Toxicological Evaluation of Ethanoic Extract of *Parquetina nigrescens* Leaves (Afzel.) on Letrozole-induced Polycystic Ovarian Syndrome in Wistar rats

By

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A DISSERTATION SUBMITTED TO THE DEPARMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCE, MOUNTAIN TOP UNIVERSITY, IBAFO, OGUN STATE, NIGERIA, IN THE FUFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN BIOCHEMISTRY

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CERTIFICATION

It is here by certified that this work was carried out by Komolafe, Marvellous Olaoluwa with matriculation number 17010102006, Department of biochemistry, Biological science, College of Basic And Applied Science, Mountain Top University, Ibafo, 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, 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, Komolafe, Marvellous Olaoluwa, hereby declare that this project titled "Toxicological evaluation of ethanoic extract of *Parquetina nigrescens* leaves on letrozole-induced Polycystic Ovarian Syndrome in female albino rats" is a record of my research work.

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Komolafe, M.O. **Date**

DEDICATION

This project is dedicated to my parent, Mr and Mrs. Komolafe, and most importantly my lord Jesus for making it possible for me to complete this project.

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ABSTRACT

Polycystic ovarian syndrome is a prevalent endocrine condition that affects roughly 5-7% of women of reproductive age and is the leading cause of infertility in women. Although various drugs like metformin have been used in the treatment of PCOS, side effects like lactic acidosis and gastrointestinal symptoms have brought about limiting effect of this drug. Thus, the need to explore alternative option like medicinal plants such as *Parquetina nigrescens* and evaluate its phytoconstituents and toxicity of ethanolic fraction of the leaves. The biochemical and toxicological effects of ethanoic extract of *Parquetina nigrescens leaves* (EEPNL) was investigated at doses of 50 and 100 mg/kg body weight on letrozole-induced Polycystic Ovarian Syndrome (PCOS) using Wistar rats. Twenty female Wistar rats (170.81 ± 5.25g) were randomly assigned into 5 groups (A - E) of four animals. Animals in group A recieved 1 ml of distilled water orally on daily basis for a period of 21 days while the rats administered with letrozole (1mg/kg body weight) in group C was treated with, 7.14mg/kg of metformin and 2mg/kg clomiphene citrate (reference drug) for 21 days and the rats in groups D and E were treated with extract (50 and 100 mg/kg body weight) of EEPNL was administered 14 days respectively after which the rats were sacrificed and the levels of some biochemical and toxicological indices were determined by assessing the liver function indices, kidney function indices, and enzyme assay. Data was subjected to analysis of variance and Duncan multiple range test. The toxicological evaluation of the ethanoic evaluation of *P. nigrescens* on letrozole induced polycystic ovarian syndrome in female albino rats has given an insight to the effect of this leaf in the normal function of the kidney and liver of the animal.

Keywords: Letrozole, toxicity, *Parquetina nigrescens*, Polyscystic Ovarian Syndrome, Rat

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ADB: Absorbance of direct bilirubin

AES: Androgen Excess Society ALP: Alkaline Phosphatase

ALT: Alanine aminotransferase

AST: Aspartate transaminase

ATB: Absorbance of total bilirubin

BMI: Body Mass Index

BUN: Blood Urea Nitrogen

CC: Clomiphene Citrate

CHF: Congestive Heart Failure

DNP: Dinitrophenylhydrazine

EEPNL: Ethanoic Extract of *Parquetina nigrescens leaves*

FSH: Follicle Stimulating Hormone

GC-MS: Gas Chromatography Mass Spectroscopy GFR: Glomerular Filtration Rate.

HCC: Hepatocellular Carcinoma

LDL: Low Density Lipoprotein

LH: Luteinizing Hormone

MET: Metformin

NAC: N-acetyl-cysteine

NAFLD: Non-Alcoholic Fatty Liver Disease

NIH: National Institute of Health

PCOS: Polycystic Ovarian Syndrome

POF: Premature Ovarian Failure

TNF: Tumor Necrosis Factor

UV: Ultraviolet

CHAPTER ONE

1.0 Introduction

Polycystic ovary syndrome is one of the most common endocrine disorders affecting approximately 5-7% in reproductive age (Delcour *et al.,* 2019) which is the main cause of infertility in women. It was discovered by Michael Leventhal and Irving Stein in 1935 when they saw an association between obesity, amenorrhea and hirsutism in seven patients with amenorrhea, infertility and enlarged multicystic ovaries (Alpanes *et al.,* 2013). PCOS is regarded to be a multisystem metabolic reproductive disorder that has developed over the years and is expected to be understood better over the coming years (lizneva *et al.,* 2016). It implies various consequences to female health, which includes high rates of infertility (Rojas *et al.,* 2014). It can be diagnosed clinically in women with hyperandrogenism, obesity and oligomenorrhea.

There are various illnesses that can be affected by PCOS, including hypertension and dyslipidemia, as well as obesity and metabolic syndrome. (Rojas *et al.,* 2014). A research done by R.A Wild in 2002 showed a huge spike in the level of this mentioned disorders when comparing women without PCOS to women with PCOS. Since the etiology of PCOS is through insulin resistance, the approach to treating PCOS will involve the use of insulin sensitizers such as clomiphene citrate and metformin (Shivali *et al*., 2018).

Since time immemorial plant has been used as a form of medication and has contributed majorly to health care all over the world. Plants having medicinal purposes being used for treatment of diseases are called medicinal plant. Medicinal plants are important elements of various indigenous medical systems in all countries of the world (Joshia *et al.,* 2010). Various plants with anti-obesity and hypoglycaemic activities have been used in the management of PCOS (Pachiappan *et al.,* 2017). A very good example of this is the *Parquetina nigrescens.*

Parquetina nigrescens (Afzel) also known as Mgbidim Gbe (Igbo), Kwankwanin (Hausa), ewe Ogbo (Yoruba) is a plant commonly found in West African countries such as Ghana and Nigeria (Femi-olabisi *et al.,* 2020)

This plant is such a medicinal plant which inhibits inflammation, improved parameters and has analgesic property (Owoley*e et al* 2016). It has been used in the treatment of menstrual disorders (Femi-olabisi *et al* 2020). Extract in the aqueous form and isolated compounds from *Parquetina nigrescens* have been evaluated in both in-vivo assay for PCOS treatment potentials (Paul *et al*., 2018). It is on this basis this study that this research is being carried out to determine the bioactive component and toxicity in *parquetina nigrescens.*

1.1 Statement of the problem

The study is done in order to discover the safe dose of the ethanolic extract of *Parquetina nigrescens* leaves that will be used for the treatment of polycystic ovarian syndrome.

1.2 Justification of the Study

Research has established on the usefulness of *parquetina nigrescens* in the treatment of PCOS, therefore it is important to determine the toxicological effect of this leave at different dose in PCOS rats.

1.3 Aim of the study

This study aims at evaluating the phytoconstituents of EEPNL to determine its toxicological effect on the PCOS rats

1.4 Objective of the study

1. To evaluate the active phytochemical constituents found in the ethanoic extract *Parquetina nigrescens* leaves,

2. To determine the toxicological effect of the solvent extract of *Parquetina nigrescens* in the treatment of PCOS in rat tissue in relation to:

i. Liver function indices ii.

Kidney function indices

iii. Enzyme assay

CHAPTER TWO

2.0 Literature Review

2.1 Historical perspective of Polycystic Ovarian Syndrome

Although Stein and Levanthal were regarded as the first to discover PCOS; however vallisneri, an Italian scientist in 1721 identified an infertile woman with shiny ovaries the size of a pigeon egg with white surfaces (Vallisneri *et al* 2016). In 1844 another report on case was discovered by Rokitansky and chereau when they described sclerotic and fibrous lesions in the ovaries of a degenerative character with hydrops follicle (Szydlarska *et al* 2017). In 1879 lawson tait presented the need for oophorectomy for the treatment of cystic degeneration of the ovaries. In 1902 von Khaled established the the implications of these ovaries. Then in 1935 stein and levanthal presented 7 women with menstrual disturbance hirsutism and enlarged ovaries with the presence of small follicle in them (Shivali *et al*., 2018). They also described lack of menstruation amongst these women and an increase in the amount of ovaries which they suggested ovarian wedge resection (Szydlarska *et al* 2017).

In 1953, scientists suggested using cortisone therapy or treating sclerocystic ovaries with exogenous testosterone, regardless of the source of androgens in PCOS (Gulherme *et al* 2015). In 1958, three investigators were the first to identify an elevated level of luteinizing hormone (LH) and 17-ketosteroids in the urine of women with bilateral cystic ovaries. Gonadotropin release, LH/FSH ratio, and androgens were all found to be irregular later on. Finally, the use of abnormal gonadotropin concentrations to diagnose PCOS was dismissed (Muha*s et al* 2018). Increased circulating levels of androgens in women with PCOS were demonstrated shortly after the description of a method of testosterone level measurement in plasma in 1961. Many women met the clinical requirements for PCOS despite the lack of evidence of hormone secretion disorders in laboratory studies due to the limitations of laboratory tests in calculating total androgenic hormone levels *(*Azziz *et al.,* 2006*).* Since the pituitary gland and gonads secrete hormones in a pulsatile manner, maximal and minimal concentrations can vary significantly during the day, making a single measurement misleading, particularly in women who have lower androgen levels than men. Researchers were searching for a diagnostic technique to replace roentgenography and reconnaissance laparotomy, which were previously used to diagnose

polycystic ovaries. The laparoscopic technique popularized by Gjoanness H. has resurrected surgical treatment of resistant anovulation. Ultrasound testing of the reproductive system was a significant advancement in clinical practice (Semins *et al*., 2010). The advantages of this research approach were immediately recognized, including its non-invasive nature, repeatability, ease of use, and precision in assessing the ovary stroma and ovary follicles. Swanson was the first to use ultrasonography to explain the anatomy of the ovaries in women with PCOS. As technology progressed and ultrasound became more widely used in medicine, the ultrasound concept of polycystic ovaries emerged, based primarily on the morphology and number of small antral follicles (*Fox et al 1991*).

A study conducted by Fox in 1991 comparing the use of transvaginal and transabdominal ultrasound revealed the presence of falsely negative results in the case of examination through the abdominal lining in as many as 30% of the women examined (Julie et al., 2005). Advances in ultrasound diagnosis enabled the verification of the ultrasound criteria (Szydlarska *et al.,* 2017) had been the best condition of a PCOS diagnosis When examined ultrasonically, about a quarter of the population had the presence of polycystic ovaries, but more than half of these had no clinical signs or symptoms. PCOS may be diagnosed: symptoms of androgen overload (clinical or biochemical), rare ovulations, and absence of other disorders with similar clinical symptoms. In 2004, a set of standards established by a group of experts during a 2003 conference in Rotterdam, the Netherlands became mandatory. The "Rotterdam requirements" that followed integrated the size and morphology of the ovary as determined by ultrasound into the diagnostic criteria. According to them, two out of three of the following criteria must be present in order to diagnose PCOS (Szydlarska *et al.,* 2017): Ovulations that is uncommon or nonexistent, Excessive androgen activity, as determined by a clinical or laboratory analysis, After ruling out other pathologies characterized by hyperandrogenism, such as adrenocorticotropic hormonedependent or independent hypercortisolemia, thyroid gland disorders, a classical and nonclassical type of congenial adrenal gland hypertrophy, tumors of the adrenal glands or ovary tumors producing androgens, as well as the influence of recessive genes, ultrasound features of polycystic ovaries may be shown. Then in 2006 the Androgen Excess Society (AES) issued a statement – criteria attempted to establish hyperandrogenism as a sine qua non diagnostic condition in combination with other signs of the syndrome. Many years has pass and the etiology of PCOS still remains a puzzle.

