.01 ± 0.82^a	1.56 ± 0.55^a	1.28 ± 0.11^a
PCOS+ distil. H_2O	6.84 ± 0.08^a	5.07 ± 0.15^a	1.77 ± 0.23^a	1.29 ± 0.68^c	2.28 ± 0.28^a	1.36 ± 0.05^a
PCOS + Met+ CC	7.12 ± 0.12^b	6.97 ± 0.11^a	0.16 ± 0.20^b	8.56 ± 0.52^b	0.31 ± 0.16^a	1.02 ± 0.00^b
PCOS + 50mg/kg b.w. of EEOGL	7.50 ± 0.44^a	6.04 ± 0.43^a	1.47 ± 0.01^a	4.35 ± 0.58^a	1.19 ± 0.48^a	1.25 ± 0.01^a
PCOS + 100mg/kg b.w. of EEOGL	8.03 ± 0.41^a	6.75 ± 0.89^a	1.28 ± 0.47^a	0.47 ± 0.12^c	6.74 ± 2.37^b	1.23 ± 0.10^a

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

4.5.2 Kidney Function Indices and PCOS

4.4.2.1 Effect of ethanolic extract of *Ocimum gratissimum* leaves on some kidney function indices of Letrozole-induced female rats.

The result obtained from creatinine concentration (table 6) increased significantly ($p<0.05$) in Letrozole-induced rats orally administered distilled water, clomiphene citrate and metformin, 50mg/kg body weight of the extract while creatinine concentration decreased significantly ($p<0.05$) in Letrozole-induced rats orally administered 100mg/kg body weight of the extract compared to that of the control animals.

Urea concentration decreased significantly ($p<0.05$) in Letrozole-induced rats orally administered distilled water while urea concentration increased significantly ($p<0.05$) in clomiphene citrate and metformin, 50mg/kg body weight of the extract and 100mg/kg body weight of the extract compared to that of the control animals.

Uric acid concentration increased significantly ($p<0.05$) in Letrozole-induced rats orally administered distilled water, clomiphene citrate and metformin, 50mg/kg body weight of the extract and 100mg/kg body weight of the extract compared to that of the control animals.

Blood urea nitrogen-creatinine ratio concentration decreased significantly ($p<0.05$) in distilled water while blood urea nitrogen-creatinine ratio concentration increased significantly ($p<0.05$) in clomiphene citrate and metformin, 50mg/kg body weight of the extract and 100mg/kg body weight of the extract compared to that of the control animals.

Table 6: Effects of administration of ethanolic extract of *Ocimum gratissimum* leaves on some kidney function indices of Letrozole-induced female rats

Groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Blood urea nitrogen ratio (mg/dl)
Control	0.34 ± 0.38^a	1.05 ± 0.26^a	0.64 ± 0.00^a	2.98 ± 0.44^a
PCOS+ distil. H_2O	0.53 ± 0.00^b	0.89 ± 0.00^a	2.27 ± 0.00^b	1.68 ± 0.00^a
PCOS + Met +CC	0.50 ± 0.00^b	3.27 ± 0.00^c	3.34 ± 0.00^c	6.54 ± 0.00^a
PCOS + 50mg/kg b.w. of EEOGL	0.80 ± 0.01^c	2.53 ± 0.00^b	9.39 ± 0.00^d	33.74 ± 4.87^b
PCOS + 100mg/kg b.w. of EEOGL	0.22 ± 0.00^d	6.12 ± 0.34^d	20.61 ± 0.00^e	28.48 ± 1.98^b

Data are means of four determinations $\pm$ SEM. Values with different superscripts for each group are significantly different ($P<0.05$).

4.5.3 Enzyme Assay

4.5.3.1 Alkaline phosphatase activity in serum, liver of Letrozole-induced rats following oral administration of ethanolic extract of *Ocimum gratissimum* leaves

The result obtained from alkaline phosphatase activity in the liver (figure 7) decreased significantly ($p<0.05$) in Letrozole-induced rats orally administered distilled water, clomiphene citrate and metformin, 50mg/kg per body weight of the extract and 100mg/kg per body weight of the extract compared to that of the control animals.

Alkaline phosphatase activity in the serum increased significantly ($p<0.05$) in Letrozole-induced rats orally administered distilled water, clomiphene citrate and metformin, 50mg/kg per body weight of the extract and 100mg/kg per body weight of the extract compared to that of the control animals.

Figure 7: ALP activity in serum and liver of Letrozole-induced rats following oral administration of ethanolic extract of *O. gratissimum* leaves

4.5.3.2 Alanine aminotransferase activity of the serum and liver of Letrozole-induced female rats after oral administration of ethanolic extract of *Ocimum gratissimum* leaves

The result obtained for alanine aminotransferase activity in the serum (figure 8) decreased significantly ($p < 0.05$) in Letrozole-induced rats orally administered clomiphene citrate and metformin and 50mg/kg per body weight of the extract while alanine aminotransferase activity in the serum increased significantly ($p < 0.05$) in Letrozole-induced rats orally administered distilled water and 100mg/kg per body weight of the extract compared to that of the control animals

Alanine aminotransferase activity in the liver increased significantly ($p < 0.05$) in Letrozole-induced rats orally administered distilled water, clomiphene citrate and metformin and 50mg/kg per body weight of the extract while alanine aminotransferase activity in the liver decreased significantly ($p < 0.05$) in Letrozole-induced rats orally administered 100mg/kg per body weight of the extract compared to that of the control animals.

Figure 8: ALT activity in the serum and liver of Letrozole-induced female rats after oral administration of ethanolic extract of *Ocimum gratissimum* leaves

4.5.3.3 Aspartate aminotransferase activity of the liver and serum of Letrozole-induced female rats following oral administration of ethanolic extract of *Ocimum gratissimum* leaves

