"COMBINATION THERAPEUTIC POTENTIAL OF *GYMNEMA SYLVESTRE* AND *PERGULARIA DAEMIA* ON ESTRADIOL-VALERATE INDUCED POLYCYSTIC OVARY SYNDROME IN WISTAR RATS"

A Dissertation submitted to

THE TAMIL NADU Dr. M. G. R. MEDICAL UNIVERSITY

Chennai-600032

In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

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This work is original and has not been submitted earlier for the award of any other Degree or Diploma of this or any other university.

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DECLARATION

This is to certify that the Dissertation entitled on **"Combination therapeutic potential of *Gymnema sylvestre* and *Pergularia daemia* on Estradiol Valerate induced Polycystic Ovary Syndrome in Wistar Rats"** submitted to **The Tamil Nadu Dr. M.G.R Medical University**, Chennai, is a bonafide project work carried out by myself **M.SUGANESWARI (Reg. No: 261525554)** in the Department of Pharmacology, Swamy Vivekananda College of Pharmacy, Tiruchengode, for the partial fulfillment for the degree of Master of pharmacy under the guidance and direct supervision of **Mr. P.SUDHAKAR, M. Pharm.,** in the Department of Pharmacology during the academic year of 2016-2017.

Hereby I declare that this work embedded in the thesis is original and not submitted in part or full for any other degree of this or any other university.

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(261525554)

EVALUATION CERTIFICATE

This is to certify that the Dissertation entitled on **"Combination therapeutic potential of *Gymnema sylvestre* and *Pergularia daemia* on Estradiol Valerate induced Polycystic Ovary Syndrome in Wistar Rats"** submitted to **The Tamil Nadu Dr. M.G.R Medical University**, Chennai, in partial fulfillment for the degree of Master of Pharmacy. This was carried out by **M.SUGANESWARI (Reg. No: 261525554)** under the guidance and direct supervision of **Mr. P. SUDHAKAR, M. Pharm.,** in the Department of Pharmacology, Swamy Vivekananda College of Pharmacy, Tiruchengode for the during the academic year of 2016-2017.

Internal Examiner **External Examiner**

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M.SUGANESWARI

(Reg.No: 261525554)

ABSTRACT

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Title	:	**Combination therapeutic potential of *Gymnema sylvestre* and *Pergularia daemia* on Estradiol Valerate induced Polycystic Ovary Syndrome in Wistar Rats**
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Year	:	2016-2017

Aim:

The aim of this study was to evaluate the Combination therapeutic potential of *Gymnema sylvestre* and *Pergularia daemia* on Estrodiol Valerate induced Polycystic Ovary Syndrome in Wistar Rats.

Materials and Methods:

In this study was PCOS treatment Gymnema sylvestre, Pergularia daemia and its combinations of plant powder against estradiol valerate induced rats**.** 12 weeks aged thirty Female Wistar albino rats (180-230gm) were divided in to five

groups of six animals per each. Treatment were designed as Group - I Normal Control, Group II Esradiol valerate (4mg/kg/i.p.) alone, Group III *Gymnema Sylvestre* (400mg/kg/orally) + Esradiol valerate (4mg/kg/i.p.), Group IV *Pergularia daemia* (250 mg/kg/orally) + Esradiol valerate (4mg/kg/i.p.), Group V Esradiol valerate (4mg/kg/i.p.) + Gymnema *Sylvestre* (400 mg/kg/orally) + *Pergularia daemia* (250 mg/kg/orally) (EV+GS+PD). The potency of *Gymnema sylvestre, Pergualria daemia* treatment against Estradiol Valerate induce PCOS was examine by following parameters biochemical, hormonal analysis, scan analysis and histopathological study of ovaries.

Results:

Estradiol Valerate induced groups of animals exhibited significant increased in serum Blood Glucose, Total cholesterol, Triglycerides, Body weight. The number of cystic and artretic follicles were higher in the EV-treatment group compared with the Normal control and Hormonal analysis significantly increased in LH, Estrogen, Testosterone and decreases the Progesterone & FSH level, which were reversed to normal levels in the treatment plant powder of *Gymnema sylvestre, Pergularia daemia* and its combinations.

Conclusion:

The present work was concluded the individual administration of *G.sylvestre* reduce the insulin resistance, decrease androgen production and *P.daemia* have profound beneficial effect on anovulation and menstrual irregularity. But the combination of *G.sylvestre+P.daemia* has potent synergistic activity to correct the hyperinsulinemia, anovuation and hyperandrogenism associated with PCOS. Further Isolation of active constituents and scope full clinical studies data are needed to initiate these combination for the better treatment and management of PCOS.

ABBREVIATIONS

ALP	Alkaline phosphatise
BMI	Body mass index
BPA	Bonneville Power Administration
CC	Clomiphene citrate
CVD	Cardiovascular disease
DHT	Dihydrotestosterone
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulphate
dm	Diameter
DM2	Dabeties mellitus 2
EV	Estradiol Valerate
Ee	Ethinyl estradiol
FDA	Food and Drug Administration
FSH	Follicule Stimulating hormone
G.sylvestre	*Gymnema sylvestre*
G.D+P.D	*Gymnema sylvestre+Pergularia daemia*
GnRH	Gonadotropin-releasing hormone
HDL	High Density Lipoprotein
HSD17B6	7β-hydroxysteroid-dehydrogenase type 6
IGT	Imapaired Glucose Tolerance
IU/L	International Units/liter
IAEC	Institutional Animal Ethical Committee
Kg/m2	Kilogram-meter Squared
LHR	Laser Hair Reduction
LSH	Luteinizing hormone
mm	Milimeter
ng/dl	Nanograms per decilitre
NIH	National Institutes of Health
nm	nano meter
OC	Oral contraceptives

OCP	Oral contraceptive pill
PCOS	Polycystic Ovary Syndrome
P.daemia	*Pergularia daemia*
PPAR	Peroxisome Proliferator Activated Receptor
Pvt.Ltd	Private limited
SHBG	Sex hormone-binding globulin
SD	Standard Deviation
SEM	Standard Error Mean
SGOT	Serum Glutamic-Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
T	Testosterone
TP	Testosterone Propionate
USG	Ultrasonographic
U.S.	United States
WHO	World Health Organization

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INTRODUCTION

CHAPTER-1

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrinal disorders in women, among that 4-12% of women affecting at the reproductive age. It was first described by stein and Leventhal in 1935 and hence it also known as Stein Leventhal syndrome [1]. The reproductive features of PCOS include the increased production of androgen and disordered gonadotropin secretion leading to the menstrual irregularity, hirsutism, and infertility [2]. PCOS is characterized by hyperandrogenism, elevated androgen levels, acne, acanthosis nigricans, insulin insensitivity, and chronic anovulation [3]. Long term consequences lead to cancer, type-II diabetes mellitus, dyslipidemia, hypertension, and cardiovascular disorder. The reproductive and metabolic features of PCOS are sometimes reversible with lifestyle modifications such as weight loss and lack of exercises. The etiology of PCOS is not clearly understood, but lipid imbalance, oxidative stress, insulin resistance and genetics are some of the contributing factors [4].