2.2 Overview on PCOS

PCOS (polycystic ovary syndrome) is one of the most common female endocrine disorders, affecting 5-7% of women (Ruta *et al.*, 2014). It is characterized by a highly irregular menstrual cycle that does not include ovulation (Jones *et al.*, 2015). The hypothalamus, pituitary gland, ovaries, adrenal gland, and peripheral adipose tissue are the main endocrine glands involved in PCOS, and they all work together to establish a general imbalance. The majority of signs occur at the start of menstruation in adolescence (Patakko *et al*., 2017). However, some women do not experience symptoms until their early to mid-twenties. Stein-Leventhal syndrome, or hyper androgenic anovulation, is another name for it. Over nutrition, insulin overproduction, ovarian confusion, and ovulatory disruption are all symptoms of syndrome "O" (Jones *et al.*, 2015). It has been linked to the development of type 2 diabetes and the occurrence of recurrent miscarriage (Nagarathna *et al.*, 2014). On ultrasound, the ovaries of a PCOS patient contain more than ten follicles. The layer of the polycystic ovary has more follicles and a dense center than the regular ovary. The stroma is the part of the body that produces testosterone (Nagarathna *et al.*, 2014). PCOS is defined as the presence of all three of the following criteria: oligo-anovulation, clinical or biochemical evidence of hyperandrogenism, and the presence of polycystic ovaries on ultrasound. Polycystic ovaries, on the other hand, are a non-specific finding that may occur in people who don't have any endocrine or metabolic problems. (Ameet *et al.*, 2014)PCOS is the most common endocrine problem in women of reproductive age and one of the leading causes of female subfertility. Cysts are not dangerous, but they can cause hormonal imbalances, which can cause period issues and make it difficult to conceive. PCOS is a medical disorder in which female sex hormones are out of control. That is, high testosterone, androstenedione, prolactin, and LH levels, as well as normal, high, or low estrogen levels. Hyperinsulinemia, insulin resistance, and reduced glucose tolerance are all normal in PCOS women, but insulin resistance can also occur in thin PCOS women (Pratab *et al.*, 2015).

The concomitant involvement of anovulation and evidence of hyperandrogenaemia was recommended by the National Institute of Health (NIH) as diagnostic criteria for PCOS (Gerard *et al.*, 2014).

2.2.1 Ovarian dysfunction

Compared to women with normal ovaries women with PCOS have 2-6 fold more primary, secondary and small anteal follicles. Abnormal androgen signaling is responsible for the great increase in the number of follicles; follicles also grow extremely slowly which are due to the deficient growth signals form the ovaries (*Maharaj et al 2014*).

PCOS being a form gonadotropin-dependent hyperandrogenism, the concentration of intraoverian androgen is elevated. There is also an increase in the formation of androstenedione and 17α hydroxyprogesterone in response to luteinizing hormone (*Tasoula et al 2004*)

2.2.2 Metabolic disturbance in PCOS

In order to be fully able to standardize the diagnosis of PCOS various statement has been produced through the years which resulted in the combination of the fundamental characteristics of the syndrome that is hyperandrogenism, hyperandrogenemia, menstrual dysfunction and the morphology of polycystic ovarian syndrome which was identified using ultrasound (*Muhas et al 2018*) . PCOS is stated to not be a specific disease but syndrome so therefore no single criteria can define it diagnosis, it is a diagnosis of exclusion.

2.2.2.1 Insulin resistance

Insulin resistance appears to be linked to excessive serine phosphorylation of the insulin receptor in at least half of PCOS women. This abnormality is caused by an extrinsic factor, most likely a serine/threonine kinase, and is an example of a significant new mechanism for human insulin resistance linked to factors regulating insulin receptor signaling. Phosphorylation of serine appears to influence the function of P450c17, a central regulator of androgen biosynthesis. It's possible that in some PCOS women, a single flaw causes both insulin resistance and hyperandrogenism.

2.2.2.2 Obesity

Weight gain and obesity are common clinical and biochemical manifestations in women who are genetically susceptible to develop PCOS (barber et al 2006). As a result, obesity and PCOS have a close relationship. The majority of women with PCOS are overweight or obese (38% - 88%).

At all ages, there is a substantial link between body mass index (BMI) and PCOS symptoms, according to studies (Ramezani-Binabaj *et al*., 2014). Furthermore, even small weight loss (about 5) can lead to clinically significant changes in PCOS' reproductive, hyperandrogenic, and metabolic characteristics(olila *et al*., 2016).

2.3 Diagnostic criteria of PCOS

PCOS is often characterized by the presence of insulin resistance and associated hyperinsulinemia and most of the patient show sign of obesity or overweight (*Unfer et al 2014*). PCOS is often usually very hard to diagnose due to it intrinsic characteristics. It is marked by infertility, oligomenorrhea, presence of cystic ovaries and amenorrhea. One of the most common symptoms of PCOS is the presence of excess androgens which can be diagnosed by looking for increased levels of androgens in the serum (*Ruta et al 2014*).

Throughout the years different criteria of diagnosing PCOS has been postulated (*Ruta et al 2014*). Criteria of the national institute of health (NIH) 1990: Chronic anovulation, clinical and biochemical hyperandrogenism. Rotterdam criteria 2003: polycystic ovaries imaging, oligo or anovulation , biochemical signs of hyperandrogenism. AES criteria 2009: Hyperandrogenism including hirsutim and or hyperandrogenaemia, Ovulatory dysfunction including oligo or anovulation and or PCOS exclusion of any other androgen excess or related disorders. Diagnostic information can further be obtained by the measurementof the LH, FSH, prolactin, testosterone level, thyroid stimulating hormone and dehydropiandrosterone to detect the hormonal imbalance (*Jones et al 2015*).

2.4 Prevalence of PCOS

PCOS was divided into four different phenotype which are the phenotype A, phenotype B, phenotype C, and phenotype D.

Phenotype A and B: PCOS phenotypes A and B are sometimes referred to as the "complete" PCOS phenotype in the classic and NIH PCOS terminology (Azziz, 2018). A woman with typical PCOS has more hairs, is obese, has irregular menstrual cycles, and is more likely to develop insulin-resistant, dyslipidemic fatty liver with a higher risk of metabolic syndrome than a woman with ovulatory PCOS (C or D) (Lizneva et al., 2016).

PCOS Phenotype C: Ovulatory PCOS patients have somewhat higher insulin, atherogenic lipids, and androgen levels than those with classic or non-hyperandrogenic PCOS phenotypic C patients (Guastella et al., 2010).

PCOS Phenotype D: In 2003, Rotterdem criteria defined phenotypic D as having ovarian dysfunction, polycystic ovaries, and no evidence of hyperandrogenism. For more information, see Lizneva et al. (2016).

The prevalence of Rotterdam characteristics varied between 8% and 13%. (Jones *et al.,* 2005). Obese women were more likely to exhibit phenotypes A and B, which were linked to hyperandrogenism, insulin resistance, and a poor cardiometabolic profile. Metabolic syndrome was the most prevalent trait in the study (Jones *et al.,* 2005).

2.5 Symptoms of PCOS

The Endocrine Society recommends that clinicians diagnose PCOS based on the Rotterdam criteria of 2003, but other guidelines varied in their recommendations for this. Rotterdam criteria requires at least two of the following three findings: hyperandrogenism, ovulatory dysfunction, and polycystic ovaries to establish a diagnosis of PCOS (Shivali *et al*., 2018)

Ultrasonography or other imaging is not usually required to make a diagnosis. On the clinical side, hyperandrogenism can be identified by the presence of excessive acne, male pattern hair growth (hirsutism), or increased serum levels of total, bioavailable, or free testosterone, or dehydroepiandrosterone sulfate, respectively. If there is a suspicion of an androgen-secreting tumor, measuring androgen levels can be useful (Shivali *et al*., 2018).

2.6 Etiology of PCOS

Even till now scientist do not know the exact cause of PCOS. All that has been established is the fact that high levels of male hormone prevent the ovaries from producing hormones and egg normally (Szydlarska *et al* 2017). Although, based on research carried out by Ajmaal *et al (2019)* he stated that genetic and environmental factor contributes to the etiology of this condition.
Unhealthy lifestyle, diet or any infectious mediators increase the risk of PCOS

2.7 Management options of PCOS

Since there is no treatment that reverses the hormonal disturbances of PCOS, medical management is targeted at individual symptoms and can be managed using drugs.

2.7.1 Use of drugs: various drugs have been used to manage the hormonal disturbances as described by *Muhas et al 2018.*

2.7.2.1 Metformin:

Insulin sensitizing drugs including metformin and troglitazone counteract certain hyperandrogenic symptoms by lowering total and free testosterone levels. It promotes ovulation and decreases infertility. Insulin resistance is an issue that controls androgen levels that are too high. The menstrual cycle, ovulation, and fertility are all restored (*Legro et al 2015*).

2.7.2.2 Clomiphene citrate:

In PCOS patients, it is the first-line therapy for ovulation induction. The oestrogen receptor antagonist interferes with the oestrogen signaling pathway's negative feedback, resulting in increased FSH availability (Muhas *et al*., 2018). Follicular growth is induced by increased FSH. It incorporates the first half of the menstrual cycle Infertility is also treated with it (*Legro et al 2015*).

2.7.2.3 N-acetyl-cysteine (NAC):

It contains antioxidants that are essential for the body's glutathione development, inhibiting oxidative stress and preventing hyperinsulinemia (Gayatri *et al.*, 2010). An anti-oxidant, NAC (N-acetyl-cysteine) is derived from L-cysteine. Because of its ability to influence insulin receptor activation and secretion, it has the potential to improve glucose utilization (Gayatri *et al.*, 2010). Furthermore, NAC inhibits cellular apoptosis and lowers homocystein levels. However, NAC can be obtained as a nutritional supplement (Ventura *et al*., 1999). It was found that NAC can have an influence on insulin levels and insulin sensitivity in PCOS women with hyperinsulinemia, according to the Fulghesu study (Fulghesu *et al*., 2002). If you have PCOS, NAC can be administered as an adjuvant to Clomiphene Citrate for ovulation induction or enhancement (Badawy *et al*., 2007).

2.8 Medicinal plants of PCOS

In the treatment of polycystic ovarian syndrome various plant have been used to manage and treat this disorder due to their hypoglycemic and anti-obesity effect.

2.8.1 *Pergularia Daemia* (Veli paruthi):

Pergularia daemia (Asclepiadaceae) is known in Tamil as "Veliparuthi" and in Sanskrit as "Uttaravaruni." Pergularia daemia has long been used for its medicinal properties (Nivetha *et al*., 2016). It has the potential to regulate the estrous cycle and normalize menstrual abnormalities. As a result, restoring the estrous cycle lowers the risk of follicular cyst formation (Poormima *et al*., 2015). PCOS-induced albino wistar rats had lower levels of LDL, FSH, LH, Estrdiol, Progesterone, and testosterone, according to Poornima et al. (Baskar *et al*., 2009). LH and FSH levels were restored after supplementation with Pergularia deamia.

2.8.2 *Foeniculum vulgare* (Shatapushpa):

The seeds of Foeniculum vulgare (Apiaceae) are a useful supplement for PCOS control. Phytoestrogens are abundant in them. Fennel's phytoestrogen concentration aids in the reduction of insulin resistance and inflammation in PCOS. It is also aids in the reduction of cellular imbalance, which causes metabolic problems in PCOS (Jungbauer *et al*., 2014).

Different portions of this plant are being utilized to treat a variety of ailments, notably those affecting the digestive system. Diabetes, bronchitis, chronic cough, kidney stones, nausea, and vomiting can all be treated with it (Wesam *et al*., 2015).

2.8.3 *Grifola frondosa* (Maitake Mushroom):

Grifola frondosa (Meripilaceae) is a fungus that has a hypoglycemic impact and may help with diabetes management. In animal tests, Grifola frondosa extract was able to promote ovulation in women with polycystic ovarian syndrome (PCOS) (Tori 2011). Grifola frondosa's mechanism of action regulates blood glucose levels and improves insulin sensitivity (Talpur 2003).

2.8.4 *Galega officinalisi* (Goats Rue):

Galega officinalisi has been used to relieve the symptoms of diabetes mellitus since the Middle Ages, and it has now been discovered to contain guanidine, a chemical that lowers blood sugar through reducing insulin resistance (Mobeen *et al.,* 2016). It is, nevertheless, the natural source of guanidine, a biguanides-class anti-diabetic medication. Metformin, a biguanide, is one of the most often prescribed drugs for PCOS. This relationship alone should prompt a second look at this herb for the treatment of polycystic ovarian syndrome (Nowak *et al.,* 2007).

2.8.5 *Parquentina nigrescence*

Parquetina is a monotypic genus with Parquetina nigrescens being the only species in it. It is commonly found in secondary forests and around villages in Senegal and Nigeria (Sopeyin *et al., 2016*).