The result obtained from aspartate aminotransferase activity in the liver (figure 9) decreased significantly ($p<0.05$) in Letrozole-induced rats orally administered distilled water, clomiphene citrate and metformin, 50mg/kg per body weight of the extract and 100mg/kg per body weight of the extract compared to that of the control animals.
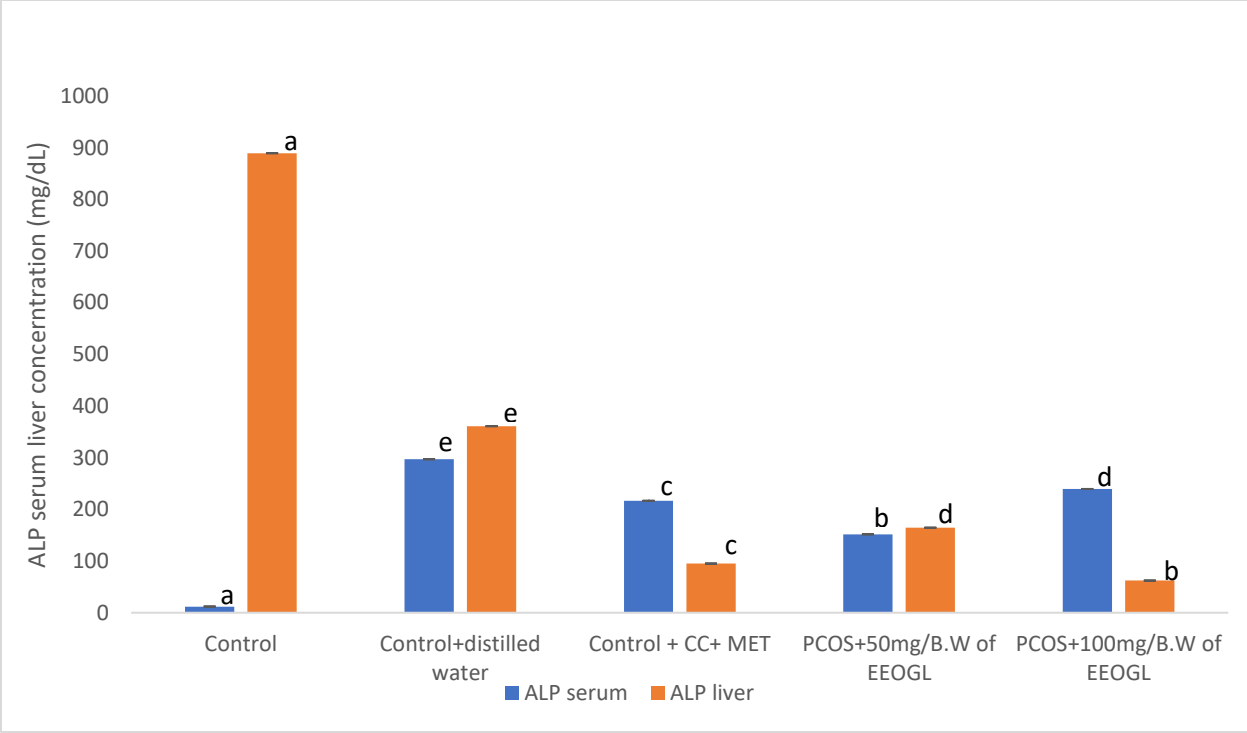
Aspartate aminotransferase activity in the serum decreased significantly ($p<0.05$) in PCOS-induced rats orally administered distilled water, clomiphene citrate and metformin, 50mg/kg per body weight of the extract while aspartate aminotransferase activity in the serum increased significantly ($p<0.05$) in PCOS- induced rats orally administered 100mg/kg per body weight of the extract compared to that of the control animals.
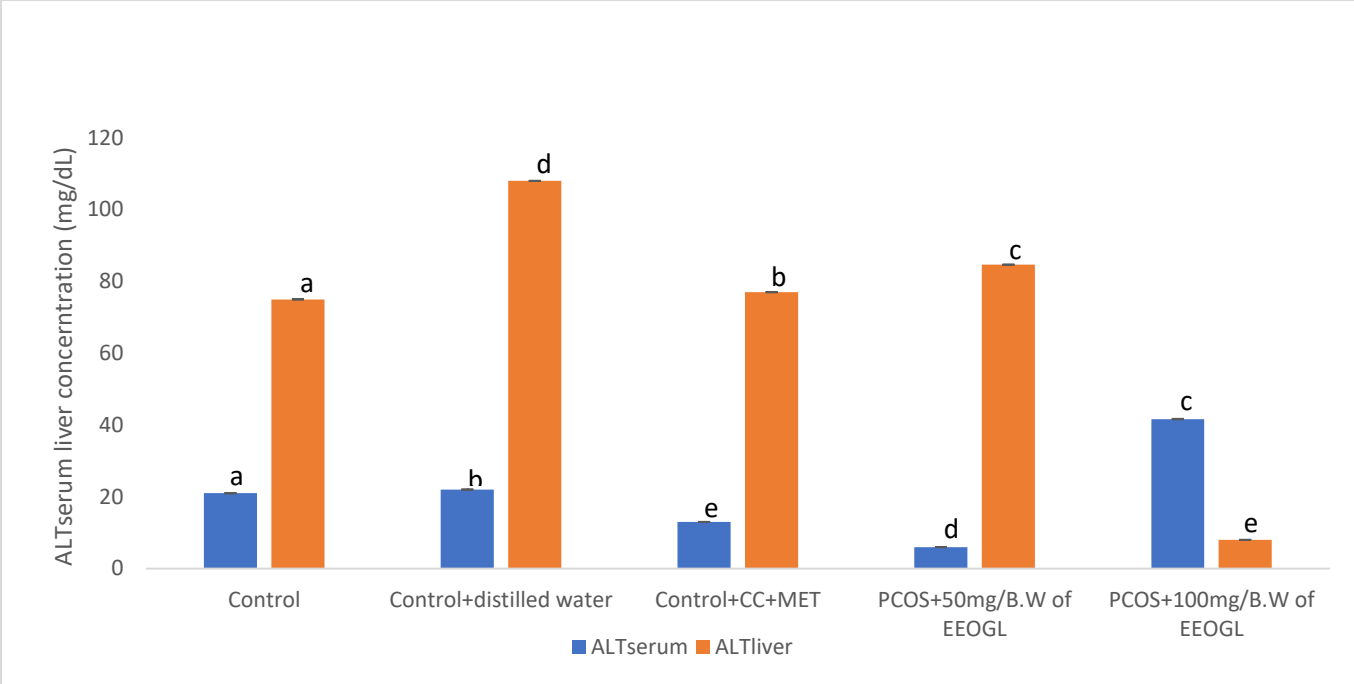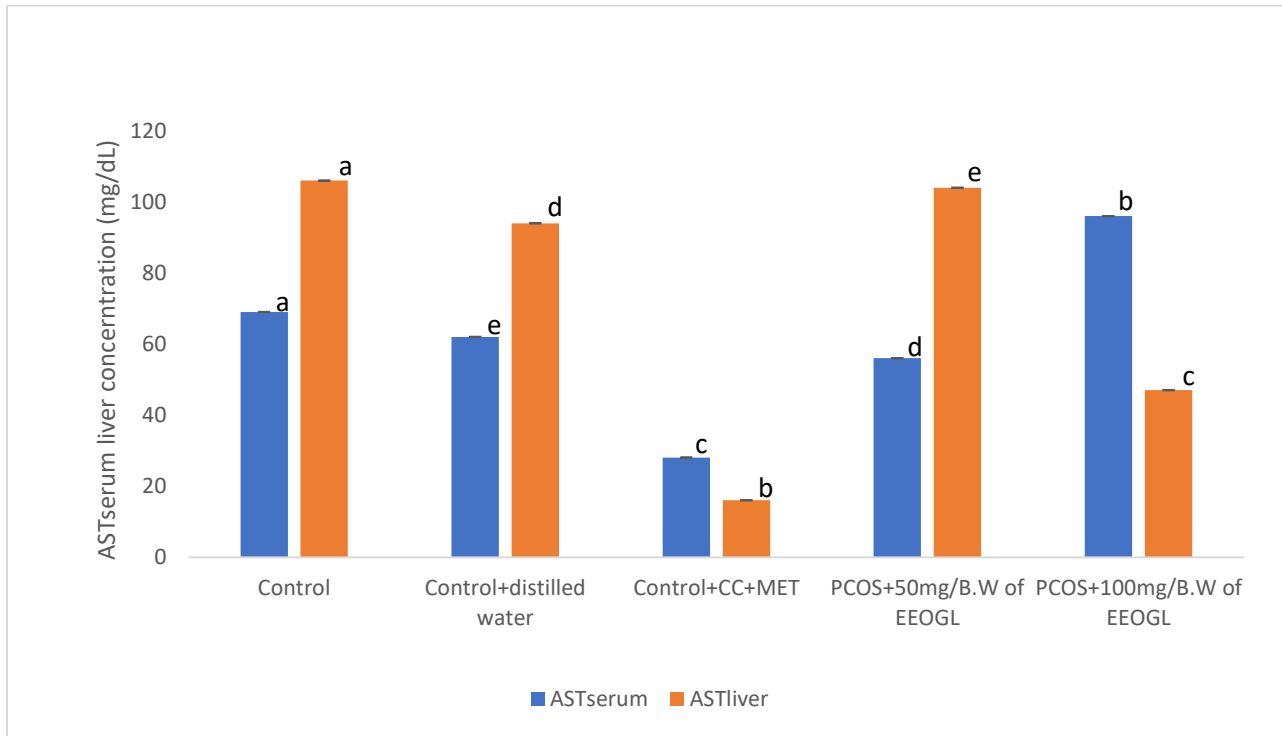
Figure 9: AST activity in serum and liver of Letrozole-induced female rats following oral administration of ethanolic extract of *Ocimum gratissimum* leaves