The pathophysiology of PCOS involves primary defects in the hypothalamic–pituitary axis, insulin secretion and ovarian function. It has been associated with insulin resistance and obesity [5]. The association with insulin resistance leads to increased production of androgen in theca cell by in the level of leutinizing hormome was increased and also the inhibition of hepatic synthesis of SHBG synthesis in liver cell. It prevents the normal follicular development in granulose cell by decrease in the level of follicular stimulating hormones which leads to follicular arrest.

Different procedures have been developed to produce PCOS model including prenatal or pre-pubertal androgen exposure, aromatase inhibitor or

estradiol administration, Testosterone Propionate (TP), Antiprogestin RU486 and Transgenic models. Exposure to a single dose of Estradiol Valerate (EV) in adult rat can cause irregular cycles, lack of ovulation and polycystic ovaries with high number of atretic follicles and cysts [6, 7]. In the present study were aimed to develop PCOS model through the EV-treated Rat.

Various treatment modalities have been employed to manage PCOS. This can be treated with medications such as clomiphene citrate (CC) is a triphenylethylene derivative that is commonly used to induce ovulation in women [8] and also used for the PCOS treatment. The various drugs including tamoxifen, aromatase inhibitors, metformin, glucocorticoids and surgically by laparoscopic ovarian drilling [9]. However, therapeutic approaches to PCOS remain an ongoing source of debate.

Many plants like *Asparagus Racemosus, Grifola frondosa, Lepidium meyenii, Tinospora Cordifolia, Curcuma longa* etc., have been highly esteemed sources which have the advantages to reduce PCOS and also having hypoglycaemic and anti-obesity effect. Nowadays some of the familiar medicinal plants and their bioactive extracts which plays a crucial role in treatment or prophylaxis of PCOS are summarized. It is concluded that the easily available beneficial herbs along with the lifestyle management is much effective in the prevention of PCOS than allopathic treatments [10].

Gymnema sylvestre (Asclepiadaceae) commonly known as “Gudmar” is a large woody, much branched climber with pubescent young parts in dry forest up to 600 mts height. *Gymnema sylvestre* leaves have been widely used in Ayurvedic traditional medicine. Leaves of the plant are used as antidiabetic, antiinflammatory, antiarthritic antiobesity, woundhealing astringent, bitter, acrid,

thermogenic anodyne, digestive and liver tonic. Tannins, flavonoids and saponins are the chief Gymnemic acid chemical constituents present in *Gymnema sylvestre.* This plant possesses many more bioactive properties such as antimicrobial, larvicidal, antiviral, hypolipidemic, anticancer, antioxidant activity [11]. *Gymnema* also indicates the treatment of PCOS, due to its insulin modulating activity and the added benefits of reducing the elevated triglycerides associated with PCOS [12].

Pergularia daemia (Asclepiadaceae) known as “Uttaravaruni” in Sanskrit, is a perennial herb growing widely along the roadsides of India. Traditionally, the plant is useful as an anthelmintic, laxative, anti-pyretic, expectorant, and used in infantile diarrhea, Phytochemically the plant has been investigated for cardenolides, alkaloids, triterpenes and saponins. The plant has been documented for anti-inflammatory, anti-pyretic and analgesic activities, antifertility, antidiabetic, wound healing, antibacterial, and hepatoprotective activity [13]. It has potential effect on normalizing menstrual irregularities and regularizing the estrous cycle. So the restoration of the estrous cycle reduces the development of follicular cyst and regained normal level of LH and FSH upon the supplementation of *Pergularia deamia* [14].

Hence the individual administration of *Gymnema sylvestre* increase the insulin sensitivity and decrease insulin resistance and also decreases the androgen production, administration of *Pergularia daemia* regulates the irregular estrous cycle and hormonal levels and also it decreases the follicular cyst development. Hence this study was aimed to evaluate the combination therapeutic potential of *Gymnema sylvestre* and *Pergularia daemia* on Estradiol Valerate (EV) induced Polycystic Ovary Syndrome in rats.

CHAPTER -2

LITERATURE REVIEW

CHAPTER-2

LITERATURE REVIEW

2.1. Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is one of the most common endrocrine disorder frequently characterised by the accumulation of numerous cysts (fluid-filled sacs) on the ovaries associated with high male hormone levels (hyperandrogenism), ovulatory dysfunction, abdominal obesity, and other metabolic disturbances [15]. Initially called the Stein and Leventhal syndrome after its discovery in the 1935s, the term also deals with the multisystem involvement including hyperinsulinism, hyperlipididaemia, increased androgens, endometrial hyperplasia, diabetes mellitus, obesity, anovulation, cardiac disease and infertility [16].

2.1.1. TWO MAIN TYPES OF PCOS

2.1.1.1. Insulin-Resistant PCOS

Insulin-Resistant PCOS is also referred to as Type 1 PCOS, and it is most often associated with the classic symptoms of PCOS. These include weight gain, ovulatory interruptions, facial hair, hair loss and acne. Those with Insulin-Resistant PCOS also exhibit a greater potential for developing diabetes and increased testosterone levels both of which are actually caused by the underlying insulin resistance. Insulin resistance alters the hypothalamic pituitary ovarian axis leads to stimulation of theca cell to produce the excess amount of androgen and reduce in the level of SHBG synthesis in liver cells resulting hyperandrogenism and anovulation leads to polycystic ovarian syndrome.

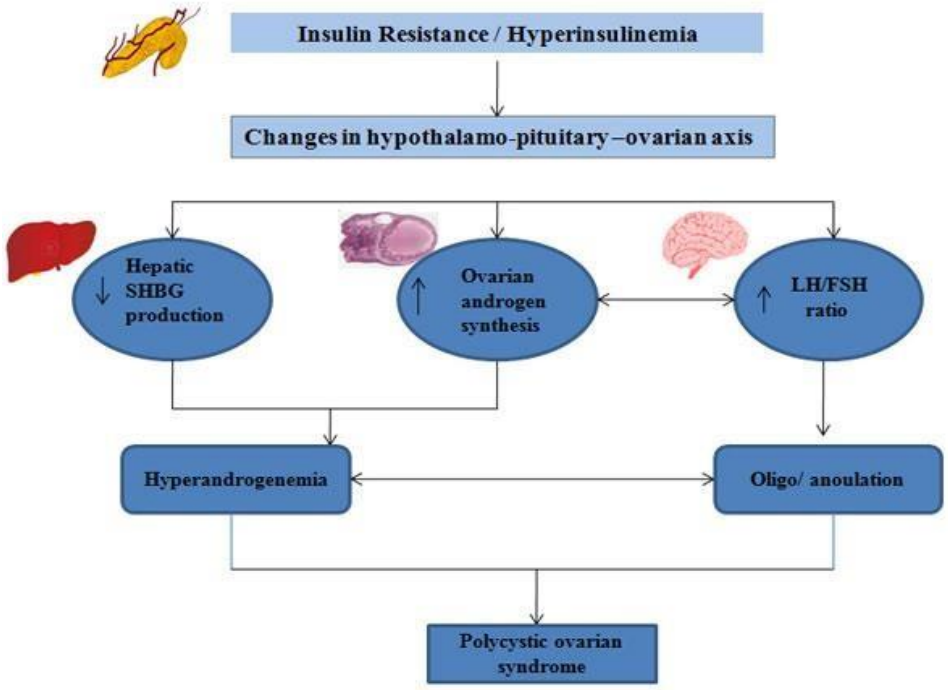


Figure No.1: Insulin Resistant Polycystic ovary syndrome

2.1.1.2. Non-Insulin Resistant PCOS

In this type of PCOS caused by Vitamin D or Iodine deficiency, hormone-disrupting toxins, thyroid disease, and adrenal stress. For women experiencing Non-Insulin Resistant PCOS, anti-Diabetic drugs will have no effect on the condition, and neither will help in reducing the weight which is gained due to hormonal imbalance. The treatment options in this case contribute to be more natural. Patients may be influenced to avoid dairy while also being prescribed supplements such as Iodine, Vitamin D, Magnesium, and Zinc, along with herbal formulas to reduce testosterone. Natural progesterone may also be prescribed in order to improve the hormonal imbalance and induce ovulation [17].