2.8.5.1 Taxonomy of *parquentina nigrescence*

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Gentianales

Family: Apocynaceae

Sub-family: Periplocoideae

Genus: *Parquetina*

Species: *nigrescens*

2.8.5.2 Traditional usage of parquentina nigrescens

Parquentina nigrescens has been used in the treatment of helminthiasis, insanity, gonorrhea, menstrual disorders (*sopeyin et al 2016*). It has also been used in managing sexual dysfunction (*Omowumi et al 2017*), in the treatment of diabetes mellitus (*Saba et al 2016*). It has been used to treat against microbes and protect gastrointestinal tract (*Odetola et al 2016*)

Figure 1: *Parquetina nigrescens* leaves

Source:www.africanplants.senckenberg.de

2.9 Letrozole

Letrozole whose chemical structure is (4,4′-[(1H-1,2,4-triazol-1-yl) methylene] bis-benzonitrile) is a highly potent inhibitor of aromatase in vitro, in vivo in animals, and in humans.

Letrozole is an aromatase inhibitor used to induce ovulation in anovulatory infertile women with an endometrial thickness of more than 56 mm (*Franik et al 2018*). It works by suppressing the enzyme aromatase, which decreases estrogen production. [*Rajan et al 2017*] Letrozole has been shown to reduce estrogen levels by at least 97 percent to 99 percent. [*pavone et al 2016*] Letrozole was also found to be successful in clomiphene-resistant individuals, resulting in ovulation in 62 % of instances and pregnancy in 14.7% (*wang et al 2018*). It is used to stimulate ovulation in women in PCOS (Rachel., 2020)

In a study conducted at a tertiary referral infertility clinic in Dhaka, 35 anovulatory infertile patients who were nonresponsive to CC were included (Begum *et al*., 2009). Begum *et al*. (2009) reported a high success rate of 77.77 percent for follicular formation when using letrozole at a dose ranging from 2.5 to 5 mg. Another study conducted by Badawy *et al.* (2007) found that taking 5 mg letrozole in PCOS patients who had previously been treated with CC resulted in an effective induction rate of 62 percent in the majority of cases.

2.9.1 Mechanism of action of letrozole

Mechanism of action of letrozole can be summarized into the following stages (Paloma et al 2016)

1. Blockage of the conversion of androgens to estrogens.
2. Acute hypoestrogenic state: Levels of estrogen are low.
3. Hypothalamic-pituitary axis from the estrogenic negative feedback: GnRH release from pituitary gland more pulsatile.
4. Increased and prolonged secretion of FSH and LH.
5. Ovarian follicle maturation → monofollicular growth.

6. Ovulation induction

2.10 Phytochemical constituents and PCOS

Phytochemicals, which are non-nutritive chemical substances generated from plants, have an important role in illness prevention in humans. Secondary metabolites and antioxidants are examples of phytochemicals with important therapeutic characteristics. Secondary metabolites and antioxidants are examples of phytochemicals with important therapeutic characteristics.

Alkaloids, tannins, saponins, flavonoids, phenols, steroids, carotenoids, and other phytochemicals found in medicinal plants have a variety of disease-prevention properties (Murri *et al*., 2013). These plant-derived chemical compounds have important anti-inflammatory, antidiabetic, anti-aging, antibacterial, antiparasitic, antidepressant, anticancer, antioxidant, and wound healing properties (Mertens-Talcott *et al*., 2003). They also play a vital role in plant stress tolerance and the accumulation of numerous critical bioactive chemicals in fruits and vegetables.

The most prevalent bioactive chemicals discovered in medicinal plants are flavonoids. They have a variety of antibacterial, antioxidant, anticancer, anti-inflammatory, and wound-healing properties in humans. A variety of fruits and vegetables have been discovered to contain anticarcinogenic flavonoids. Apples and berries were discovered to have cardioprotective effects and to have a favorable effect on blood pressure.

Anthocyanins are flavonoid components prevalent in cell vacuoles that are important for coloring in flowers, fruits, and vegetables and are generally produced during plant stress. Anthocyanins have been shown to have antioxidative properties in cell culture systems such as colon, liver, breast, leukemic cells, and keratinocytes.

Carotenoids are a type of natural antioxidant present in fruits and vegetables. They include xanthophyll and carotenes, both of which scavenge peroxyl radicals. Lycopene can be found in tomatoes and berries, whereas -carotenes are orange-colored carotenoids found in yellow-orange and dark-green leafy vegetable .

The use of antioxidants in the treatment of women with PCOS has sparked a lot of interest recently (Mertens-Talcott *et al*., 2003). Obesity and abdominal adiposity, androgen excess, and insulin resistance are all hallmarks of PCOS that might lead to oxidative stress in these people.

Indeed, PCOS is a disorder that causes a considerable fall in serum antioxidant and vitamin levels, and these women are at a higher risk of oxidative status properties (Murri *et al*., 2013). Oxidative stress and antioxidant deficiency may raise the risk of cardiovascular disease, insulin resistance, hypertension, central obesity, and dyslipidemia in these wome .

2.10.1 GC-MS (Gas Chromatography – Mass Spectroscopy) Principle and application

The hyphenated analytical technique of Gas Chromatography–Mass Spectrometry (GC-MS) combines the separation capabilities of gas-liquid chromatography with the detection feature of mass spectrometry to identify various compounds within a test sample (Sahil *et al.,* 2011). The volatile and thermally stable substitutes in a sample are separated by GC, whilst the analyte is fragmented by GC-MS and identified based on its mass. GC-MS/MS is created by adding a mass spectrometer. Single and triple quadrupole modes (Jenke *et al*., 1996) provide better performance.

Using gas chromatography–mass spectrometry, many of congenital metabolic abnormalities known as inborn errors of metabolism may now be detected in newborns. Even in low concentrations, GC-MS can identify chemicals in urine (Alon *et al.,* 2006). These molecules aren't ordinarily present, but they show up in people who have metabolic problems. This is a simple, effective, and efficient technique to identify a condition, such as when a urine test at birth is used to determine inherited metabolic abnormalities. The GCMS is used to determine metabolic activity in conjunction with isotopic tagging of metabolites (stein 1994).

For active pharmaceutical ingredients (API), bulk pharmaceuticals, and formulations, GC-MS is widely utilized in the pharmaceutical industry for analytical research and development, quality control, quality assurance, production, and pilot plant departments. It is utilized in the creation of processes and methods, as well as the detection of contaminants in APIs. It is an important component of research in medicinal chemistry (compound synthesis and characterisation), pharmaceutical analysis (impurity profiling, stability testing), pharmacognosy, pharmaceutical process control, pharmaceutical biotechnology, and other fields (Priya *et al*., 2012).

2.10.2 UV-Visible spectrometry

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 Organ study

2.11.1 Ovaries and PCOS

The ovary is a highly organized composite of germ cells (oocytes or eggs) and somatic cells (granulosa cells, thecal cells, and stromal cells) whose interactions determine the formation of oocyte-containing follicles, ovulation, and the formation of the corpus luteum (the endocrine structure that forms from the ovarian follicle after ovulation and is required for establishing and maintaining pregnancy) (Fulghesu et al 2017). Follicle-stimulating hormone (FSH) and luteinizing hormone (LH), secreted from the anterior pituitary gland under the control of pulses of gonadotropin-releasing hormone (GnRH) from the hypothalamus, control many events in the adult ovary (Mujan 2015). Low-frequency GnRH pulses cause a slight increase in FSH levels early in a woman's menstrual cycle, promoting follicle growth, while high-frequency GnRH pulses cause a sharp rise in LH levels just before mid-cycle (known as the "LH surge"), triggering ovulation and the formation of the corpusluteum (Fritz et al 2012). By directing feedback mechanisms to the hypothalamus and pituitary, the ovary ensures the timely release of fertilizable oocytes and the maintenance of luteal cell function, which is required for pregnancy. For example, estrogen produced by developing follicle cells both inhibits and elicits elevated GnRH pulses in the hypothalamus, triggering the mid-cycle LH surge that initiates ovulation (Sudhu et al 2017). Fertility is thus dependent on a complex set of endocrine events involving multiple organ systems.

Premature ovarian failure (POF), polycystic ovarian syndrome (PCOS), ovarian hyperstimulation syndrome, ovulation defects, poor oocyte quality, and cancer can all be caused disruption of this meticulously maintained network (Yahia 2013).

The ovaries are a pair of tiny oval-shaped structures found in the pelvic cavity. Their primary responsibility is to assist ladies in becoming pregnant (wallers et al 2016). Each month, they lay

an egg. When the egg is ripe, it is extracted from the ovary and pushed down the fallopian tube to be fertilized (ovulation). The eggs do not fully mature in women with PCOS. This is the primary reason behind infertility. In some women diagnosed with PCOS, ultrasound imaging of the ovaries reveals numerous follicles: these are not cysts, but partially produced eggs within the ovaries that have not developed fully (Yan et al 2016). They are caused by the body's excessive production of male-type hormones.

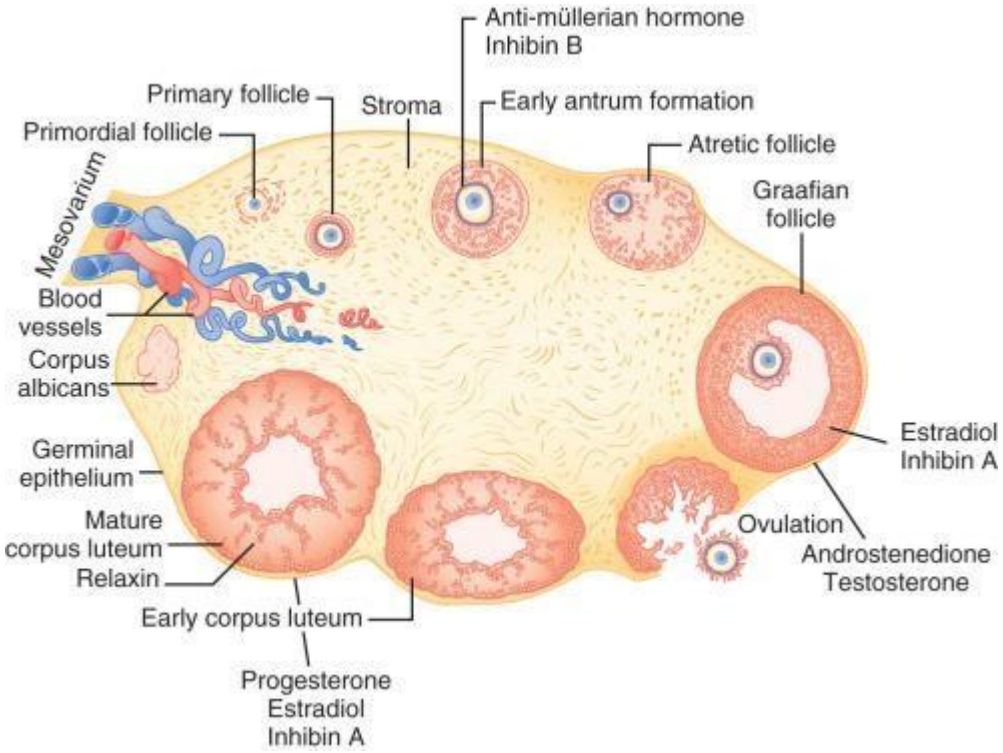

Figure 2: Diagram of the ovary

Source: www.newsmedical.com

2.11.2 Liver

The liver is the largest solid organ, the largest gland, and one of the most important organs in the body, serving as a hub for nutritional metabolism and waste metabolite excretion [Ozougwu et al 2014]. Its main job is to regulate the flow and safety of substances absorbed from the digestive system before they are distributed to the systemic circulatory system [allen 2002]. A complete loss of liver function can result in death in minutes, highlighting the liver's critical role. The liver weighs around 1500g and accounts for around 2.5 percent of adult body weight [moore et al 2006].

Lipids are thought to have a major role in the etiology of polycystic ovarian syndrome (PCOS) and fatty liver disease (Tsouma et al 2014). Both PCOS and fatty liver disease causes multiple amount of problems (spritzer et al 2016). PCOS has been linked to hirsutism and reproductive problems Fatty liver disease is linked to an increase in liver damage that leads to cirrhosis and hepatocellular cancer whereas fatty liver disease is linked to an increase in liver damage that leads to cirrhosis and hepatocellular carcinoma (HCC) (Donati et al 2010).