CHAPTER FIVE

5.0 Discussion

Polycystic Ovarian Syndrome (PCOS) or Stein- Leventhal syndrome is a metabolic endocrine disorder that is very common in women of reproductive age affecting approximately 2-10% of them (Moran *et al.,* 2010). It is a highly prevalent metabolic disorder with several consequences in female health including alarming rate of infertility (Rojas *et al.*, 2014). Medicinal plants, contain bioactive chemicals that differ in kind and concentration both within and across species. Traditional medicine has explored these pharmacological active principles for the treatment of various diseases. Plants are potentially hazardous due to their chemical contents: as a result, certain plants employed in traditional medicine are fundamentally harmful (Merlin *et al.,* 2019).

The study investigated the toxicological effects of *Ocimum gratissimum* leaves on letrozole-induced polycystic ovarian syndrome in female rats. Letrozole which is a non-steroidal aromatase inhibitor was used to induce PCOS orally (Cohen *et al.,* 2002). The toxicological evaluation of *Ocimum gratissimum* leaves on letrozole-induced polycystic ovarian syndrome in female wistar rats has provided additional information of its effects on the liver and kidney and organ body weight ratio of the animals.

The importance of assessing the total protein levels, levels of albumin, globulin, and bilirubin (Total and direct) in the serum of animals together with the administration of chemical compounds such as the ethanolic extract of *Ocimum gratissimum* cannot be overemphasized as they are useful criteria for evaluating not only the secretory ability and or functional capacity of the liver (Femi-olabisi *et al.,* 2020).

The detection of some phytochemicals revealed the potential therapeutic properties in the ethanolic extract of *Ocimum gratissimum* leaves. The phytochemical screening displayed tannins, saponins, flavonoids, alkaloids, quinones, phenols, terpenoids, cardiac glycosides and coumarins as positive indicating that those components exist in the ethanolic extract of *Ocimum gratissimum* leaves. Carbohydrates, glycosides, ninhydrin, anthraquinones, steroids, Phlobatannins, anthracyanin were negative indicating their absence in the ethanolic extract of *Ocimum gratissimum* leaves.

In the UV-spectroscopy analysis, it was discovered that the leaf extract had its peak absorbance near the 245nm and 285nm. The absorbance of the leaf extract was highest at round 245nm which then decreased significantly until it reached the 285nm region then peaked again. Then a stable absorbance occurred between 295nm till 600nm. Absorbance varied significantly up until 295nm region.

The GC-MS analysis identified several compounds present in the ethanolic extract of *Ocimum gratissimum* with Ethanone, 1-(6,6-dimethylbicyclo [3.1.0] hex-2-en-2-yl)- as the major phytocomponent of *Ocimum gratissimum* having the highest peak in the GC-MS chromatogram. Compounds like Cyclopropane, 1-methyl-2-(3-methyl pentyl)- is an organic component present in *Ocimum gratissimum* leaves having the highest percentage (% of total).

Benzene, 4-ethenyl-1,2-dimethyl is an organic component present in *Ocimum gratissimum* with the lowest peak having a retention time of 0.01.

Albumin, globulin, and total bilirubin which make up the cell's protein content can be used to analyze the liver's functional capacity (Femi-olabisi *et al.,* 2020). Furthermore, changes in the concentration of albumin, globulin, and bilirubin in animal serum indicates the liver's synthetic and secretory functions as well as the type of liver damage (Femi-olabisi *et al.,* 2020). The reduction in the level of computed albumin-globulin ratio by letrozole in this present study may be attributed to letrozole diminishing the biosynthesis of the albumin-globulin ratio and it may reflect overproduction of globulins, such as seen. The unaltered total protein level by letrozole was also maintained by all the doses of the ethanolic extract which suggests normal functioning of the liver in relation to total bilirubin (Femi-olabisi *et al.,* 2020).

Effects of ethanolic extract of *Ocimum gratissimum* on serum total protein, albumin, globulin, and albumin: globulin ratio was examined in this study. From the result obtained, there was a significant increase in albumin and globulin levels of letrozole-induced animals administered orally 50 and 100mg/kg body weight extract ($P<0.05$). In contrary to Adeleke et al., (2013) there was a decrease in the serum albumin compared to the control animals. The elevated albumin level by letrozole was further aggravated by the administration of the ethanolic extract suggesting a possible synergistic action between the drug and the extract in this study.

The kidney is known for its ability to remove metabolic waste, maintain balance at optimum pH and maintain chemical balance in the blood (Taubert *et al.,* 2007). Creatinine urea and uric acid are considered to be metabolic waste thus high level of this in blood indicates kidney dysfunctions, damage, or diseases (Shafee *et al.,* 2013). In this study, there are elevated level of creatinine, urea, uric acid as well as BUN- creatinine ratio concentration in Letrozole-induced rats orally administered 50mg/kg per body weight of the ethanolic extract and 100mg/kg per body weight of the ethanolic extract when compared with the control animals indicating that the doses have an increase effect of letrozole on the function of the kidney.

AST enzyme is found mostly in the liver, but also in muscles. If liver damage occurs, it releases AST into your bloodstream. Dose at 50mg/kg showed a reduced level in the concentration reversing the effect of letrozole which causes a spike in the level of AST concentration showing that both deses poses no damage and even reverses any damage caused while dose at 100mg/kg showed an increased level in AST concentration increasing the effect of letrozole.

 ALP enzyme helps to denote the state of the plasma membrane and an increase in the level of this enzyme denotes damage done to the plasma membrane (Femi-olabisi *et al.,* 2020). Administration of letrozole shows a diminished level of ALP activities in the liver and after administration of 50mg/kg and 100mg/kg of the ethanolic extract there was a noticed decrease in the level of the ALP activity in the liver and serum showing that the extract inhibits the action of ALP activity similar to that of letrozole at the cellular molecular level (Akanji *et al.,* 1993).

ALT enzyme denotes the metabolic conditions of the hepatocytes, and a high level of this enzyme shows damages done to the hepatocytes. ALT levels were increased significantly after administration of letrozole in the serum and liver showing a positive effect while there was an increase in the level of ALT activity in the liver and a decrease in the serum after the administration of 50mg/kg of the ethanolic extract of the extract while there was a decrease in ALT activity in the liver and an increase in animals administered 100mg/kg of the ethanolic extract.