2.2. EPIDIMIOLOGY OF PCOS:

In the United States, polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders of reproductive-age women, with a prevalence of 4-12%. Up to 10% of women are diagnosed with PCOS during gynecologic visits. In some European studies, the prevalence of PCOS has been reported to be 6.5-8%.

A great deal of ethnic variability in hirsutism is observed. For example, Asian (East and Southeast Asia) women have less hirsutism than white women given the same serum androgen values. In a study that assessed hirsutism in southern Chinese women, investigators found a prevalence of 10.5%. In hirsute women, there was a significant increase in the incidence of acne, menstrual irregularities, polycystic ovaries, and acanthosis nigricans.

PCOS affects premenopausal women, and the age of onset is most often perimenarchal (before bone age reaches 16 y). However, clinical recognition of the syndrome may be delayed by failure of the patient to become concerned by irregular menses, hirsutism, or other symptoms or by the overlap of PCOS findings with normal physiologic maturation during the 2 years after menarche. In lean women with a genetic predisposition to PCOS, the syndrome may be unmasked when they subsequently gain weight.

The prevalence of hirsutism, acne, female pattern hair loss, acanthosis nigricans, seborrhea, striae, and acrochordons was found to be 78%, 48%, 31%, 30%, 29%, 13%, and 9%, respectively [18].

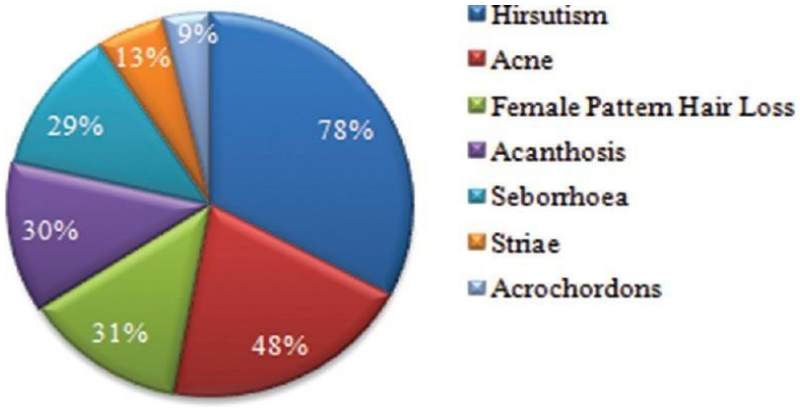


Fig No. 2: Prevalence of cutaneous manifestations of PCOS patients

2.3. PATHOGENESIS AND RISK FACTORS OF PCOS [19]

2.3.1. Genetics: PCOS is believed to be a complex disorder, with genetic as well as environmental factors contributing to development of the disease. 20-40% of female first-degree relatives of women with PCOS also have the syndrome, suggesting that the disease is partially heritable and clusters in families. Prevalence and severity of presentation vary with ethnicity, with South Asians at a higher risk of disease. Some candidate genes have been identified as contributing to risk of the disease, including 7β-hydroxysteroid-dehydrogenase type 6 (HSD17B6).

2.3.2. Intrauterine exposures: exposures to testosterone in utero may predispose to the later development of PCOS. Animal studies have demonstrated that in utero exposure is correlated with development of a PCOS-like syndrome including hyperinsulinemia, hyperandrogenism, oligoanovulation, and polycystic ovaries. Exposure to androgens may impair estrogen and progesterone inhibition of GnRH, contributing to increased pulse frequency.

2.3.3. Environment/lifestyle: several lifestyle factors and environmental exposures have been associated with a more severe PCOS phenotype. Sedentary lifestyle is associated with increased metabolic dysfunction, and weight gain is associated with oligoanovulation and hyperandrogenism. BPA and other environmental androgen-disrupting chemicals may accumulate to a greater extent in individuals with PCOS because of decreased hepatic clearance; these also induce androgen production and insulin resistance.