While the process appears to be more explanatory in terms of additional fat buildup in the liver (Margini et al 2016), data suggests that PCOS phenotypic diversity allows for variable correlation with fatty liver disease. According to Macut et al, people with elevated serum testosterone, a symptom of PCOS, had a two-fold greater risk of fatty liver (Kauffman et al 2010). Rocha et al found that the prevalence of PCOS with NAFLD varies, but that the combined presence of PCOS and NAFLD leads to a higher degree of clinical and biochemical hyperandrogenism than female subjects only demonstrating PCOS, with the former category being more associated with metabolic derangements Contrary to popular belief, there is evidence that both fatty liver disease and PCOS share a same etiology (Macut et al 2010). Vassilatou et al research has shown that lipid abnormalities are fundamental to the development of PCOS and NAFLD; nonetheless, he classified data from PCOS patients as either obese or not obese. Similarly, Kauffman et al. have shown that PCOS and NAFLD can both be caused by the same pathogenic processes, such as androgen excess and insulin resistance.

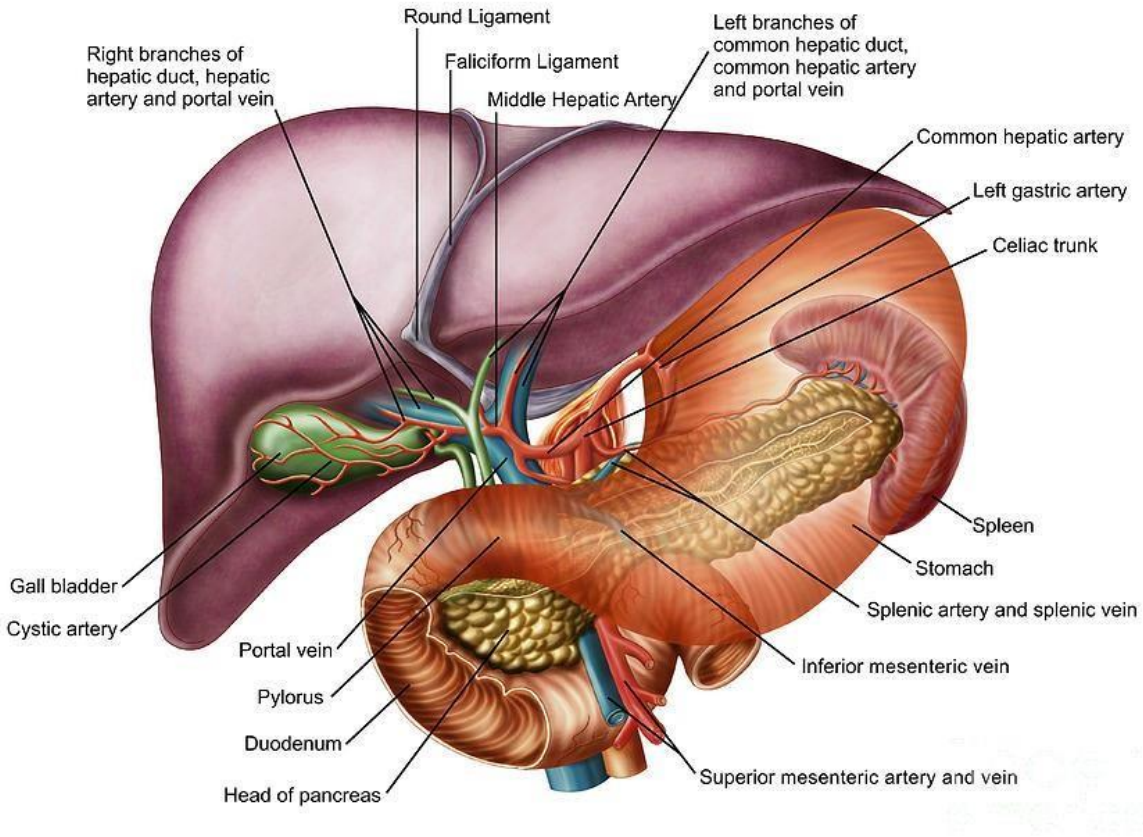

Figure 3: diagram of the liver

Source: www.leogenic.com

2.11.3 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.

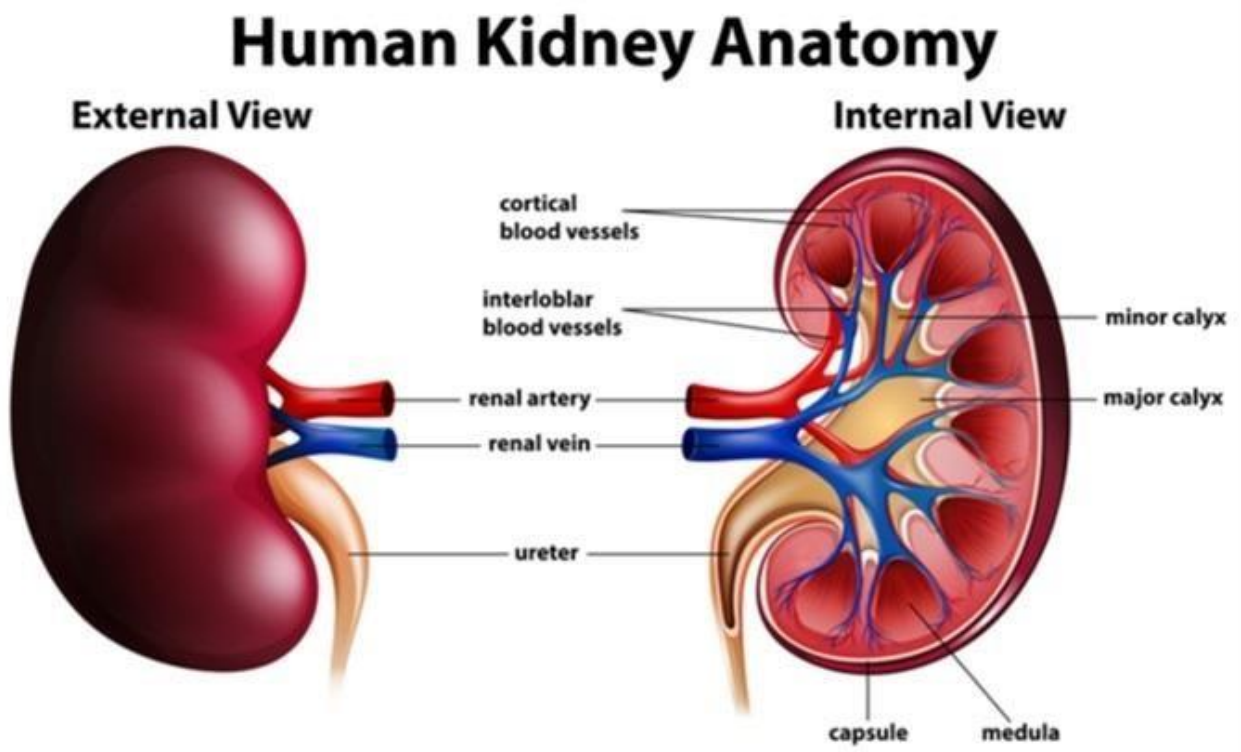

Figure 4: Diagram of the kidney

Source: www.newsmedical.com

2.11.4 Percentage organ body weight ratio

Ratio of organ to body weight is the ratio of organ-to-body-weight of an animal. Weight varies widely between organs in the body, depending on their location and function (Nirogi *et al*., 2014). The greater the organ, the more functions it can do.

It is expresses as:

$$\frac{\text{Weight of the organ}}{\text{Total weight of the animal}} \times 100$$

An important quantitative endpoint for many toxicology investigations is comparing organ weights between treated and untreated groups of animals. Significant differences in organ weight between treated and untreated (control) animals can occur in the absence of any morphological alterations, making organ weight one of the most sensitive markers of a test (Bailey *et al.,* 2004).

2.12 Biochemical assessment

2.12.1 Kidney function indices

Biochemical markers play a critical role in proper diagnosis, risk assessment, and treatment selection. There has been a dramatic change in biomarker research and application over the past few decades. As defined by NIH in 2001, a biomarker is "a trait that is reliably tested and analyzed as an indicator of normal biology, pathologic processes, or pharmacologic responses to therapeutic intervention (Yuegang *et al*., 2008)." It is routine to test for renal function indicators such as creatinine, urea, and uric acid and BUN creatinine ratio.

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. The National Kidney Disease Education Program advises that 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 (Edmund *et al*., 2006)

Produced by the liver, 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 non-renal factors, including food and urea cycle, urea clearance does not provide a good indication of the glomerular filtration rate (GFR).

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*., 2005). Molecularly, inflammation and insulin resistance are also linked to this condition (Gonzalez *et al*., 2006). PCOS is associated with elevated levels of circulating proatherogenic inflammatory mediators (Diamanti-Kandarakis *et al*., 2006). Serum uric acid was associated positively with interleukin 6 (IL-6), CRP and tumor necrosis factor- alpha (TNF-α) and negatively with IL-1 beta (IL-1β). These results suggest that uric acid contributes to systemic inflammation in humans and is in line with experimental data showing that uric acid triggers sterile inflammation (Lohsoonthorn *et al*., 2006). It is also known that hyperuricemia is an independent risk factor for renal dysfunction in the normal population (Lyngdoh *et al*., 2011).

2.12.2 Liver function indices

Biochemical liver function indices can be determined by running biochemical assays such as serum total protein, albumin, total and direct (conjugated) bilirubin, globulin and albuminglobulin 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 Drugs, assay kits and chemicals

Letrozole (2.5mg), Metformin (500mg), Clomiphene Citrate (50mg), Absolute Ethanol, Dimethyl Sulfoxide (DMSO), Sucrose solution, Sodium Chloride, 0.4N Sodium Hydroxide, Albumin, Total Protein, Creatine, Alkaline Phosphatase (ALP). Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Urea, Uric acid,

3.1.2 Plant materials and authentication

Fresh leaves of *Parquetina nirescens* were obtained from the premises of the union bank of Nigeria (UBA) in Mountain of fire and miracles ministries, ibafo, Ogun State, Nigeria. The plant was authenticated at the Department of Plant Biology, University of Ilorin, Ilorin , Nigeria. A voucher specimen UH 001/0980 was deposited at the Herbarium of the Department.

3.1.3 Experimental animal specimen

Twenty healthy female albino rats (*Rattus novergieus*) with an average weight of 180.00g was obtained from the animal holding unit of the Mountain Top University, Ogun State, Nigeria. The animals were kept in a well-ventilated house condition and fed with rat pellets and water.

3.2 Methods

3.2.1 Preparation of aqueous extract of *parquetina nigrescens* leaves

Reagent/Materials

Parquetina nigrescens, Whattman's No. 1 filter paper, jute bag, Beakers, electric blender, rotatory evaporator, funnel measuring cylinder, plastic container, hot air oven and absolute ethanol

Procedure

The identified sample (2,141.37g) was toughly rinsed under running water to remove contaminants , oven dried at 50 degree Celsius when it attains a constant weight of 544.17g. It was blended with the aid of an electric blender. The Pulverized flower (500g) was weighed and extracted using 2500ml of ethanol, it was then kept in a container with a lid and placed in a cupboard for 48hours, sieved with a jute bag. The extract was poured in a container, watthman's filter paper and funnels was then used to further separate the extract. The extract was subjected to rotatory evaporation to separate the solvent (ethanol) from the extract collected in beakers and placed in the oven to concentrate the extract which yielded 13.2%. The concentrates was then refrigerated at −4°C.

3.2.2 Phytochemical screening of the extract

3.2.2.1 Qualitative screening of secondary metabolites

Parquetina nigrescens leaves was screened for secondary metabolites present as described according to Arvindganth *et al.*, (2015).

3.2.2.1.1 Qualitative screening of secondary metabolites

a. **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).
b. **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). .
c. **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 indicates the presence of saponins (Arvindganth *et al*., 2015)..
d. **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).
e. **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).
f. **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).
g. **Test for Quinones:** 1ml of plant extract was added with 1ml of concentrated sulphuric acid in a test tube. Formation of red colour solution indicates the presence of quinones (Arvindganth *et al.,* 2015).
h. **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).
i. **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).
j. **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).

k. **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).

l. **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).

m. **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 indicates the presence of anthraquinones (Arvindganth *et al.,* 2015).

n. **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).

o. **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 indicates the presence of phlobatannins (Arvindganth *et al.,* 2015).