5.1 Conclusion

In conclusion, the toxicological evaluation of the ethanolic extract of *Ocimum gratissimum* leaves reveals that both doses of 50mg/kg and 100mg/kg per body weight of the extract causes a significant increase or decrease ($p < 0.05$) in the biochemical assessments (liver function indices, kidney function indices) and also percentage organ-body weight when compared to the control animals without causing any obvious toxic effect on the animals. Although they were changes in the size of the studied organs (liver, kidney), mild alterations were noticed in the Letrozole-induced female rats when compared to the control animals.

5.2 Recommendation

The result on this study reveals that further studies should be carried on the toxicological effect of *Ocimum gratissimum* leaves on Letrozole-induced female rats of which the long-term effect should be thoroughly investigated.

REFERENCES

Agarwal, K., Varma R. Ocimum gratissimum L (2014). A Medicinal Plant with Promising Antiurolithiatic Activity. *Int J Pharma Sci Drug Res*; 6:78-81.

Ajiboye, A. A., Fadimu O. Y., Ajiboye M. D., Agboola D. A., Adelaja A. B., Bem A. A. (2014). Phytochemical and Nutritional Constituents of Some Common Vegetables in South-West, Nigeria. *Global J Sci Front Res: C Biological Sci*;14: p 1-7.

Akinmoladun, A. C., Ibukun E. O., Afor E., Obuotor E. M., Farombi E. O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. *Sci. Res. Essay.* 2:63-166.

American College of Obstetricians and Gynecologists. Polycystic ovary syndrome. Washington, DC: American College of Obstetricians and Gynecologists; (2009). ACOG practice bulletin; no. 108.

Ayurvedic Medicines for PCOS | PCOD- Poly Cystic Ovarian Syndrome & Disease. https://curaayurveda.wordpress.com. 12 April, (2014).

Azziz, R., Carmina E., DeWailly D., et al. (2006). Position statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. *J Clin Endocrinol Metab*.; 91:4237–4245.

Baldeon, M.E., Castro, J., Villacres, E., Narvaez, L., Fornasini, M. (2012). Hypoglycemic effect of cooked *Lupinus mutabilis* and its purified alkaloids in subjects with type-2 diabetes. *Nutricon. Hospitalaria,* 27(4), 1261-1266.

Baranova, A. *et al.* (2013). Molecular signature of adipose tissue in patients with both non-alcoholic fatty liver disease (NAFLD) and polycystic ovarian syndrome (PCOS). *J. Transl. Med.* **11**, 133.

Baranova, A., Tran, T. P., Birerdinc, A. and Younossi, Z. M. (2011). Systematic review: Association of polycystic ovary syndrome with metabolic syndrome and non-alcoholic fatty liver disease. *Aliment Pharmacol. Ther.* **33**, 801–814.

Barber T, M., McCarthy M, I., Wass J, A., Franks S. (2006). Obesity and polycystic ovary syndrome. *Clin Endocrinol* (Oxf). 65(2):137-45.

Bargiota, A., and D. Kandarakis, (2012) "The effects of old, new emerging medicines on metabolic aberrations in PCOS," *Therapeutic Advances in Endocrinology and Metabolism,* vol.3, no.1, pp.27– 47,

Bency Baby T, Smitha Rani, Remya K., Shebina Rasheed P., Azeem A, K. (2016). Polycystic ovarian syndrome: Therapeutic potential of herbal remedies- A review. *International Journal of Herbal Medicine;* 4(5):91-96.

Chandrasekaran C, V., Vijayalakshmi M, A., Prakash K, Bansal V, S. (2012). Meenakshi J, Amit A. Herbal Approach for Obesity Management Review Article. *American Journal of Plant Sciences.* 3(No.7A):1003-1014.

Cheang K, I., Huszar J, M., Best A, M., Sharma S, Essah P, A., Nestler J, E. (2009). Long-term effect of metformin on metabolic parameters in the polycystic ovary syndrome. *Diab Vasc Dis Res.* 6(2):110-9.

Chetia J, Upadhyaya S, Saikia L, R. (2014). Phytochemical analysis, antioxidant and antimicrobial activity and nutrient content analysis of Ocimum gratissimum linn. from Dibrugarh, N.E. India. *Int J Pharm Sci Rev Res*; 25:229-35.

Cohen, M.H., Johnson, J.R., Chen, N.L and Pazdur, R (2002) Approved summary: Letrozole in the treatment of postmenopausal women with advanced breast cancer. *Clin cancer Res;* 8(3):665-9. PMID: 11895893.

Cora M, C., Kooistra L & Travlos G. (2015). Vaginal Cytology of the Laboratory Rat and Mouse: Review and Criteria for the Staging of the Estrous Cycle Using Stained Vaginal Smears. *Toxicol Pathol*, 43, 776-93.

Costello, M., Shrestha, B., Eden, J., Sjoblom, P., and Johnson, N. (2007). Insulinsensitising drugs versus the combined oral contraceptive pill for hirsutism, acne and risk of diabetes, cardiovascular disease, and endometrial cancer in polycystic ovary syndrome. *Cochrane Database Syst. Rev*. CD005552. doi: 10.1002/14651858.CD005552.pub2

Cristiana M, Murbach F, M'arcia O, M., Mirtes C. (2006). Effects of seasonal variation on the central nervous system activity of Ocimum gratissimum (L) essential oil. *J Ethnopharmacol.* 105: 161-66

de Leo V, Musacchio M, C., Cappelli V, et al (2016). Genetic, hormonal, and metabolic aspects of PCOS: an update. *Reprod Biol Endocrinol.* 14(1):38. 10.1186/s12958-016-0173.

 Diamanti-Kandarakis E, Paterakis T, Alexandraki K, Piperi C, Aessopos A, Katsikis I, et al. (2006). Indices of low-grade chronic inflammation in polycystic ovary syndrome and the beneficial effect of metformin. *Hum Reprod.* 21(6):1426–1431. [PubMed] [Google Scholar].

Dunaif A, Wu X, Lee A, Diamanti-Kandarakis E (2001). Defects in insulin receptor signaling in vivo in the polycystic ovary syndrome (PCOS). *Am J Physiol Endocrinol Metab.* 281(2): E392-9.

Dunaif, A. (1999). Insulin action in the polycystic ovary syndrome. *Endocrinol. Metab. Clin. North Am.* 28, 341–359. doi: 10.1016/S0889-8529(05)70073-6.

E. Diamanti-Kandarakis and A. Dunaif, (2012). "Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications," *Endocrine Reviews*, vol. 33, no. 6, pp.981–1030,2012.

Edmund L., David J. (2006) Kidney function tests. In: Carl AB, Edward R, David E, editors. Tietz Textbook of clinical chemistry and molecular diagnostics. 4th ed. New Delhi: Elsevier Inc pp. 797–808. [Google Scholar].

Effraim K, D., Jacks T, W., Sodipo O, A. (2003). Histopathological studies on the toxicity of Ocimum gratissimum leaves extract on some on some organs of rabbit. *Afr J Biomed Res* 6:21-25.

Ehrmann, D.A. Barnes, R.B., Rosenfield, R.L. and Cavaghan, M.K, (1999). Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes Care*, 22, 141-146.