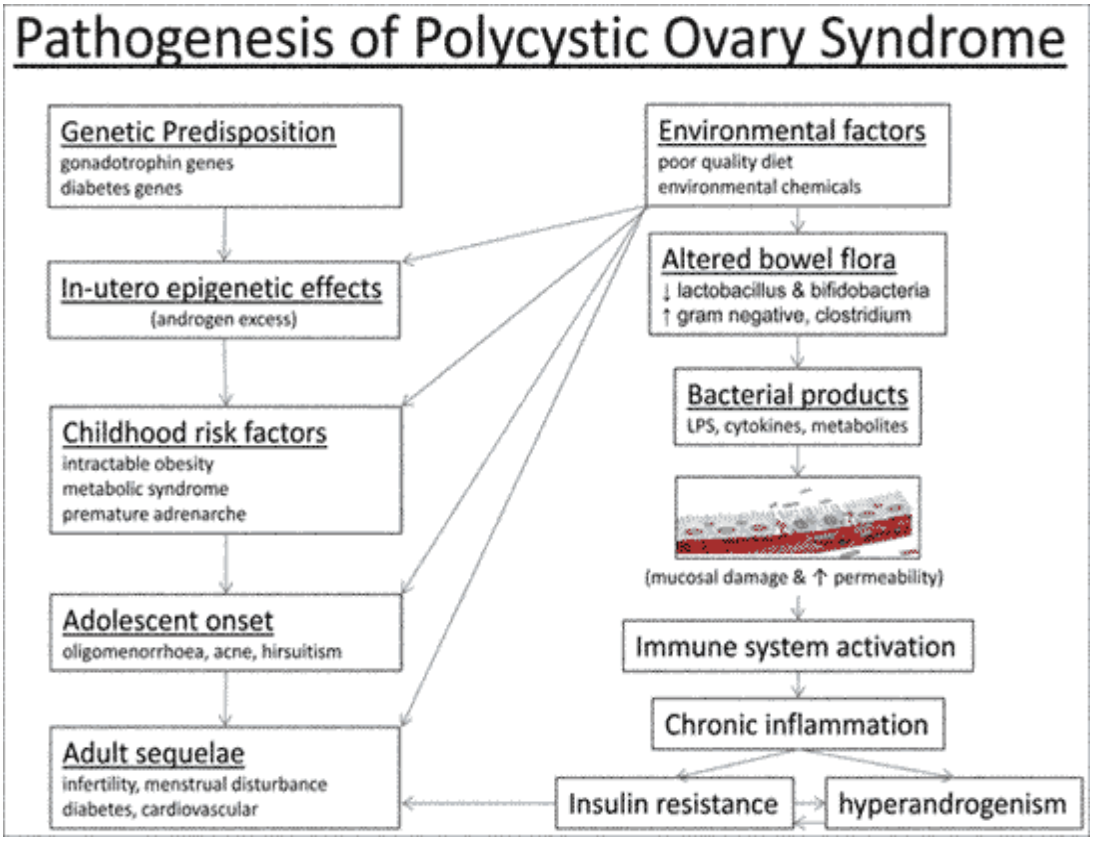


Figure No.3: Pathogenesis of Polycystic Ovary Syndrome

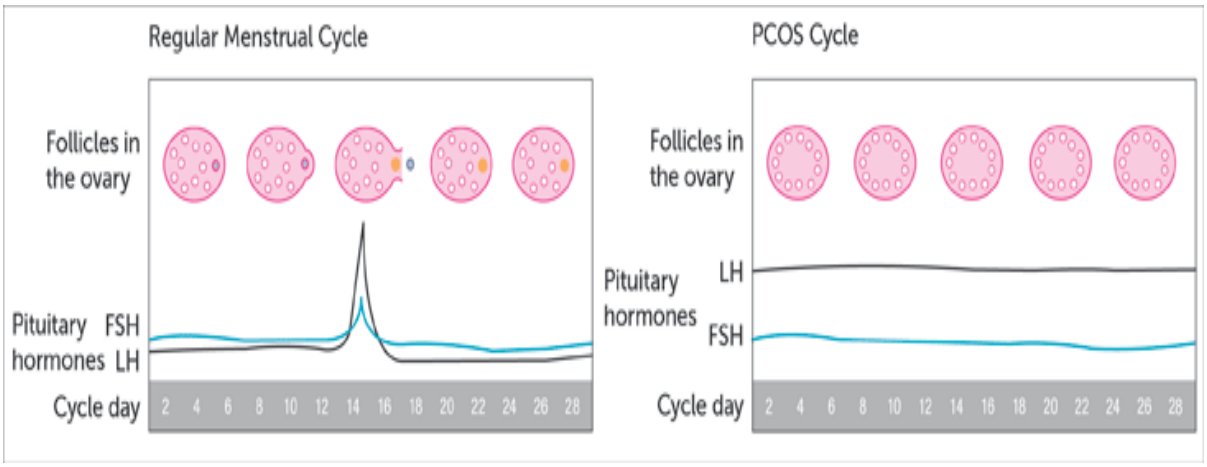
2.3.4. Obesity: although obesity is not believed to cause PCOS, it is known to exacerbate the symptoms of the disease. Obesity is present in 30-75% of women with PCOS. Adipose dysfunction contributes to the development of glucose intolerance and hyperinsulinemia, which in turn can exaggerate the

manifestations of hyperandrogenisms. Obese women with PCOS are at increased risk of anovulation and consequent sub fertility.

2.4. DIFFERENCE BETWEEN NORMAL MENSTURAL CYCLE AND POLYCYSTIC OVARIAN SYNDROME MENSTRUAL CYCLE

2.4.1. Normal Menstrual Cycles

The menstrual cycle starts when the brain sends LH and FSH to the ovaries. A big surge of LH is the signal that causes the ovaries to ovulate, or release an egg. The egg travales down the fallopian tube and into the uterus. Progesterone from the ovary causes the lining of the uterus to thicken. If the egg isn't fertilized the lining of the uterus is shed. This is a menstrual period. After the menstrual period, the cycle begins all over again. The diagram right side shows a regular menstrual cycle, and the diagram on the left side shows a PCOS cycle with no ovulation.



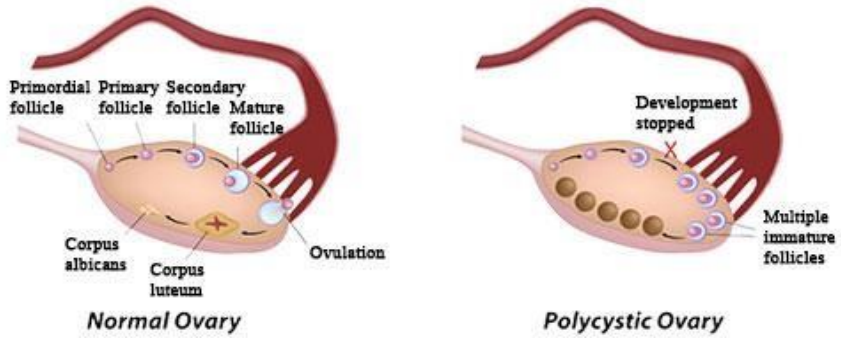


Figure No.4: Difference between Normal Menstrual Cycle and PCOS Menstrual Cycle

2.4.2. Menstrual cycle in PCOS

In women with polycystic ovary syndrome (PCOS), multiple small follicles (small cysts 4 to 9 mm in dm) accumulate in the ovary, hence the term polycystic ovaries. None of these small follicles are capable of growing to a size that would trigger ovulation. As a result, the levels of estrogen, progesterone, LH, and FSH become imbalanced. Androgens are normally produced by the ovaries and the adrenal glands. Examples of androgens include testosterone, androstenedione, dehydroepiandrosterone (DHEA), and DHEA sulfate (DHEAS). Androgens may become increased in women with PCOS because of the high levels of LH but also because of high levels of insulin that are usually seen with PCOS [20].

2.5. PCOS AETIOLOGY

2.5.1. Insulin Resistance

Insulin is a hormone produced by the pancreas to control the amount of sugar in the blood. It helps to move glucose from blood into cells, where it's broken down to produce energy. Insulin resistance means the body's tissues are

resistant to the effects of insulin. High levels of insulin cause the ovaries to produce too much testosterone, which interferes with the development of the follicles and prevents normal ovulation. Insulin resistance can also lead to weight gain. Insulin resistance is a pathophysiological contributor in around 50% to 80% of women with PCOS, especially in overweight women. Conversely, lean women and women with milder PCOS appear to have less severe hyperinsulinaemia and insulin resistance. Insulin resistance contributes to metabolic features but also to reproductive features through augmenting androgen production and increasing free androgens by reducing sex hormone binding globulin (SHBG) [21].

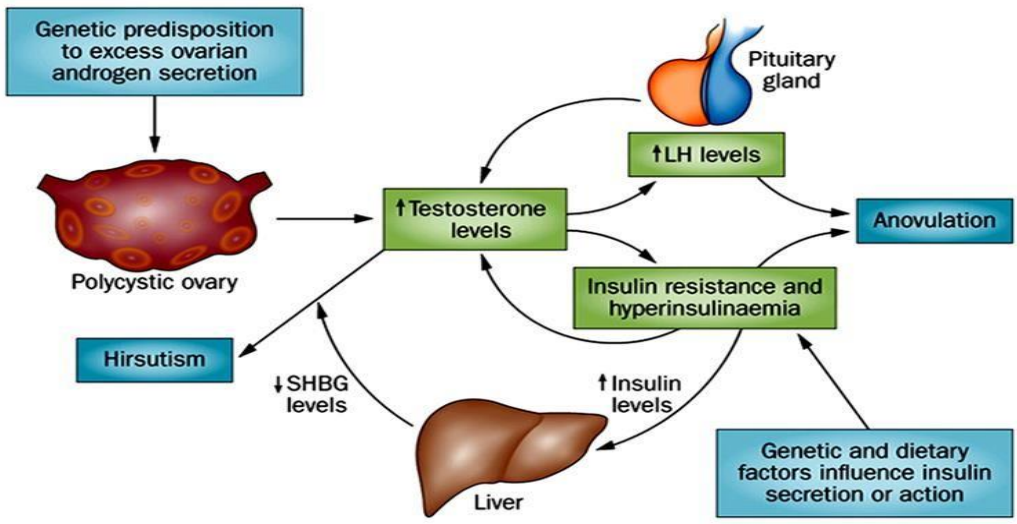


Figure No.5: Schema of aetiology and features of PCOS

2.5.2. Hormone imbalance

Many women with PCOS are found to have an imbalance in certain hormones. It includes.