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

3.2.3 UV-Visible spectroscopy

Principle

Ultraviolet and visible absorption spectroscopy is a method of spectroscopy that involves calculating the attenuation 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 (Arvindganth *et al.,* 2015).

Reagent/materials

UV-Visible Spectrophotometer, cuvette, 40 ml distilled water, 0.48g *Parquetina nigrescens,* filter paper, conical flask.

Procedure

1.18g of *Parquetina nigrescens* 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 spectroscopy and read at different wavelengths between 200-600nm with increment of 5 nm to get the different absorbance.

3.2.4 GC-MS analysis

Procedure

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, 1.0 μm 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).

3.2.5 Induction of PCOS

3.2.5.1 Animal Grouping and Extract Administration

A total of 20 female rats with an average weight of 180g was acclimatized for one week to standard health conditions. They were placed into 5 different groups based on their similarity in body weight.

PCOS was induced in 16 female albino rats in groups designated B – E with 2 mg of Letrozole. The extract administration according to the groups was carried out as follows:

a. **Group 1** (non-PCOS-induced control) received 1ml of distilled water
b. **Group 2** (PCOS-induced) received 1 ml of distilled water
c. **Group 3** (PCOS-induced) received 0.5ml of 7.14 mg/kg body weight of metformin and 0.5ml of 2mg/kg body weight clomiphene citrate
d. **Group 4** (PCOS-induced) received 0.5ml of ethanoic extract of *Parquetina nigrescens* leaves
e. **Group 5** (PCOS-induced) each received 1ml of ethanoic extract of *Parquetina nigrescens* 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.

3.2.5.2 Confirmation of PCOS

The estrous cycle of the rats were 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.

3.2.5.3 Vaginal cytology

Vaginal smears were obtained from the rats between the period of 8am and 9am daily using cotton buds and distilled water. The buds was 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.

3.2.6. 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 ^{o} frozen (4C) 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 ^{o} bottles, stored frozen (4C) overnight before being used for the various biochemical assays (Yakubu *et al*., 2008).

3.2.7 BIOCHEMICAL ASSESSMENT

3.2.7.1 Enzyme assay

3.2.7.1.1 Alanine aminotransferase assay

This method described by Reitmann and Frankel (1957) was used for assaying the activity of alanine aminotransferase. **Principle** ἀ-oxoglutarate + L-alanine ——> L-glutamate + pyruvate

Alanine Aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4- dinitrophenyl-hydrazine.

Reagents

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

Reagent 2: 2,4-dinitrophenylhydrazine (DNP)

Reagent 3: 0.4N Sodium Hydroxide

Test tubes, micropipette, syringe and needle, hot-air oven, enzyme source (serum/liver), beaker and distilled water

Procedure

28 sterile test tubes which was labelled appropriately was used, 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 ^{o}37C. 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 (25^{o}C). 2.5ml of Reagent 3 was also added to both samples test tubes and blank test tube, which the solution was mixed and the absorbance of the samples was read against the blank using UV-Visible spectroscopy at 546nm wavelength. **3.2.7.1.2 Aspartate aminotransferase activity (AST)**

Principle

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

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

Reagent

Reagent 1, Reagent 2, Reagent 3 (0.4N Sodium Hydroxide), test tubes and test tube racks, micropipette, syringe and needle, hot-air oven, enzyme source (serum/liver), beaker and distilled water.

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 37oC. 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 (25C). 2.5ml of Reagent 3 was also added to both samples test tubes and blank test tube, which the solution was mixed and the absorbance of the samples was read against the blank using UV-Visible spectroscopy at 546nm wavelength.

3.2.7.1.3 Alkaline phosphate activity (ALP)

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

Principle:

The hydrolysisproduct para-nitrophenol has a characteristic yellow colour in acid solution. The initial phosphate incubation of the enzymewith acetate buffer served to inactivate microsomal phosphate. Acid phosphate catalyzes the hydrolyses of para-nitrophenyl liberating paranitrophenol and phosphate. The para-nitrophenol couples with diazotitized 2-amino-5chloroacetate to form diazo dye. The increase in absorbance is directly proportional to the level of acid phosphate in the sample

Reagent/materials:

Standard, Reagent 1, test tubes and test tube racks, micropipette, syringe and needle, distilled water and enzyme source (serum).

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.

3.2.7.2 Liver function indices

3.2.7.2.1 Total protein concentration

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

Reagent/materials

Standard, Reagent 1, test tubes and test tube racks, micropipette, syringe and needle, distilled water and enzyme source (serum, kidney and liver).

Procedure

20µl of the sample (serum/liver/kidney) 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 ^{o} minutes at 25C. The absorbance of the samples was read against the blank using UV-Visible spectroscopy at 546nm wavelength.

3.2.7.2.2 Serum albumin (ALB) concentration

This procedure was described by Doumas *et al* (1971) in the determination of the serum albumin concentration

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.

Reagent/materials

Standard, Reagent 1, test tubes and test tube racks, micropipette, syringe and needle, distilled water and enzyme source (serum).

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 37$_0$C, which the absorbance of the samples was read against the blank using UV-Visible spectroscopy at 630nm wavelength.

Calculation:

$$\text{Concentration of albumin} = \frac{(A_{sample} \times \text{concentration of standard})}{A_{standard}}$$

Asample=Absorbance of sample

Astandard=Absorbance of standard

Concentration of standard=4.68g/dl

3.2.7.2.3 Serum globulin concentration

The globulin concentration was determined by using the formula

Globulin= Total protein - Albumin

The result was expressed in g/dl

3.2.7.2.4 Serum total and direct bilirubin concentration

This method was described by Evelyn and Malloy (1938) to be used to determine the serum bilirubin concentration

Principle:

Direct bilirubin in the sample reacts with diazotized sulphanilic acid in alkaline medium to form blue coloured complex

Total bilirubin in the sample was determined by the presence of caffeine benzoate and acetate as accelerator to form azobilirubin.

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℃ 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 = 10.8 × ATB

Direct bilirubin = 14.4 × ADB

ADB = Absorbance of direct bilirubin

ATB = Absorbance of total bilirubin

3.2.7.2.5 Serum albumin-globulin concentration ratio

This was derived using the method described by melnick *et al* (1940) by dividing the concentration of albumin by the concentration of globulin

3.2.7.3 Kidney function indices

3.2 7.3.1 Creatinine concentration

This method was described by Bohmer and Bartels (1972) in the determination of the creatinine concentration

Principle

Creatinine reacts with picric acid to form a red coloured complex. The amount of complex formed is directly proportional to the creatinine read on the UV spectrophotometer at 490nm

Reagent/materials

Standard, Reagent, test tubes and test tube racks, micropipette, syringe and needle, distilled water and enzyme source (serum).

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 =

Asample = Absorbance of the test sample

Astandard = Absorbance of the standard

3.2 7.3.2 Serum urea concentration

This procedure was described by Vakirtzi and Veniamin (1970) to determine the serum urea concentration **Principle:**

Urea in the serum is hydrolyzed to ammonia in the presence of urease. The ammonia in then determined using a UV spectrophotometer

$$Urea + H20 \longrightarrow 2NH3 + CO2$$

$$NH3 + Hypochlorite + Phenol \longrightarrow Indophenols$$

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

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

Concentration of standard = 50mg/dl

3.2 7.3.3 Uric acid concentration

The concentration was determined using the method described by Tiez (1995)

Principle:

Uric acid is concerted by uricase to allantoin and hydrogen peroxide which under the catalytic influence of peroxidase, oxidizes 3,5-dichloro-2-benzenesulforic acid and 4-aminophenazone to a red violet quinoneimine compound.

Procedure:

0.5ml or 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℃ 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

$$\text{Concentration of uric acid (mg/dl)} = \frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$$

Concentration of standard =

3.2 7.3.4 Serum blood urea nitrogen (BUN) - creatinine ratio

This was determined by dividing the blood urea concentration by creatinine concentration. This method was described by Tiez (2006)

$$\text{BUN-RATIO (mg/dl)} = \frac{\text{Serum urea}}{\text{Serum creatinine}}$$

3.2.8 Percentage organ-body weight ratio

This was obtained by dividing the weight of the organ by the weight of the rat. The result was the multiplied by 100 to determine the percentage (Bailey *et al.*, 2004).

CHAPTER FOUR

4.0 Results

4.1 Chemical constituents of aqueous extract of *Parquetina nigrescens* leaves

4.1.1 Secondary metabolites

Qualitative analysis of the aqueous extract of *Parquetina nigrescens* revealed the presence of the following secondary metabolites in the table below

The peresence of the following metabolites was exhibited: saponins, alkaloids, flavovoids, quininones, terpenoids, phenols, cardiac glycosides, steroids,While the following metabolites were absent carbonhydrates, tanins, anthracyanine, Ninhydrin, anthraquinones, glocosides, phlobatamins.

Table 1: metabolites screened in the ethanoic extract

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

4.2 UV-Spectroscopy analysis of ethanoic extract of *P.nigrescens*

The result of the UV-spectroscopy analysis of *Parquentina nigrescens* showed different absorbance of the plant at different wavelength as shown in the Figure below.

Using various wavelengths the extract showed different absorbance for each wave length reaching a peak of 2.5 from 310 to 420nm then a sudden drop in the absorbance was noticed for 425 to 600nm.

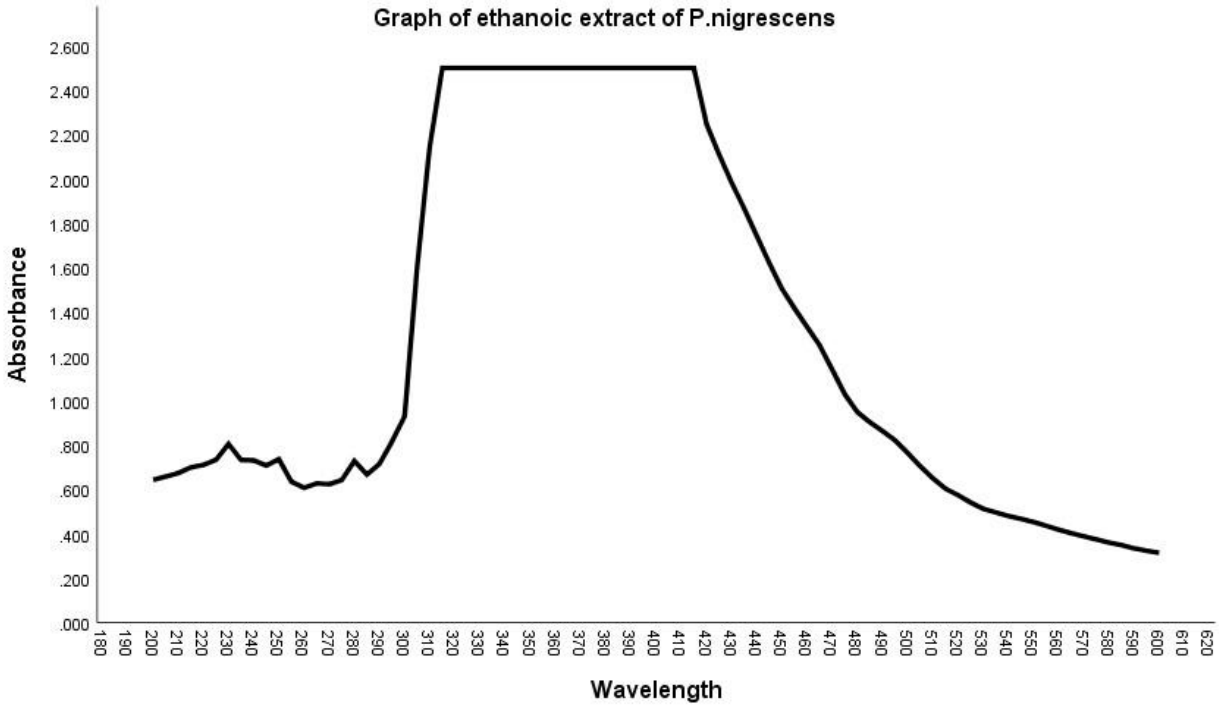

Figure 5: UV-spectroscopy of ethanoic extract of *P. nigrescens*

4.3 Gas chromatography/Mass spectrometry (GC/MS) analysis

4.3.1 Chromatogram of ethanoic extract of *P. nigrescens*

The result below shows the phytochemical components present in *P. nigrescens*

The GC-MS result showed different different compounds by showing their retention time