Fauser, B. C., Tarlatzis, B. C., Rebar, R. W., Legro, R. S., Balen, A. H., Lobo, R., et al. (2012). Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam

ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil. Steril*. 97, 28–38. e25. doi: 10.1016/j.fertnstert.2011.09.024.

Fauser, B.C., B. C. Tarlatzis, R. W. Rebar et al., (2012). "Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponred 3rd PCOS Consensus Workshop Group," *Fertility and Sterility,* vol.97, no.1, pp.28–38, 2012.

Femi-olabisi, F., Faokunla, O., Oluyemi, A.A and Moses, I (2020). Biochemical and toxicological evaluation of aqueous extract of *Parquentina nigrescens* (AFZEL.) leaves on mifepristone induced polycystic ovarian syndrome in rats. *Journal of drug and delivery and therapeutics.* 10(2):94-101. DOI: 10.22270/jddt. v10i2-s.3956.

G. M., Reaven, (2011). "Insulin resistance: the link between obesity and cardiovascular disease," *Medical Clinics of North America,* vol. 95, no.5, pp.875–892,2011.

G. Reaven (2008). "Insulin resistance and compensatory hyperinsulinemia: the linchpin between obesity and cardiovascular disease". *Official Journal of the Chair on Cardio metabolic Risk*, vol. 1, no.2, pp.3–10,2008.

Gonzales G. F., Cordova A, Vega K., Chung A, Villena A, Gonez C et al (2002). Effect of Lepidium meyenii (MACA) on sexual desire and its absent relationship with serum testosterone levels in adult healthy men. 34(6):36772.

Gonzalez F, Minium J, Rote N, S., Kirwan J, P. (2005). Hyperglycemia alters tumor necrosis factor-alpha release from mononuclear cells in women with polycystic ovary syndrome. *J Clin Endocrinol Metab.*90(9):5336–5342. [PubMed] [Google Scholar]

Gonzalez F, Rote N, S., Minium J, Kirwan J, P. (2006). In vitro evidence that hyperglycemia stimulates tumor necrosis factor- alpha release in obese women with polycystic ovary syndrome. *J Endocrinol.* 188(3):521–529. [PubMed] [Google Scholar]

Grusak, M. A. (2002). Enhancing mineral content in plant food product. *Journal of American college of nutrition,* 21(3), 178S-183S.

H. Teede, A. Deeks, and L. Moran (2015). "Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan," BMC Medicine, vol.8, article41,2010.

Hart, R., and Doherty, D, A. (2015). The potential implications of a PCOS diagnosis on a woman's long-term health using data linkage. J. *Clin. Endocrinol. Metab.* 100, 911–919. doi: 10.1210/jc.2014-3886.

Hart, R., and Norman, R. (2006). Polycystic ovarian syndrome–prognosis and outcomes. Best Pract. Res. *Clin. Obstet. Gynaecol*. 20, 751–778. doi: 10.1016/j.bpobgyn.04.006.

Hu WH, Qiao J, Zhao SY, Zhang XW, Li MZ (2006). Monocyte chemoattractant protein-1 and its correlation with lipoprotein in polycystic ovary syndrome. *Beijing Da Xue Bao.* 38(5):487–491. [PubMed] [Google Scholar].

Hubschmann, H.J. (2015). Handbook of GC-MS: Fundamental and Application (John Wiley and Sons).

I. Kaygusuz, O. F. Karatas, H. Kafali, E. Cimentepe, and D. Unal. (2013). "Is polycystic ovarian syndrome a risk factor for urolithiasis?" *Urolithiasis*, vol. 41, no. 4, pp. 361-362.

Ijeh II, Omodamiro O. D., Nwanna I. J. (2005) Antimicrobial effects of aqueous and ethanolic fractions of two spices, Ocimum gratissimum and Xylopia aethiopica. *Afr J Biotech* 4:953-56

Iweala E, E, J., Liu f, Cheng R, Li Y, Omonhinmin C, A., Zhang Y. (2015). Anticancer and free radical scavenging activity of some Nigerian food plants in vitro. *Int J Cancer Res;* 11:41-51.

J. A. Marcondes, C, R. Barcellos, and M.P. Rocha (2011). "Armadilhas e dificuldades no diagnʹostico da sʹındrome dos ovʹarios policʹısticos," Arquivos Brasileiros de Endocrinologia & Metabologia, vol.55, pp.6–15.

J. Miranda, V. Herrero, J. Chirinosetal., "Major cardiovascular risk factors in Latin America: a comparison with the United States: the Latin American consortium of studies in obesity (LASO)," PLoSONE, vol.8, no.1, ArticleIDe54056,2013.

Johnson T, KaplanL, Ouyang P, Rizza P. (2012). National Institutes of Health Evidence-Based Methodology Workshop on Polycystic Ovary Syndrome. NIH EbMW Reports. Bethesda, MD:

National Institutes of Health, ;1:1–14. Available at: https://prevention.nih.gov/sites/default/files/2018-06/FinalReport.pdf. Accessed 17 February 2019.

Jungbauer, Alois, Svjetlana Medjakovic. (2014). Phytoestrogens and the metabolic syndrome. The *Journal of steroid biochemistry and molecular biology*; 139:277-289.

Kabir O, A., Olukayode O., Chidi E, O., Christopher C, I., Kehinde A, F. (2005). Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant Staphylococcus aureus activity. BMC *Complement Altern Med* 5:1-7

Katulski, K., Czyzyk, A., Podfigurna-Stopa, A., Genazzani, A. R., and Meczekalski, B. (2015). Pregnancy complications in polycystic ovary syndrome patients. *Gynecol. Endocrinol.* 31, 87–91. doi: 10.3109/09513590.2014.974535.

Kumarendran, B. *et al.* (2018). Polycystic ovary syndrome, androgen excess, and the risk of nonalcoholic fatty liver disease in women: A longitudinal study based on a United Kingdom primary care database. *PLoS Med.* **15**, e1002542.

Lane, D., E. (2006). "Polycystic ovary syndrome and its differential diagnosis. *Obstetrical and Gynecological Survey,* vol.61, no.2, pp. 125–135.

Legro, R, S., Arslanian, S. A., Ehrmann, D. A., Hoeger, K. M., Murad, M. H., Pasquali, R., et al. (2013). Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *J. Clin. Endocrinol. Metab*. 98, 4565–4592. doi: 10.1210/jc.2013-2350.

Lerchbaum, E., Schwetz, V., Giuliani, A., and Obermayer-Pietsch, B. (2013). Assessment of glucose metabolism in polycystic ovary syndrome: HbA1c or fasting glucose compared with the oral glucose tolerance test as a screening method. Hum. Reprod. 28, 2537–2544. doi: 10.1093/humrep/det255.

Lidegaard, Ø., Løkkegaard, E., Jensen, A., Skovlund, C.W., and Keiding, N. (2012). Thromboticstrokeandmyocardialinfarctionwithhormonalcontraception. N. Engl. J. Med. 366, 2257–2266. doi: 10.1056/NEJMoa1111840.

Lizneva D., Suturina L., Walker W., Brakta S., Gavrilova-Jordan L., Azziz R. (2016). Criteria, prevalence, and phenotypes of polycystic ovary syndrome. *Fertil Steril*;106(1):6–15.

Lord J, M., Flight I, H., Norman R, J. (2003) Metformin in polycystic ovary syndrome: systematic review and meta-analysis. BMJ. 2003 Oct 25. 327(7421):951-3.