- ✓ Raised levels of Testosterone (T) – a hormone often thought of as a male hormone, although all women usually produce small amounts of it.

- ✓ Raised levels of Luteinizing hormone (LH) – this stimulates ovulation, but may have an abnormal effect on the ovaries if levels are too high. Low levels of Sex hormone-binding globulin (SHBG) – a protein in the blood, which binds to testosterone and reduces its effect.
- ✓ Raised levels of Prolactin– hormone that stimulates the breast glands to produce milk in pregnancy (only in some women with PCOS)

2.5.3. Genetics

PCOS is a multi-factorial disease sometimes runs in families. If any relatives, such as your mother, sister or aunt, have PCOS, then the risk of developing it is often increased. This suggests there may be a genetic link to PCOS, although specific genes associated with the condition are still under research. The several genes proposed and investigated as the main and possibly PCOS-related genes include those that regulate the HPO axis and those associated with peripheral insulin resistance and its sequelae. Genetic and environmental contributors to hormonal disturbances combine with other factors, including obesity, ovarian dysfunction and hypothalamic pituitary abnormalities to contribute to the aetiology of PCOS [22].

2.6. CLINICAL FEATURES OF PCOS [23]

Women with PCOS may present with a variety of clinical upshots including

- ✓ **Reproductive manifestations** like Menstrual irregularaties, Hirsutism, Infertility and Pregnancy complications
- ✓ **Metabolic implications** like Insulin resistance, Obesity, Metabolic syndrome, IGT, DM2 and Potentially CVD

- ✓ **Psychological problems** include reduced quality of life, poor self-esteem, depression, anxiety etc. **(Table.1 & Figure.8)** are shows the clinical manifestations of PCOS.

Features of PCOS may manifest at any age, ranging from childhood (premature puberty), teenage (Hirsutism, Menstrual irregularities), early adulthood and middle life (infertility, Glucose intolerance) in later life (Diabetes Mellitus and Cardiovascular disease).

Manifestations of Ovarian dysfunction	Manifestations of Hyperandrogenism	Associated conditions
Oligomenorrhea Amenorrhea USG-Polycystic ovaries	Hirsutism Acne Alopecia Seborrhea Acanthosis Nigricans (Excess Circulating antigens)	Obesity Insulin resistance Impaired fasting glucose Type 2 diabetes mellitus Dyslipidemia Metabolic syndrome Mood disorders Arterial hypertension

Table No.: 1 Clinical manifestations and associated conditions

2.6.1. Reproductive Manifestations of PCOS

2.6.1.1. Ovarian dysfunction

Ovarian dysfunction usually manifests as oligomenorrhoea/amenorrhoea resulting from chronic oligo-ovulation/anovulation. However, prolonged anovulation can lead to dysfunctional uterine bleeding which may mimic more regular menstrual cycles. The majority of PCOS patients have ovarian dysfunction, with 70% to 80% of women with PCOS presenting with

oligomenorrhoea or amenorrhoea. Oligomenorrhoea occurs usually in adolescence, with onset later in life often associated with weight gain. Menorrhagia can occur with unopposed oestrogen and endometrial hyperplasia, further exacerbated by elevated oestrogen levels in obesity.

2.6.1.2. Infertility

It is the most common cause of anovulatory infertility. It accounts for 90% to 95% of women attending infertility clinics with anovulation. However 60% of women with PCOS are fertile, although time to conceive is often increased. Obesity independently exacerbates infertility, and induces a greater risk of miscarriage.

2.6.1.3. Hyperandrogenism

The clinical and biochemical signs of androgen excess in PCOS result from increased synthesis and release of ovarian androgens. Clinical hyperandrogenism primarily includes Hirsutism, Acne & seborrhea and Male pattern alopecia.

2.6.1.3.1. Hirsutism

PCOS is a common cause of hirsutism occurring in approximately 60% of cases; however this varies with race and degree of obesity. Hirsutism is defined as the presence of excessive terminal hair in areas of the body that are androgen-dependent and usually hairless or with limited hair growth, such as the face, upper lip, chin chest, abdomen, back, areolas, thighs and arms. It refers to a male pattern of body hair (androgenic hair).Normally, in females after pubarche the

major androgenic molecules are Dehydroepiandrosterone sulfate (DHEAS), Androstenedione, Dehydroepiandrostenedione, Testosterone, and Dihydrotestosterone (DHT), in descending order of serum concentration. Only the Testosterone and DHT can bind to the androgen receptor and promote hair follicle changes. Terminal hair should be differentiated from vellus hair. Terminal hair development requires androgen stimulation—as seen in pubarche, where androgens trigger vellus to mature into terminal hair and thus, hirsutism can be seen as the result of the interaction hyperandrogenemia and its influence in the hair follicle unit.

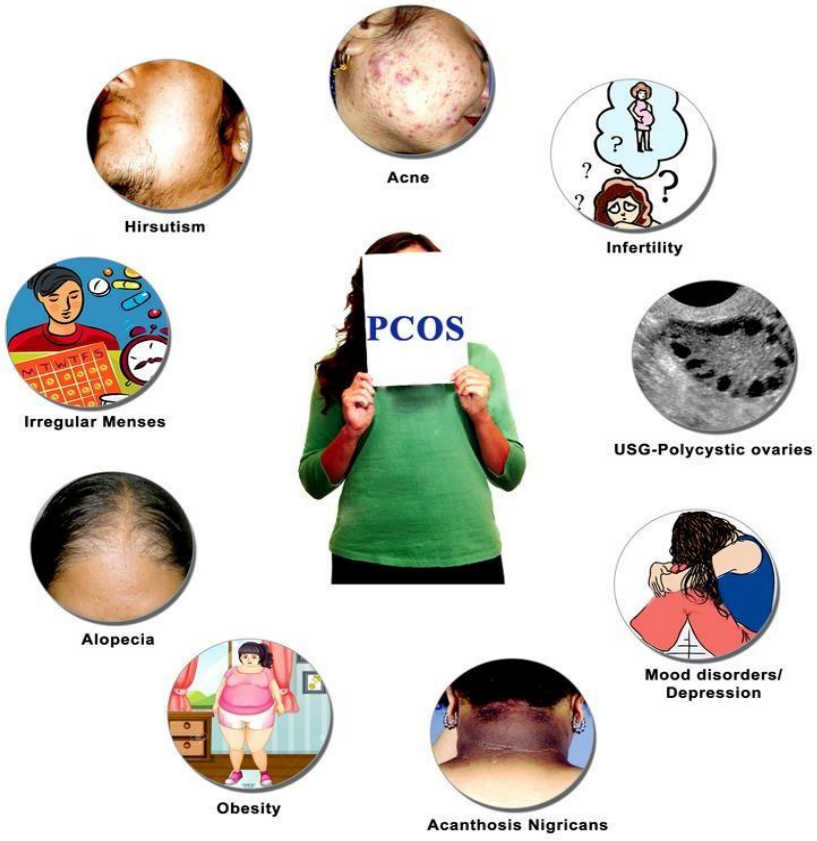


Figure No.6: Clinical manifestations of PCOS

2.6.1.3.2. Acne and Seborrhea

Acne affects one third of cases and is not particularly specific for PCOS. Sebaceous glands are also androgen-dependent structures, with sebocytes being highly sensitive to androgen signalling, which is exacerbated in PCOS, leading to the development of acne, oily skin and seborrhea. Androgens stimulate sebocyte proliferation—especially in the mid-back, forehead, and chin and secretion of sebum. Local bacteria further complicate the process by secreting lypolytic enzymes which break down triglycerides produced in the sebocyte.