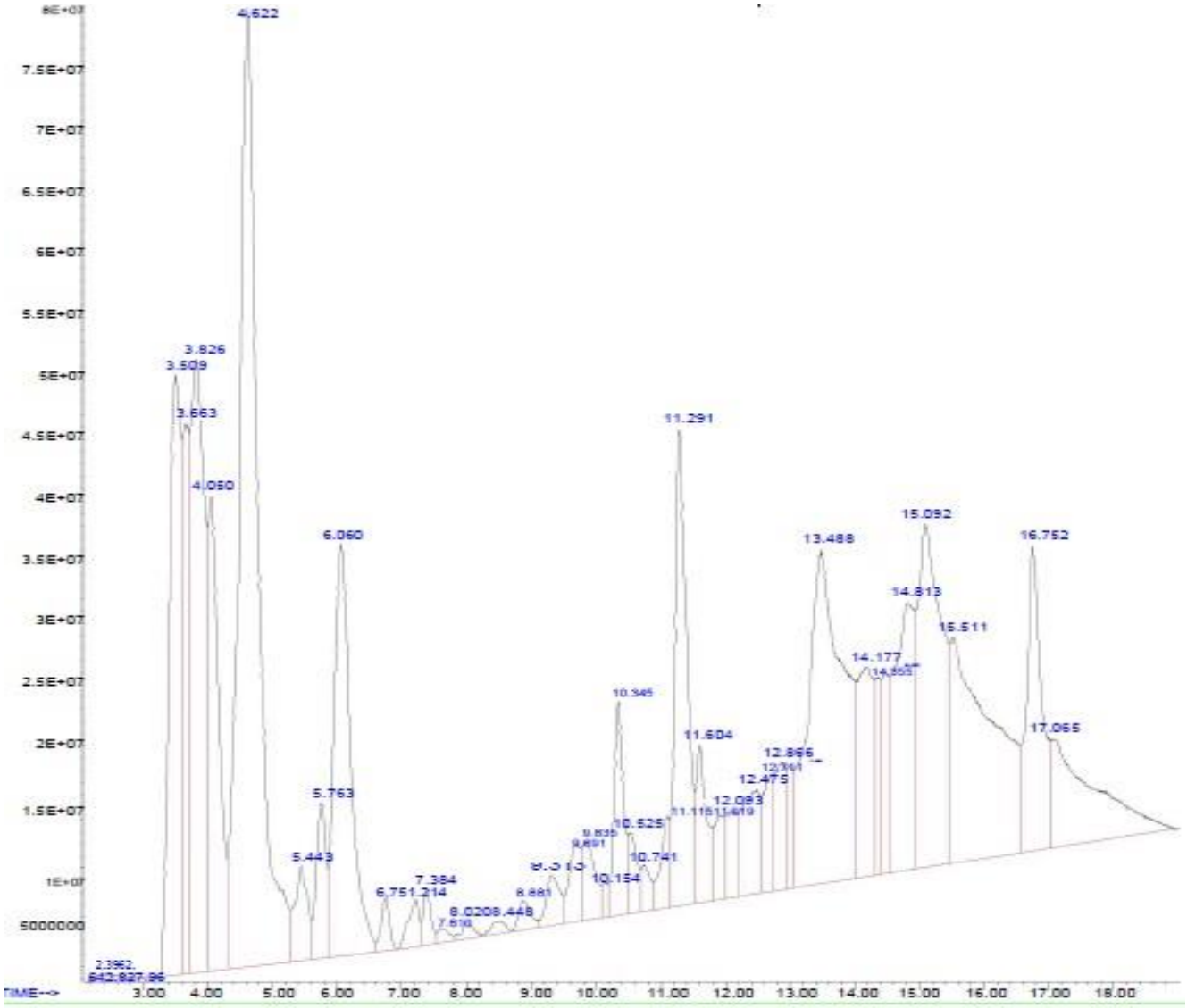

Figure 6: GC-MS chromatogram of ethanoic extract of *Parquetina nigrescens*

4.3.2 Phytochemical component of ethanoic extract of *P.nigrescens*

The result of the GC/MS analysis showed the presence of various compounds by showing their peak. The compounds were identified by their retention time, percentage of total and area percentage in the table below. It had the highest peak Cyclopropane, 1-methyl-2-(3methylpentyl) with a retention time of 4.620 and the lowest peak Glycerol triethyl ether with a retention time of 2.393. A total of 44 metabolites were identified from the leave extract.

Table 2: phytochemical components of ethanoic extract of *P.nigrescens*

Peak	**Name of Compound**	**% Area**	**Retention Time**	**Chemical Compound formula**
1	Glycerol triethyl ether	0.07	2.393	$C_{12}H_{20}O_6$
2	propriomazine	0.04	2.644	$C_{20}H_{24}N_2OS$
3	Benzo[b][1,4]diazepine-2(1H,3H)-one	0.01	2.825	$C_{15}H_{12}N_2O$
4	Fumaric acid, 2-ethoxyethyl isobutyl ester	0.01	2.975	$C_{10}H_{16}O_4$
5	D-Fructose	5.57	3.507	$C_6H_{12}O_6$
6	Hydroxylamine, O-(3-methylbutyl)-3-Buten-2ol	2.61	4.663	$C_{10}H_{18}O_3$
7	2-Hexene, 5-methyl-,	6.74	3.826	C_7H_{14}
8	Carbonyl sulfide	4. 18	4.051	COS
9	Cyclopropane, 1-methyl-2-(3-methylpentyl	14.60	4.620	$C_{10}H_{20}$
10	Furfural	1.02	5.446	$C_5H_4O_2$
11	Peroxide, dimethyl	1.19	5.765	$C_{14}H_{10}O_3$
12	Cyclopentane, 1,2-dimethyl-	5.53	6.059	C_7H_{14}
13	Pentane, 1-(1-ethoxyethoxy)-	0.35	6.753	$C_9H_{20}O_2$
14	2-Furancarboxaldehyde, 5-methyl-	0.40	7.216	$C_6H_6O_2$
15	3,4-Difluoroanisole	0.35	7.385	$C_7H_6F_2O$
16	Triethylphosphine	0.13	7.610	$C_6H_{15}OP$
17	2-[2-[2-[2-[2-[2-[2-[2-(2-Acetyloxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl acetate	0.16	8.023	$C_{10}H_{20}O5$
18	Benzeneacetaldehyde	0.14	8.449	C_8H_8O
19	t-Butyldichlorophosphine	0.30	8.880	$C_4H_9Cl_2P$
20	5-Methylhexane-2,4-dione, enol	0.56	9.312	$C_7H_{12}O_2$
21	p-Dioxane-2,3-diol	0.69	9.693	$C_4H_8O_4$
22	Octanoic acid, 2-methyl-, methyl ester	0.87	9.837	$C_{10}H_{20}O_2$
23	2,4(3H,5H)-Furandione, 3-methyl-pyridine	0.15	10.156	$C_6H_4F_4O_3$
24	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6methyl	1.59	10.344	$C_6H_8O_4$
25	Ethanamine, N-ethyl-N-nitroso	0.56	10.525	$C_8H_{10}N_2O$
26	6-Methoxy-3-pyridazinethiol	0.33	10.744	C_6H_7NOS
27	Pyrrolidine, 1-methyl-	0.70	11.13	$C_5H_{11}N$
28	5-Hydroxymethylfurfural	4.55	11.288	$C_6H_6O_3$
29	2-Methoxy-4-vinylphenol	1.27	11.601	$C9H_{10}O_2$

30	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone	0.70	11.920	$C_{13}H_{18}O_2$
31	Glutaric acid, heptyl 2-naphthyl ester	0.81	12.095	$C_{18}H_{34}O_4$
32	Succinic acid, 3-pentyl tridec-2-ynyl ester	1.44	12.744	$C_{22}H_{38}O_4$
33	4-Methyl-2-pentyl acetate	0.90	12.708	$C_8H_{16}O_2$
34	Benzaldehyde, 3-hydroxy-	1.12	12.865	$C_7H_6O_2$
35	Acetic acid, 8a-methyl-8oxodecahydronaphthalen-1-yl ester	0.65	12.984	$C_{16}H_{26}O_2$
36	Oxaziridine, 2-methyl-3-propyl-	9.39	13.490	$C_5H_{11}NO$
37	1-Cyclohexene-1-propanol, 2,6,6-trimethyl	2.61	14.178	$C_{12}H_{22}O$
38	Benzocycloheptene, 3-hydroxy-	0.85	14.354	$C_{15}H_{24}O_2$
39	Benzenebutanamine	1.24	14.460	$C_{10}H_{16}N_2$
40	Indan, 1-methyl-	4.13	14.810	$C_{10}H_{12}$
41	Pentanoic acid, 1,1-dimethylpropylester	6.45	15.092	$C_9H_{18}O_2$
42	Hexadecanoic acid, ethyl ester	7.56	15.511	$C_{36}H_{72}O_3$
43	Phytol	3.73	16.749	$C_{20}H_{40}O$
44	Methyl 2-hydroxy-octadeca-9,12,15-trienoate	3.73	17.062	$C_{19}H_{32}O_3$

4.4 Percentage organ-body weight ratio of rats used

The result obtained from the analysis of the percentage body weight ratio showed that there was a increase ($p< 0.05$) in the percentage body weight ratio in the size of all organs administered extract showing signs of inflammation.

Table 3: Effect of ethanoic extract of *P. nigrescens* on the organ body weight ratio of letrozole induced PCOS rats

	Liver	**Kidney**	**Ovary**
Control	2.67± 0.02 [a]	0.47± 0.02 [a]	0.05± 0.00 [a]
PCOS + Distilled water	2.64 ± 0.05 [a]	0.50± 0.02 [b]	0.10± 0.01 [c]
PCOS + Met + CC	3.03± 0.18 [b]	0.57± 0.02 [b]	0.07± 0.01 [b]
PCOS + 50mg/kg b.w of EEPNL	3.14± 0.11 [b]	0.54± 0.03 [b]	0.07± 0.01 [b]
PCOS + 100mg/kg b.w of EEPNL	3.07± 0.13 [b]	0.54± 0.05 [b]	0.08± 0.01 [c]

Data are means of four determination ± SEM. Values with different superscript are significally different (P<0.05).

4.5 Biochemical assessment

4.5.1 Kidney function indices

4.5.1.1 Effect of ethanoic extract of Parquetina nigrescens leaves on selected kidney function indices of letrozole induced PCOS in female rats

In the serum creatinine level there was an aggrevated increase ($P<0.05$) concentration level 0f the PCOS induced group administered distilled and the group administered metformin and clomiphene citrate compared to the control, while there was decrease ($P<0.05$) in the concentration of the PCOS induced groups administered 50mg/kg and 100mg/kg per body weight of extract when compared to the control

A substantial drop ($P<0.05$) in both the urea concentration and the BUN creatinine ratio was observed in the PCOS-induced group that was given distilled water, while the concentration level in another PCOS-induced group was increased ($P<0.05$).

All of the PCOS-induced groups had higher serum uric acid concentrations ($P<0.05$) than the control group.

Table 4: Effect of ethanoic extract of *P. nigrescens* on kidney function indices

	Creatinine (mg/dl)	**Urea (mg/dl)**	**BUN Creatinine Ratio (mg/dl)**	**Uric acid (mg/dl)**
Control	0.34 ± 0.04^{a}	1.05 ± 0.26^{a}	2.98 ± 0.44^{a}	0.64 ± 0.00^{a}
PCOS + distilled water	0.54 ± 0.00^{b}	0.89 ± 0.00^{a}	1.68 ± 0.00^{b}	2.27 ± 0.00^{a}
PCOS+ Met + CC	0.50 ± 0.00^{b}	3.57 ± 0.00^{c}	6.54 ± 0.00^{c}	3.34 ± 0.00^{a}
PCOS + 50mg/kg b.w of EEPNL	0.20 ± 0.01^{d}	1.64 ± 0.09^{b}	8.18 ± 0.20^{b}	2.12 ± 0.53^{a}
PCOS + 100mg/kg b.w of EEPNL	0.10 ± 0.00^{c}	1.49 ± 0.09^{b}	14.90 ± 0.87^{d}	7.33 ± 0.00^{b}

Data are means of four determination ± SEM. Values with different superscript are significantly different ($P<0.05$).