Macdonald I, O., Oludare A, S., Olabiyi A. (2010). Phytotoxic and Anti-microbial activities of Flavonoids in Ocimum gratissimum. *Life Sci J*; 7:45-48.

Mahapatra S, K., Chakraborty S, P., Das S., Roy S. (2009) Methanol extract of Ocimum gratissimum protects murine peritoneal macrophages from nicotine toxicity by decreasing free radical generation, lipid and protein damage and enhances antioxidant protection. *Oxi Med Cell Long*. 2:222-30

Mann Abdullahi (2012). Phytochemical Constituents and Antimicrobial and Grain Protectant Activities of Clove Basil (Ocimum gratissimum L.) Grown in Nigeria. *Int J Plant Res*; 2:51-58.

Marcondes F, K., Bianchi F, J., Tanno A, P. (2002) Determination of the estrous cycle phases of rats: some helpful considerations. *Braz. J. Biol*. 62: 609/614.

Matasyoh L, G., Josphat C, M., Francis N, W., Miriam G, K., Anne W, T, M., Titus K, M. (2007). Chemical composition and antimicrobial activity of the essential oil of Ocimum gratissimum L. growing in Eastern Kenya. *Afr J Biotech*. 6:760-65.

Merlin, L. K., Komalaga, G., Forkuo, D. A., Firempong, C., Anning, A. K and Dickson, A. R., (2019). Toxicity and safety implications of herbal medicines used in africa. *Intechopen* DOI: 10.5772/intechopen.72437.

Michael, B. *et al*. (2007). Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. *Toxicol Pathol* **35**, 742–750.

Miller W, Myers G, Ashwood E, et al. (2005). Creatinine measurement: state of the art in accuracy and interlaboratory harmonization. *Arch Pathol Lab Med*. 129(3):297–304. [PubMed] [Google Scholar]

Misso, M., Boyle, J., Norman, R., and Teede, H. (2014). Development of evidenced based guidelines for PCOS and implications for community health. *Semin. Reprod. Med*. 32, 230–240. doi: 10.1055/s-0034-1371095.

Nabholtz J, M. (2008). Long-term safety of aromatase inhibitors in the treatment of breast cancer. *Ther Clin Risk Manag*. Feb;4(1):189-204.

Nangia-Makker P et al. (2007). Inhibition of breast tumor growth and angiogenesis by a medicinal herb: Ocimum gratissimum. *Int J Cancer*; 121:884-94.

Nwanjo H, U., Oze G, O. (2007). Hypolipidemic and Antioxidant properties of Ocimum gratissimum on Diabetic rats. *Plant Prod Res J*; 11:1-4.

Nwankwo C, S., Ebenezer I, A., Ikpeama A, I., Asuzu F, O. (2014). The nutritional and anti–nutritional values of two culinary herbs–uziza leaf (Piper guineense) and scent leaf (Ocimum gratissium) popularly used in Nigeria. *Int J Sci Eng Res;* 5:1160-63.

Nweze E, I., Eze E. (2009). Justification for the use of Ocimum gratissimum L in herbal medicine and its interaction with disc antibiotics. *BMC Compl Alter Med*,9

Obaseiki-Ebor E, E., Odukoya K., Telikepalli H., Mitscher L, A., Shankel D, M. (2015). Antimutagenic activity of extracts of leaves of four common edible vegetable plants in Nigeria (West Africa). *Mut Res 1993*; 302:109-17

Offiah V, N., Chikwendu U, A. (1999). Antidiarrhoeal effects of Ocimum gratissimum leaf extract in experimental animals. *J Ethnopharma*; 68:327- 30.

Ohadoma S, C., Amazu L, U., Enye J, C., Okolo C, E. (2015). Comparative analysis of therapeutic benefits of Ocimum gratissimum and Vernonia amygdalina. *Eur J Pharma Med Res*; 2:29-32.

Okwu D, E. (2003) The potentials of Ocimum gratissimum, Penrgularia extensa and Tetrapleura tetraptera as spice and flavouring agents. *Niger Agric J*; 3:143-48.

Oluwafemi Adeleke, Omotade Oloyede, Sunday Onikanni (2013). Toxicity studies of the crude aqueous leaves extracts of Ocimum gratissimum in albino rats. Research gate.

Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S (2009). Agroforestree Database: A tree reference and selection guide version 4.0 (http://www.worldagroforestry.org/sites/treedbs/treed atabases.asp).

Pagana, Kathleen D. Mosby's Manual of Diagnostic and Laboratory Tests. St. Louis Mosby, Inc., 1998 and Rebecca J.F Gale Encyclopedia of Medicine. 2002 [Google Scholar].

Pandey S, Shukla A, Pandey S, Pandey A. (2017) An overview of resurrecting herb 'Sanjeevani' (Selaginella bryopteris) and its pharmacological and ethnomedicinal uses. *The Pharm Innov*; 6:1114.

Pandey S (2017). Antibacterial and antifungal activities of Ocimum gratissimum L. *Int J Pharm Pharma Sci*;9 (In press).

Panidis, D., D. Macut, K. Tziomalos et al., (2012). "Prevalence of metabolic syndrome in women with polycystic ovary syndrome" *Clinical Endocrinology*.

Pasquali, R., Stener-Victorin, E., Yildiz, B.O., Duleba, A.J., Hoeger, K., Mason, H., Homburg, R., Hickey, T., Franks, S., Tapanainen, J., Balen, A., Abbott, D.H., Diamanti-Kandarakis, E. and Legro, R.S. (2011). PCOS Forum: Research in Polycystic Ovary Syndrome Today and Tomorrow. *Clinical Endocrinology (Oxf)*; 74:424-433.

R. Legro (2012). "Obesity and PCOS: implications for diagnosis and treatment," Seminars in Reproductive Medicine, vol. 30, no. 6, pp.496–506.

R. Pasquali, A. Gambineri, and U. Pagotto (2006). "The impact of obesity on reproduction in women with polycystic ovary syndrome," BJOG: *An International Journal of Obstetrics and Gynaecology,* vol.113, no.10, pp.1148–1159.

Rabelo M, Souza E, P., Soares P, M, G. (2003). Antinociceptive properties of the essential oil of Ocimum gratissimum L. in mice. *Braz J Med Biol Res* 36:521-24.

Randeva, H, S., Tan B, K., Weickert M, O., Lois K, Nestler J, E., Sattar N, Lehnert H. (2012). Cardiometabolic aspects of the polycystic ovary syndrome. *Endocr Rev.*Oct;33(5):812-41.

Riedl, K. M. and Hagerman, A. E. (2001). Tannin-protein complexes as radical scavengers and radical sinks. *Journal of Agricultural Food Chemistry*, 49, 4917-4923.

Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and longterm health risks related to polycystic ovary syndrome. *Fertil Steril.* 2004; 81:19–25.

Royal College of Obstetricians and Gynaecologists. Long-term consequences of polycystic ovary syndrome. London, UK: Royal College of Obstetricians and Gynaecologists; 2007. Green-top guideline; no. 33

S. E. Kahn, R. L. Hull, and K. M. Utzschneider. (2006) "Mechanisms linking obesity to insulin resistance and type 2 diabetes," Nature, vol.444, no.7121, pp.840–846.