2.6.1.3.3. Male pattern Hair loss (Androgenic alopecia)

Hair is miniaturized, due to an increased telogen (the resting phase of the hair growth): anagen (anagen active growth phase of hair follicles) ratio with telogen hair being at mitotical rest and anagen hair being mitotically active and associated to genetic susceptibility related to increased 5a -reductase activity in the hair follicle. This increased enzymatic activity would favor the local conversion of testosterone into DHT, a more powerful androgen. The balding pattern is dominated by the frontal and parietal scalp zones, leaving the occipital area with great hair density, as opposed to thinner and scarcer hair in the crown area.

2.6.1.3.4. Acanthosis Nigricans:

Acanthosis nigricans, a dark and hyperpigmented hyperplasia of the skin typically found at the nape of the neck, axilla, groins is a marker for insulin resistance. Many adolescents with PCOS have higher levels of insulin in their blood, excess circulating androgens leads to dark patches.

Other features of hyperandrogenism include virilisation, which, especially if presenting with clitoromegaly and rapid onset, requires exclusion of other causes including adrenal or ovarian androgen-secreting tumours.

2.6.2. Metabolic Manifestations of PCOS

2.6.2.1. Obesity

It is a key magnifying factor of PCOS. It may play a pathogenetic role in the development of the syndrome in susceptible individuals. In fact, insulin possesses true gonadotrophic function and increased insulin availability at the level of ovarian tissue may favour excess androgen synthesis. Obesity, particularly the abdominal phenotype, may be partly responsible for insulin resistance and associated hyperinsulinemia in women with PCOS. Therefore, obesity-related hyperinsulinemia may play a key role in favouring hyperandrogenism in these women. Irrespective of the pathogenetic mechanism involved, obese PCOS women have more severe hyperandrogenism and related clinical features than normal-weight PCOS women.

2.6.2.2. Dyslipidaemia

Dyslipidaemia is common in PCOS compared to weight matched controls, with higher triglycerides and lower high density lipoprotein cholesterol. It occurs independent of body mass index, however there is a synergistic deleterious effect of obesity and insulin resistance in PCOS analogous to that seen in DM2. The causes of dyslipidaemia in PCOS are again multifactorial. Insulin resistance appears to have a pivotal role mediated in part by stimulation of lipolysis and altered expression of lipoprotein lipase and hepatic lipase.

2.6.2.3. Insulin resistance and abnormal glucose metabolism

Insulin resistance occurs in around 50% to 80% of women with PCOS. Primarily in the more severe NIH diagnosed PCOS and in those who are overweight. Lean women and milder Rotterdam diagnosed PCOS appear to have less severe insulin resistance. It results in hyperinsulinaemia with its associated diverse and complex effects on regulating lipid metabolism, protein synthesis and modulation of androgen production. Women with PCOS are at increased risk of developing IGT and DM2 with prevalence rates of 31.3% and 7.5%, respectively. Also develop abnormal glucose metabolism at a younger age and may demonstrate a more rapid conversion from IGT to DM2

2.6.2.4. Cardiovascular disease risk

Alongside insulin resistance, metabolic syndrome, IGT and DM2, women with PCOS also have increased novel cardiovascular risk factors (inflammation, oxidative stress and impaired fibrinolysis)

2.6.3. Psychological Manifestation of PCOS

The challenges to familiarly identity and body image due to obesity, acne and excess hair, as well infertility and long-term health-related concerns compromise quality of life and adversely impact on mood and psychological well-being. PCOS women are more prone to depression, anxiety, low self-esteem, negative body image, and psychosexual dysfunction.

The other critical aspect of psychosocial impact in PCOS is the negative impact of mood disturbance, poor self-esteem and reduced psychological well-

being on motivation and on ability to implement and sustain successful lifestyle changes that are critical in this condition. These issues all need to be explored and addressed as part of PCOS assessment and management.

2.7. DIAGNOSTIC CRITERIA FOR PCOS

PCOS has undergone multiple sequences of Diagnostic criteria. Since 1990, various bodies have laid down criteria for the diagnosis of PCOS, based on oligo or anovulation, signs of hyperandrogenism, and ovarian sonography. Many definitions are used for diagnosis of PCOS such as National Institutes of Health (NIH) criteria, Rotterdam criteria and Androgen Excess PCOS Society criteria.

NIH 1990	Rotterdam 2003	Androgen Excess Society 2006
Must Include Chronic anovulation Clinical and/ or Biochemical signs of hyperndrogenism	Two of the following 3 1.Oligo/anovulation 2. Clinical and/ or Biochemical signs of hyperndrogenism 3. Polyctic ovaries on USG	Must Include Ovarian dysfunaction Oligo/anovulation Polyctic ovaries on USG Androgen Excess Hirsutism Hyperandrogenemia

Table No.: 2 Diagnosistic criteria for PCOS

2.7.1. NIH criteria: In 1990, a workshop sponsored by the NIH suggested that a patient has PCOS if she has oligo ovulation, signs of androgen excess (clinical or biochemical) and other entities are excluded that would cause polycystic ovaries.

2.7.2. Rotterdam criteria: In 2003, a consensus workshop held in Rotterdam indicated PCOS to be present if any 2 out of 3 criteria are met including

oligoovulation and/or anovulation, excess androgen activity and polycystic ovaries (By gynecologic ultrasound).The Rotterdam definition is wider, including many more patients, most notably patients without androgen excess. Critics say that findings obtained from the study of patients with androgen excess cannot necessarily be extrapolated to patients without androgen excess.

2.7.3. Androgen excess PCOS Society criteria: In 2006, the Androgen Excess PCOS Society suggested a tightening of the diagnostic criteria to all of the following including excess androgen activity, oligo ovulation/anovulation, polycystic ovaries and other entities are excluded that would cause excess androgen activity [24] [25].

2.8. TREATMENT OF POLYCYSTIC OVARY SYNDROME

2.8.1. Therapies aimed at treating Anovulatory Infertility

The recommended first line treatment for ovulation induction remains the antiestrogen clomiphene citrate (CC). Recommended second line intervention is either exogenous gonadotropins or laparoscopic ovarian surgery [29].There appears to be some benefit of addition of metformin to clomiphene, especially in obese subjects (modified first line treatment)**.**

2.8.1.1. Clomiphene citrate

It is a triphenylethylene derived nonsteroidal agent that is theorized to function at the level of the hypothalamus as an anti estrogen to improve gonadotropin secretion. An important concern is the relatively high rate of multiple pregnancies (7.8%) after conception, majority being twins [26].