4.5.2 Liver function indices

4.5.2.1 Effect of ethanoic extract of *Parquetina nigrescens* leaves on the liver function indices of letrozole induced female rats.

In the serum albumin the concentration of the PCOS induced group administered distilled water and the group administered clomiphene citrate it was compared favourably ($P>0.05$) when compared to the control while there was an increase ($P<0.05$) in the albumin concentration of the PCOS groups administered the ethanoic extract

In the serum globulin level the concentration was compared favourably ($P>0.05$) to all the PCOS induced groups

In the total protein concentration level there was a decrease ($P<0.05$) in the concentration of the PCOS group administered clomiphene citrate and metformin when compared to the control, while there was an increase in all other PCOS induced groups when compared to the control,

In the direct bilirubin concentration there was a decrease ($P<0.05$) in the PCOS induced group administered distilled water and the group administered 100mg of extract, while there was an increase ($P<0.05$) in the PCOS induced group administered clomiphene citrate and metformin and the group administered 50mg of extract when compared to the control.

In the total bilirubin and the albumin globulin ratio concentration there was a decrease ($P<0.05$) in the PCOS induced group administered clomiphene citrate and metformin and the group administrated 50mg of extract while there was an increase ($P<0.05$) in the PCOS induced groups administered distilled water and the group administered 100mg of extract.

Table 5: Effect of ethanoic extract of *P. nigrescens* leaves on the concentration of the liver function indices.

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 + distilled water	6.84 ± 0.08^{a}	5.07 ± 0.15^{a}	1.77 ± 0.23^{b}	1.29 ± 0.68^{b}	2.28 ± 0.28^{a}	1.36 ± 0.05^{a}
PCOS + Met + CC	7.12 ± 0.12^{a}	6.97 ± 0.11^{a}	0.16 ± 0.20^{a}	8.56 ± 0.52^{a}	0.31 ± 0.16^{a}	1.02 ± 0.00^{a}
PCOS + 50mg/kg b.w of EEPNL	9.32 ± 1.19^{b}	7.02 ± 2.16^{a}	2.31 ± 1.11^{b}	5.53 ± 2.29^{a}	0.39 ± 0.18^{a}	0.9 ± 0.29^{a}
PCOS + 100mg/kg b.w of EEPNL	8.04 ± 0.67^{b}	5.64 ± 1.86^{a}	4.48 ± 1.74^{b}	2.80 ± 0.65^{c}	5.66 ± 4.44^{a}	3.24 ± 1.97^{a}

Data are means of four determination ± SEM. Values with different superscript are significally different (P<0.05).

4.5.3 Enzyme assay

4.5.3.1 Alanine aminotransferase activity in serum, liver of letrozole-induced rats following oral administration of ethanoic extract of *Parquetina nigrescens* leaves

Result of ALT indicated and increase ($P<0.05$) in enzyme activity in the PCOD induced groups administered both 50mg/kg and 100mg/kg of extract, while there was a decrease ($P<0.05$) in the enzyme activity of the PCOS induced group administered clomiphene citrate and metformin and the PCOS induced group administered distilled water was compared favourable ($P>0.05$) to the control in the serum.

In the liver the enzyme activity of the PCOS induced group administered distilled water increased ($P<0.05$) when compared to the control, while the PCOS induced group administered 100mg/kg of extract decreased ($P<0.05$) when compared to the control. The PCOS induced group administered metformin and clomiphene citrate and the PCOS induced group administered 50mg/kg of extract was compared favourably ($P>0.05$) to the control.

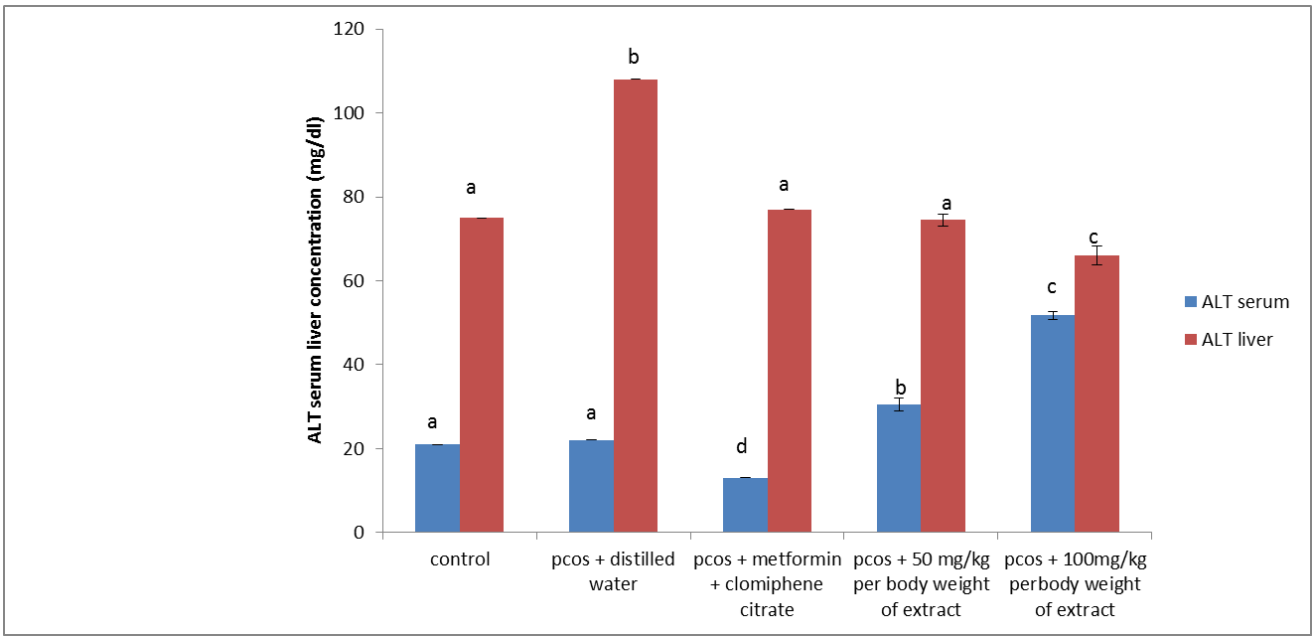

Figure 7: ALT activity in serum and liver of letrozole-induced rats following oral administration of ethanoic extract of *P. nigrescens* leaves

4.5.3.2 Aspartate aminotransferase activity of the serum and liver of letrozole-induced female rats after oral administration of ethanoic extract of *Parquetina nigrescens* leaves

The result showed a decrease ($P<0.05$) in the concentration of AST in the all the PCOS induced group both in the liver and the serum in the PCOS induced groups administered both 50mg/kg and 100mg/kg of extract .

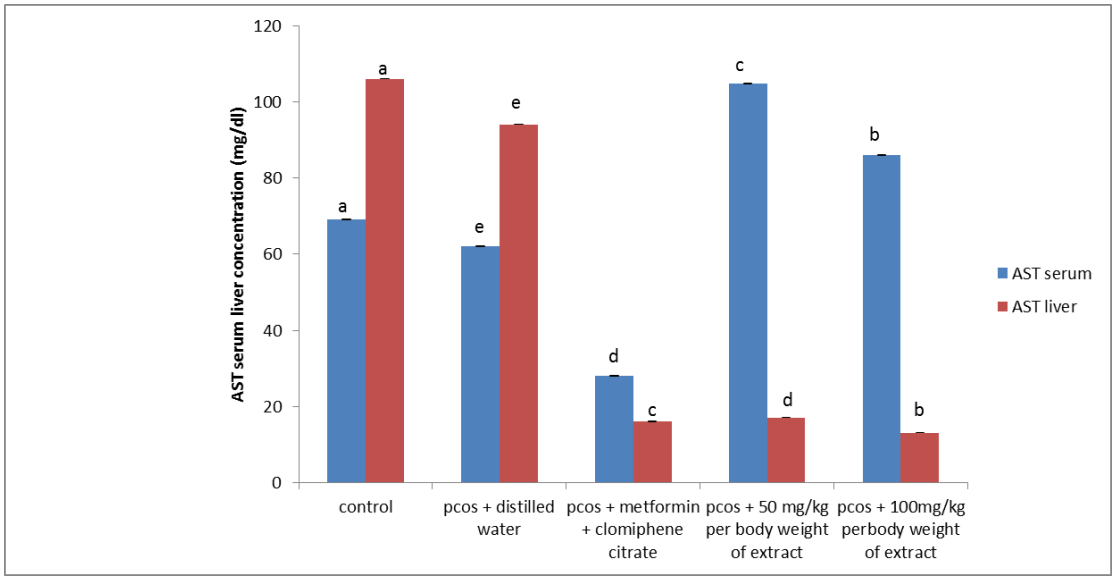

Figure 8: AST activity in serum and liver of letrozole-induced female rats following oral administration of ethanoic extract of *P. nigrescens* leaves

4.5.3.3 Alkaline phosphatase activity of the liver and serum of letrozole-induced female rats following oral administration of ethanoic extract of *Parquetina nigrescens* leaves

The result below showed a decrease ($P<0.05$) in the enzyme activity of ALP in the PCOS induced groups in the liver and serum

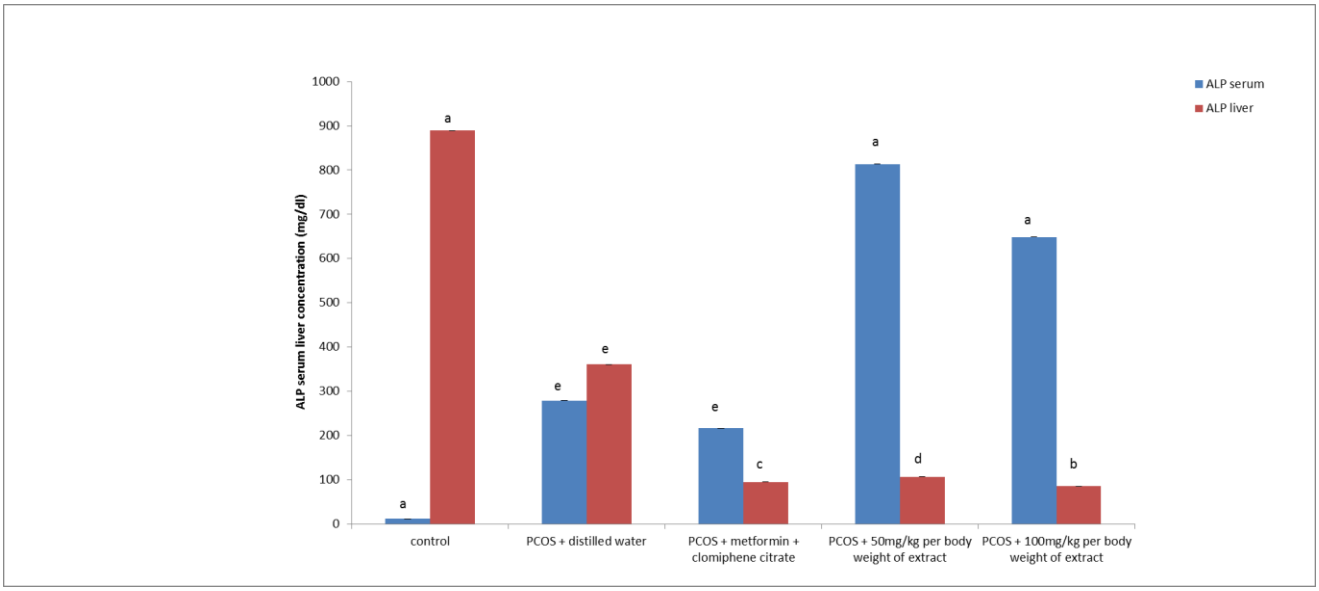

Figure 9: ALP activity in serum and liver of letrozole-induced rats following oral administration of ethanoic extract of *P. nigrescens* leaves

CHAPTER FIVE 5.0 Discussion

Phytochemical analysis of *P. nigrescens* showed the presence of secondary metabolites which has medicinal properties. The result showed secondary metabolites such as Saponins, Alkaloids, Flavonoids, Quinones, Terpenoids, Phenols, Cardiac Glycosides and Steroids. Some of the metabolite has been noted to have medicinal properties related to the treatment of PCOS.