S. J. Nath, D. Sarma, P. K. Bagchi, S. K. Baruah, R. T. Puthenveetil, and S. J. Baruah (2013). "e role of serum testosterone as a lithogenic factor and its correlation with stone and urine composition amongst male stone formers," *Uro Today International Journal*, vol. 6, no. 3, p. 37. S. Mukherjee and A. Maitra (2010). "Molecular & genetic factors contributing to insulin resistance in polycystic ovary syndrome," *Indian Journal of Medical Research*, vol.131, no.6, pp.743–760,

S. Toprak, A. Y¨onem, B. Cakiretal. (2001). "Insulin resistance in non-obese patients with polycystic ovary syndrome" *Hormone Research,* vol.55, no.2, pp.65–70,2001.

Salmela, P.I., Sotaniemi, E. A., Niemi, M. and Maentausta, O., (2004). Liver Function tests in diabetic patients. *Diabetes Care*, 7: 248 – 254.

Santi D., Poti F., Simoni M., Casarini L. (2018). Pharmacogenetics of G-protein-coupled receptors variants: FSH receptor and infertility treatment. *Best Pract. Res. Clin. Endocrinol. Metab*; 32:189–200. doi: 10.1016/j.beem.2018.01.001.

Shanmagam, G., Ramaswamy, R.R., Radhakrishnan, V., and Tao H. (2010). GC-MS method for the determination of paraben preservative in the human breast cancerous tissue. *Microchemical journal.* 6, 391-396.

Shari Lieberman (2009). A Review of the Effectiveness of Cimicifuga racemosa (Black Cohosh) for the Symptoms of Menopause. *Journal of Women's Health.*7(5):525-529.

Singh A, K. (2012). Seedling morphology of four species of Ocimum L. (Lamiaceae) and its taxonomic significance. *Bangla J Plant Taxon*; 19:89-92

Spritzer, P. M., Motta, A. B., Sir-Petermann, T., and Diamanti-Kandarakis, E. (2015). Novel strategies in the management of polycystic ovary syndrome. *Minerva Endocrinol*. 40, 195–212.

Stepto, N.K., et al., (2013). Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemichyperinsulaemic clamp. *Hum Reprod,* 28(3): p. 777-84.

Swayamjeet Satapathy, Namrata Das, Debapriya Bandyopadhyay, Sushil Chandra Mahapatra, Dip Sundar Sahu, Mruthyumjayarao Meda (2016). Effect of Tulsi (Ocimum sanctum Linn.) Supplementation on Metabolic Parameters and Liver Enzymes in Young Overweight and Obese Subjects. *Ind J Clin Biochem.* DOI: 10.1007/s12291-016-0615-4.

Tori Hudson N, D. (2011). Maitake Mushroom Extract and Polycystic Ovarian Syndrome. *Natural Medicine Journal*. 3(2):2157-6769.

Vause T, D., Cheung A, P., Sierra S, et al. (2010). Ovulation induction in polycystic ovary syndrome. *J Obstet Gynaecol* Can. 32(5):495-502.

Ververidis, F., Trantas, E., Douglas, C., Vollmer, G., George, K., Nicklas, P. (2007). Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Paer 1: chemical diversity, impacts on plant biology and human health. *Biotechnology journal,* (10), 1214.

Vilioglu Y, S., Mazza G., Gao L., Oomah B, D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J Agric Food Chem*; 46:4113-17.

Wesam Kooti, Maryam Moradi, Sara Ali-Akbari, Naim Sharafi-Ahvazi, Majid Asadi-Samani, Damoon AshtaryLarky (2015). Therapeutic and pharmacological potential of Foeniculum vulgare Mill: A review. *J Herb Med Pharmacol.* 4(1):1-9.

Y.H. Shi, D.N. Zhao, J.L. Zhao et al., (2006). "Characteristicsofglucose metabolism in non-obese and obese women with polycystic ovarian syndrome," Zhonghua Fu Chan Ke Za Zhi, vol. 45, no. 8, pp.575–577,2010.

Yager JD, Davidson NE (2006). Estrogen carcinogenesis in breast cancer. *N Engl J Med* 354:270–282.

Yang, N.S., et al., (2009). Alanine aminotransferase isoenzymes: molecular cloning and quantitative analysis of tissue expression in rats and serum elevation in liver toxicity. *Hepatology*. 49(2), 598-607.

Yildiz B, O., Azziz R. (2007). The adrenal and polycystic ovary syndrome. *Rev Endocr Metab Disord* 8:331-342.

Yildiz, B. O. (2015). Approach to the patient: contraception in women with polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.* 100, 794–802. doi: 10.1210/jc.2014-3196.

Yuan, Y., and Foley, G. L. (2002). Female reproductive system. In Handbook of Toxicologic Pathology, 2nd ed., Vol. 2. (W. M. Haschek, C. G. Rousseaux, and M. S. Wallig, eds.), pp. 847–94, Academic Press, London.

Zawadski J, K., Dunaif A. (1992). Diagnostic criteria for polycystic ovary syndrome. In: Givens JHF, Merriman G, editors. The Polycystic Ovary Syndrome. Cambridge, MA: Blackwell Scientific; 377–384.

APPENDIX

0.25M Sucrose Solution

171.15g sucrose was dissolved in 2 liters of distilled water.

Preparation of 7.14mg/kg of Letrozole

Each tablet of metformin drug contains 500mg of active ingredient is used by humans with approximately 70kg body weight. The average weight of the experimental animals was 170.81g. Therefore 7.1mg/kg weight metformin was used.

Preparation of 1mg/kg of Letrozole

Each tablet of letrozole contains 2.5mg of active ingredient letrozole 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 the experimental animals was used.

Preparation of 2mg/kg weight of Clomiphene citrate

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

Reagents for total and conjugated bilirubin

R1- 29mmol/l of sulphanilic acid and 0.17N hydrochloric acid

R2- 38.5mmol/l of sodium nitrite

R3- 0.26mmol/l of caffeine and 0.52mol of sodium benzoate

Reagent for Phosphatase

(i) Alkaline phosphatase (ALP)

Carbonate Buffer (0.1M, pH 10.1)

(a) 2.10g of sodium bicarbonate ($NaHCO_3$) was dissolved in 250ml of distilled water

(b) 7.2035g of sodium carbonate ($NaCO_3.H_2O$) was dissolved in distilled water to make 250ml solution. Solution A was added to Solution B with continuous stirring until pH of the mixture comes to 10.1

Reagent for Aspartate aminotransferase (AST)

0.4N Sodium Hydroxide

16g of sodium hydroxide was dissolved in 1litre of distilled water in one litre volumetric flask.