2.8.1.2. Gonadotropins

These are frequently used to induce ovulation in women with PCOS for whom clomiphene treatment has failed. Low dose therapy with gonadotropins offers a higher rate of ovulation, monofollicular development, with a significantly lower risk of ovarian hyperstimulation syndrome [25].

2.8.1.3. Ovarian surgery

This is primarily recommended as second line infertility therapy. Multiple pregnancy rates are reduced in those women who conceive following laparoscopic drilling. In some cases, the fertility benefits of ovarian drilling may be temporary and adjuvant therapy after drilling with clomiphene may be necessary. Ovarian drilling does not appear to improve metabolic abnormalities in women with PCOS [27]

2.8.1.4. Aromatase inhibitors

Aromatase inhibitors such as letrozole and anastrazole have been proposed as both first and secondary treatment for ovulation induction (in women with PCOS and also for unexplained infertility. Results in women with PCOS appear comparable to clomiphene from small trials. Proposed benefits include oral administration, a shorter half life than clomiphene, more favorable effects on the endometrium, potentially higher implantation rates, and lower multiple pregnancy rates due to monofollicular ovulation. Their use is still experimental at this point [28].

2.8.1.5. Thiazolidinediones

Smaller trials have shown some benefit to this class of drugs for the treatment of infertility usually in conjunction with clomiphene [29]. However the

concern about hepatotoxicity, cardiovascular risk, weight gain, and the pregnancy Category C have limited the use of these drugs in women with PCOS.

2.8.2. Therapies aimed at improving Insulin sensitivity to treat anovulation and Androgen excess

A logical approach to the management of PCOS includes using life style measures and medications that improve insulin sensitivity in target tissues, achieving reductions in insulin secretion, and stabilizing glucose tolerance.

2.8.2.1. Lifestyle modification

The gold standard for improving insulin sensitivity in obese PCOS women should be weight loss, diet, exercise. It is recommended as the first line of treatment in obese women who present with infertility. Hypo-caloric diets result in appropriate weight loss in women with PCOS. Unfortunately, there have been few studies on the effect of exercise alone on symptoms in PCOS women; although it is reasonable to assume that exercise would have the same beneficial effects in women with PCOS as it does in women with type 2 DM. However the exercise program must be tailored to the degree of obesity, and the patient's baseline fitness [30] [31].

2.8.2.2. Biguanide

Metformin may be most useful in the long term maintenance of PCOS. Metformin does lower serum androgens, and improves ovulatory and menstrual frequency. Metformin is the drug of choice to treat glucose intolerance and elevated diabetes risk in women with PCOS. Its use throughout pregnancy has shown to have beneficial effects in reducing early pregnancy loss and have a

favorable effect on plasma glucose levels and other metabolic aspects [37]. Metformin is also associated with weight loss in women with PCOS, although the results in other populations are inconsistent. The dose is usually 1500-2000 mg/day given in divided doses. Metformin carries a small risk of lactic acidosis, most commonly among women with poorly controlled diabetes and impaired renal function. Other reported side effects include vitamin B12 deficiency, peripheral neuropathy [32].

2.8.2.3. Thiazolidinediones

Pioglitazone and rosiglitazone are pharmacological ligands for the nuclear receptor peroxisome proliferator activated receptor (PPAR). They improve the action of insulin in the liver, skeletal muscles, adipose tissue and have only modest effect on hepatic glucose output. Improving insulin sensitivity with these drugs is associated with a decrease in circulating androgen levels, improved ovulation rate, and improved glucose tolerance. However the concern about hepatotoxicity, cardiovascular risk, weight gain, and the pregnancy category C have limited the use of these drugs in women with PCOS [33].

2.8.3. Therapies aimed at Treating Oligomenorrhea

If the patient does not wish to conceive, medical therapy is directed towards interruption of the effect of unopposed estrogen on the endometrium. Nonfluctuating levels of unopposed estradiol in the absence of progesterone cause irregular uterine bleeding, amenorrhea, infertility and increased risk of endometrial cancer.

2.8.3.1. Combination of oral contraceptives

Oral contraceptives have been the mainstay of long term management of PCOS. They offer benefit through a variety of mechanisms, including suppression

of pituitary LH secretion, suppression of ovarian androgen secretion, and increased circulating SHBG levels. Individual OC preparations may have different doses and drug combinations and thus have varying risk–benefit ratios. Most oral contraceptives (OC) preparations contain estrogen (ethinyl estradiol 0.030 mg) in combination with antiandrogens. Anti androgens include cyproterone acetate, drosperinone, levonorgestril, norgestimate and desogestril. The “best” oral contraceptive for women with PCOS is unknown. A low dose oral contraceptive pill is therefore recommended.

Oral contraceptives may also be associated with a significant elevation in circulating triglycerides as well as in high density lipoprotein (HDL) levels, though these do not appear to progress over time. There is no evidence to suggest that women with PCOS experience more cardiovascular events than the general population when they use oral contraceptives. If a woman is taking an oral contraceptive that contains drospirenone, a progestin with antimineralocorticoid properties, it may be necessary to reduce her dose of spironolactone if used concomitantly. Regular evaluation of potassium levels is necessary [34].

2.8.3.2. Progestin

Both depot and intermittent oral medroxyprogesterone acetate (10 mg for 10 days) have been shown to suppress pituitary gonadotropins and circulating androgens in women with PCOS. No studies have addressed the long term use of these compounds to treat hirsutism. There is also a paucity of data to address the varying risk benefit ratios of varying classes of progestins. Progestin only oral contraceptives are an alternative for endometrial protection, but they are associated with a high incidence of breakthrough bleeding [35].

2.8.4. Therapies aimed at Treating Hirsuitism

No oral contraceptive has been approved by the FDA for the treatment of hirsutism. A number of observational or nonrandomized studies have noted improvement in hirsutism in women with PCOS who use oral contraceptives, but no studies of adequate power confirm their benefit in improving hirsutism in PCOS [36].

2.8.4.1. Spironolactone

It is primarily used to treat hirsutism and appears effective, though the evidence is weak. It is a diuretic and aldosterone antagonist, also binds to the androgen receptor as an antagonist. It has other mechanisms of action, including inhibition of ovarian and adrenal steroidogenesis, competition for androgen receptors in hair follicles, and direct inhibition of 5 alpha reductase activities. The usual dose is 25–100 mg twice a day, and is titrated to balance efficacy while avoiding side effects such as orthostatic hypotension. A full clinical effect may take 6 months or more. About 20% of women using spironolactone will experience increased menstrual frequency [37]. Because it can cause and exacerbate hyperkalemia, spironolactone should be used cautiously in women with renal impairment. Rarely, exposure has resulted in ambiguous genitalia in male infants.

2.8.4.2. Cyproterone acetate

It is a progestogen with anti androgen properties. It is frequently combined in an oral contraceptive tablets and is popular in the treatment of PCOS. A newer progestin from the same class, drospirenone has been marketed in the U.S. as especially effective for the treatment of female hyperandrogenism, although the data suggesting this is superior to other formulations is not based on head to

head randomized trials [38]. When given as 100mg/day, it inhibits testosterone production resulting in upto 75% decrease in circulating testosterone levels.