Flavonoids are polyphenolic antioxidants with a low molecular weight that have an antiglycemic action. Genistein inhibits glucose uptake in both hormone-dependent and hormone-independent breast cancer lines and increases the expression of the glucose-regulated protein 78, which is important in cell survival (Mohammad *et al*., 2006). Flavonoids have been shown to improve heart function, reduce angina attacks, and lower cholesterol levels. These chemicals work by modulating inflammatory mediators (Sanchez *et al*., 2008). Flavonoid and flavonoid glycosides have been demonstrated to stimulate pancreatic beta cell regranulation and have been employed in clinical therapy of diabetes due to enhanced insulin sensitivity (Seeram *et al*., 2006). All its medicinal effect stated are management of sign and symptoms of PCOS

Diabetes, an endocrine condition, is characterized by hyperglycemia, which causes oxidative stress due to an excess of free radicals. Phenolic acids diminish streptozotocin toxicity by neutralizing free radicals generated in the pancreas by streptozotocin (Uttara *et al*., 2009). Streptozotocin is a naturally occurring alkylating antineoplastic agent that is particularly toxic to the insulin-producing beta cells of the pancreas in mammals. The reduction in toxicity and oxidative stress in pancreatic cells promotes beta cell proliferation and insulin secretion. Increased insulin secretion lowers blood glucose levels due to increased glucose consumption by extrahepatic tissues.

Terpenoids have been proven to lower diastolic blood pressure and blood sugar levels in hypertensive and diabetic patients (Grace *et al*., 2013).

Alkaloids have been shown to reduce blood glucose levels. The alkaloids tetrandine and berberine have been shown to exhibit antioxidant activity, which is responsible for the plant's many biological actions, including anti-diabetic effects (Yang *et al*., 2001). In mice, alkaloid fractions demonstrated hypoglycemic potential (Cassidy *et al*., 2000).

Saponins are plant chemicals that exist as steroid alkaloids, triterpene glycosides, or steroids. These phytochemicals have been shown to have anticarcinogenic, immunostimulant, and hypocholesterolaemic effects (Ros *et al*., 2000). Saponins are hypoglycemic because they stimulate pancreatic -cells, impede glucose transport across the brush border cells of the small intestines, and reduce glucose transfer from the stomach to the small intestines.

Results gotten from the evaluation of the organ body weight ratio shows that letrozole and the extract at both 50 and 100 mg/kg maybe a probable cause of inflammation or it was caused by other factors not tested.

The toxicological evaluation of the ethanoic evaluation of *P. nigrescens* on letrozole induced polycystic ovarian syndrome in female albino rats has given an insight to the effect of this leaf in the normal function of the kidney and liver of the animal.

The kidney is known for its ability to remove metabolic waste, maintain balance at optimum pH and maintain chemical balance in the blood. Creatinine urea and uric acid are considered to be metabolic waste thus high level of this in blood indicates kidney dysfunctions, damage or diseases. The absence of the change in the concentration of these metabolic waste indicates that kidney function was not affected by the administration of the ethanoic extract at 50mg/kg or the administration of letrozole in the rats. However there was an elevated level in the concentration of the uric acid and BUN-creatinine ratio of the group that was administered 100mg/kg showing that this dose affect the kidney and it function in the filtration of these waste hence this dose affect the metabolic process of the metabolites.

The liver function evaluation is an important factor in determining the appropriate dose in administration as the serum globulin, albumin, bilirubin concentration shows the secretory ability and the state of the liver. Changes in these concentrations may denote liver damage down to the type of liver damage. The administration of the extract at 100mg/kg showed a increase level of the serum concentration of these metabolites because, letrozole causes an increase in the level of the concentration of albumin, globulin and bilirubin hence, showing that this dose poses a probable damage to the state of the liver.

AST enzyme is found mostly in the liver, but also in muscles. If liver damage occurs, it releases AST into your bloodstream. Doses at both 50 and 100mg/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.

ALP enzyme help to denote the state of the plasma membrane and an increase in the level of this enzyme denotes damage done to the plasma membrane. Administration of letrozole shows a diminished level of ALP activities and after administration of 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 this enzyme similar to that of letrozole.

ALT enzyme denotes the metabolic conditions of the hepatocytes and a high level of this enzyme shows damages done to the hepatocytes. ALT levels was unchanged after administration of letrozole showing no negative effect while there was an increase in the level of this enzyme after the administration of the extract at both doses.

5.1 Conclusion

This study showed that in the liver function indices there was an aggravated increase in the concentration level of the group administered extract at both 50mg/kg and 100 mg/kg per body weight hence, the extract was highly toxic.

In the kidney function indices there was an aggravated increase in the uric acid, urea and BUN creatinine ratio showing high toxicity and in the creatinine concentration there was no toxicity in both 50mg/kg and 100mg/kg per body weight of extract.

The result of the ALT liver at both 50mg/kg and 100mg/kg there was a reduction in the concentration when compared to the untreated group showing that it reversed the damage done by PCOS. It was very similar to the effect done by metformin and clomiphene citrate hence, it is not toxic but it is advisable to use 50mg/kg body weight because it concentration was most similar to the control. In the serum there was an aggravated increase in the concentration of both 50mg/kg and 100mg/kg per body weight when compared to the control showing high toxicity.

The result of the AST at both 50mg/kg and 100mg/kg per body weight there was a significant increase in concentration showing high toxicity while in the liver at both 50mg/kg and 100mg/kg there was no toxicity noticed.

In the ALP serum there was an aggravated increase in level of the concentration showing very high toxicity at both 50mg/kg and 100mg/kg per body weight while in the ALP liver there was no toxicity noticed due to the decrease in the concentration level. In conclusion the extract was toxic at both doses to both the kidney and liver.

5.2 Recommendation

Further studies should be done on *Parquetina nigrescens* on toxicity at other mg/kg per body weight and the antioxidant and antidiabetic effect this leaves should be looked into.

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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 ($NaCO_3.H_20$) 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 curve

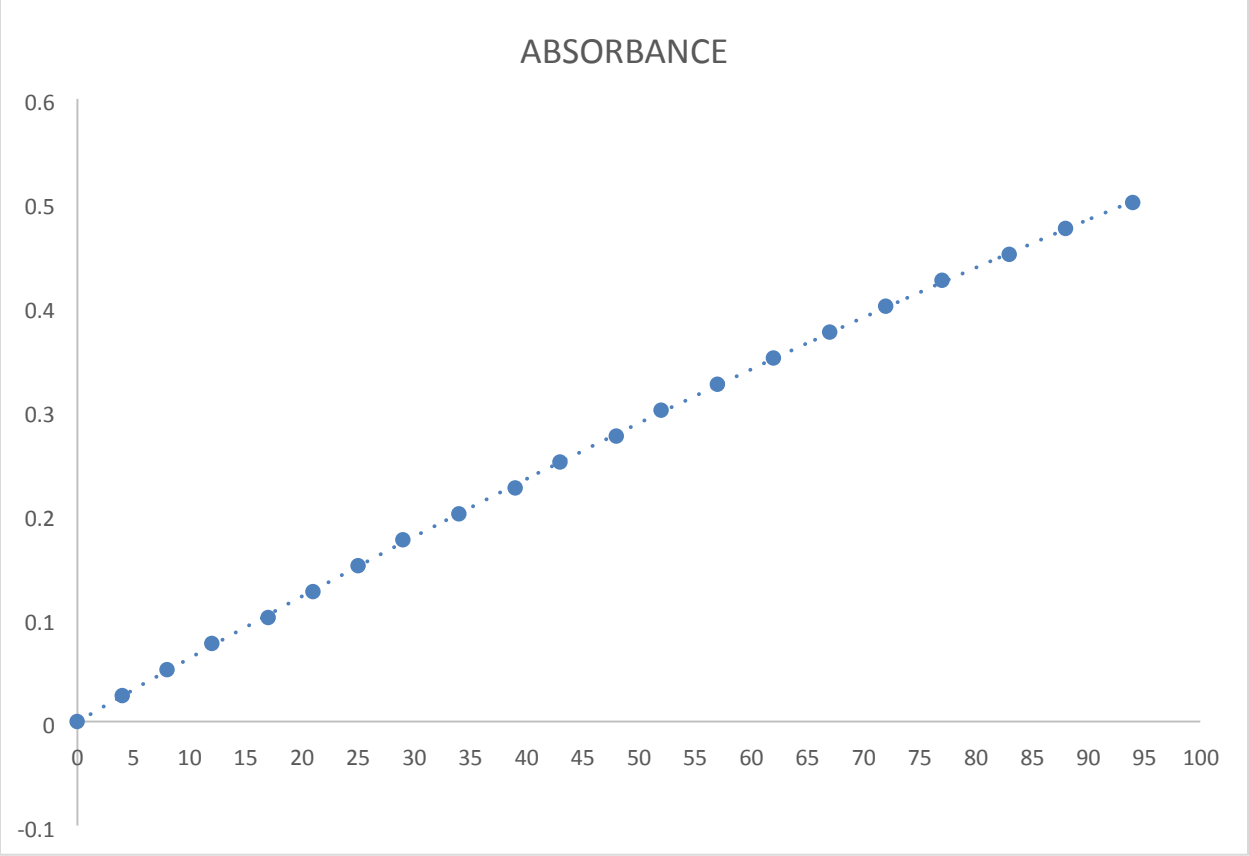

Figure 10:ALT calibration curve

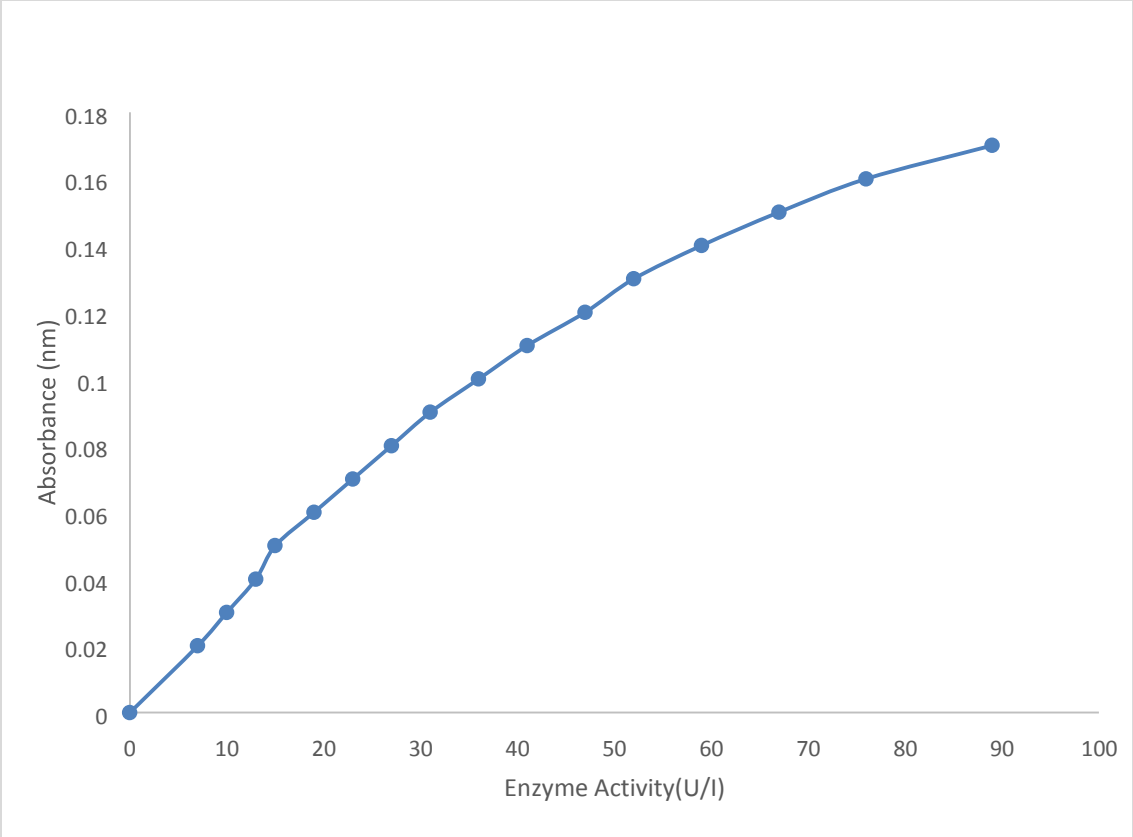

Figure 11: Calibration curve of aspartate aminotransferase