Reagent R1 composition

Tris Buffer (pH 7.8) 110mmol/l

L-Aspartate 340mmol/l

Lactate dehydrogenase ≥ 4000 U/L

Malate dehydrogenase ≥ 750 U/L

Reagent R2 composition

CAPSO 20mmol/l

2-Oxoglutarate 85mmol/l
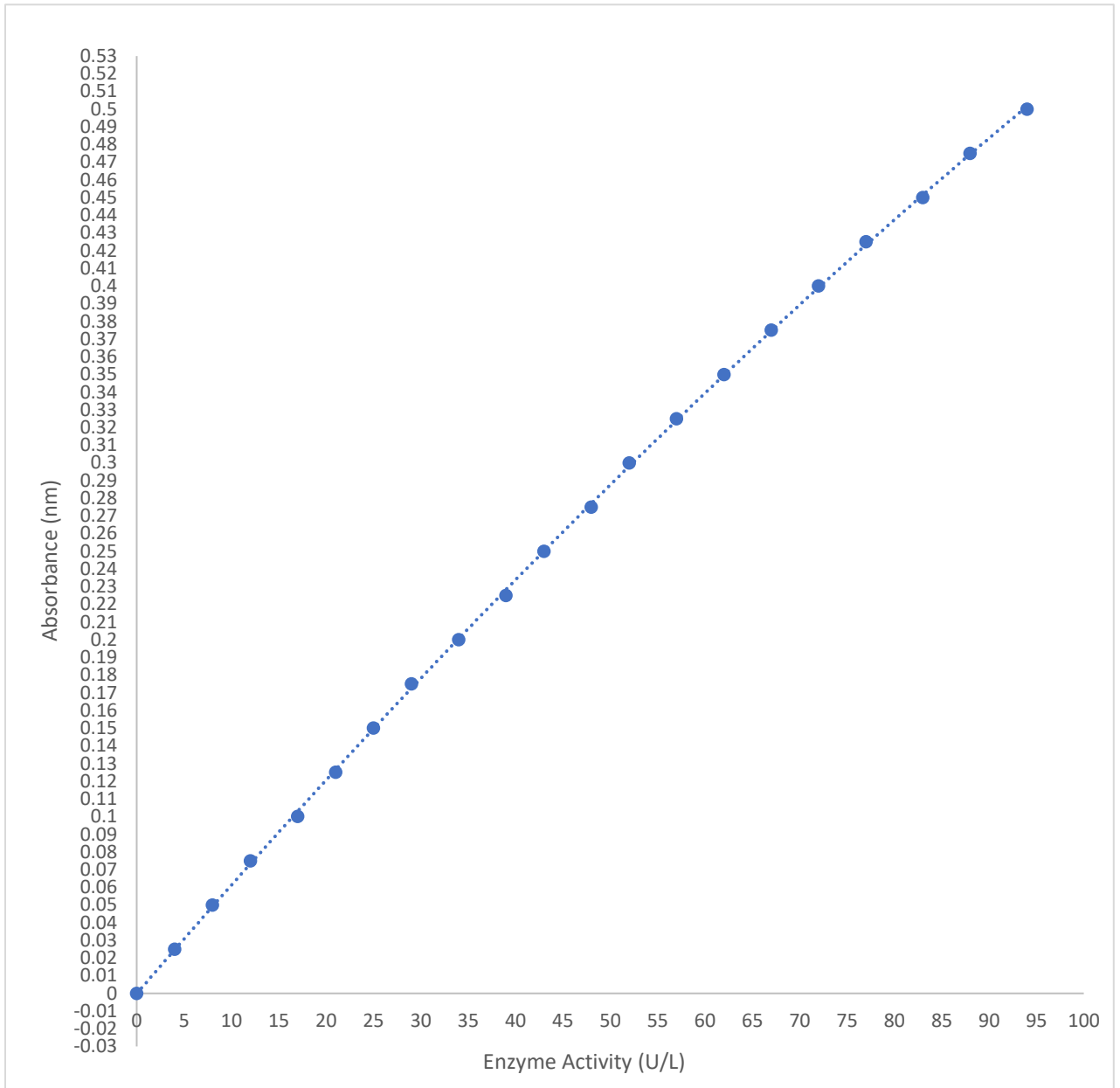
NADH 1.05mmol/l

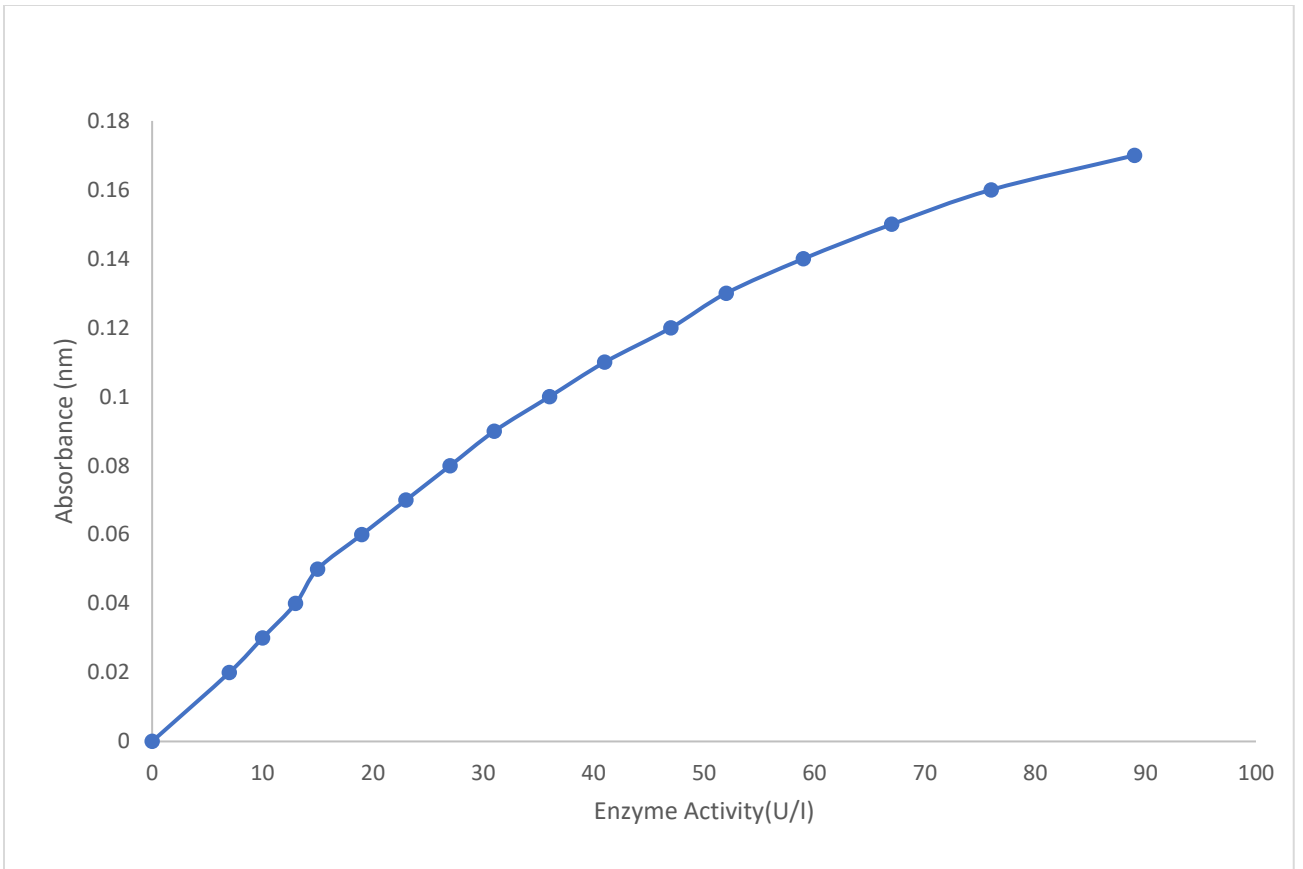# CALIBRATION CURVES

Figure 10: Calibration curve for alanine aminotransferase

Figure 11: Calibration curve of aspartate aminotransferase