2.8.4.3. Flutamide

It is an androgen receptor antagonist, and is another non steroidal antiandrogen that has been shown to be effective against hirsutism in smaller trials the most common side effect is dry skin, but its use has been associated with hepatitis in rare cases. The common dosage is 250 mg/day. The risk of teratogenicity with this compound is significant, and contraception should be used. Flutamide has also been combined with lifestyle and metformin therapy for treatment of PCOS and may have additive effects [39].

2.8.4.4. Finasteride

It is a specific inhibitor of type II 5-reductase enzyme found in the hair follicles on the top of the scalp and in the sebaceous gland ducts. Its use is restricted to women in the post-menopausal group or women with documented hyperandrogenic state in the dosage of 5 mg/day. Finasteride is better tolerated than other antiandrogens, with minimal hepatic and renal toxicity; however, it has well documented risk for teratogenicity and feminising in a male fetuses, and adequate contraception should be used. Overall, randomized trials have found that spironolactone, flutamide and finasteride to have similar efficacy in improving hirsutism [40].

2.8.4.5. Ornithine decarboxylase inhibitors

These have been developed for the treatment of female hirsutism. Ornithine decarboxylase is necessary for the production of polyamines, and inhibition of this enzyme limits cell division and function in the pilosebaceous unit. Recently a potent inhibitor of this enzyme, eflornithine, has been found to be

effective as a facial cream for the treatment of unwanted facial hair. It is available as a 13.9% cream of eflornithine hydrochloride, and is applied to affected areas twice daily.

In clinical trials, 32% of patients had marked improvement after 24 weeks compared to 8% of placebo treated women, and the benefit was first noted at eight weeks. It is pregnancy category C drug. It appears to be well tolerated, with only about 2% of patients developing skin irritation or other adverse reactions. Relapse is common after stopping [41].

2.8.5. Mechanical and Cosmetic Means of Hair Reduction and Destruction

Mechanical hair removal techniques like shaving, plucking, waxing, depilatory creams, electrolysis, and laser hair reduction (LHR) offer good cosmetic relief and often are the front line of treatment used by women. A word of caution regarding facial waxing as it cans [42]. Precipitate folliculitis. Various lasers are used for hair reduction and include the Diode (800nm), Alexandrite (755 nm).It results in reduction in density and thickness of hair but if at any point in time, there is uncontrolled androgen excess, there will be a relapse in hirsutism.

2.8.6. Statins

Another area where there is emerging support in the literature for a cardiovascular and endocrine benefit in women with PCOS is the use of statins. They have been shown to improve hyperandrogenemia, lipid levels, and reduce inflammation [43]. Statins may prove to be an additional therapeutic tool for the steroidogenic abnormalities in PCOS. However, available data are limited and should be interpreted with caution until further research has been carried out. A recent study among women receiving combined treatment of statin and OCP has

shown a significant statin-attributable attenuation of clinical and biochemical hyperandrogenism in concert with amelioration of cardiovascular risk factors. This clinical study has provided support to previous in vitro findings showing that statin inhibits proliferation and steroidogenesis of ovarian theca-interstitial cells from PCOS women. Owing to the potential fetal toxicity of statins, effective contraception is essential when statin treatment is assigned in women of reproductive age.

CHAPTER-3

PLANT PROFILE

CHAPTER - 3

PLANTS PROFILE

3.1 GYMNEMA SYLVESTRE

Botanical name	:	*Gymnema sylvestre*
Family	:	Asclepiadaceae (Milk weed family)

3.1.1 Vernacular names [44]

Tamil	:	Shirukurum Kaay, Shakkaraikolli,Kokilam
English	:	Periploca of the woods
Hindi	:	Gudmar, Gurmar
Sanskrit	:	Meshashringi, Madhunashini
Malayalam	:	Chakkarakkolli
Telugu	:	Podapatri
Kannada	:	Sannagerasehambu, Kadhasige
Marathi	:	Kavali, Kalikardori
Gujarati	:	Dhuleti, Mardashingi
Siddha	:	Kannu Minnayamkodi, Passaam, Shirukurinja
Unani	:	Gurmaar Buuti

3.1.2 Taxonomical name

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Asteridae
Order	:	Gentianales

Family	:	Asclepiadaceae
Genus	:	Gymnema
Species	:	*G.sylvestre*

3.1.3 Distribution of *Gymnema sylvester*

Figure No.7: Aerial Parts of *Gymnema Sylvester &* Powder of *Gymnema sylvester*

Woody, large climber running over tops of high trees; young branches slender and pubescent; leaves opposite, simple, petioles 0.6-1.2 cm, stout or slender, lamina 2.5-6.25 cm in length, elliptic or ovate, thinly coriaceous, upper surface rarely pubescent; cymes sub globose, ± 1.25 cm in diameter; flowers yellow, ±0.2 cm in diameter; follicles slender, ±5-7.5 by 0.8 cm; seeds pale brown, flat, along with thin broad marginal wing. Distributed in: Asia, tropical Africa and Australia [45].

3.1.4. Phytoconstituents of *Gymnema sylvestre*

Plant constituents are flavones, anthraquinones, hentri-acontane, pentatriacontane, α and β-chlorophylls, phytin, resins, *d*-quercitol, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin related glycosides, stigmasterol, gymnemic acids, tartaric acid, gurmarin, calcium oxalate, glucose, saponins, stigmasterol, quercitol. The plant extract also tests positive for alkaloids. Leaves of this species yield acidic glycosides and anthroquinones and their derivatives and the amino acid derivatives betaine, choline and trimethylamine [46].

3.1.5. Pharmacological action of *Gymnema sylvestre:*

3.1.5.1. Antidiabetic Activity

The herb accounts for its sweet inactivation property to the presence of triterpene saponins known as gymnemic acids, gymnemasaponins, and gurmarin. Experimental trials confirmed the hypoglycemic effect of *G. sylvestre* on beryllium nitrate and streptozotocin treated rats. There was a slight increase in body weight and protein and a significant decrease in fasting blood glucose in diabetic rats treated with *G. sylvestre, C. auriculata, E. jambolanum*, and *S. reticulata* and the effects were quite similar to insulin and glibenclamide treatment [47].

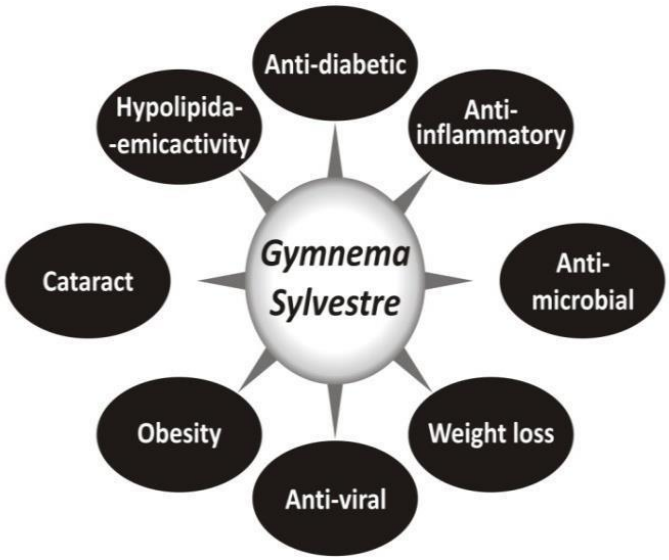


Figure No.8: Pharmacological action of *Gymnema sylvestre*

3.1.5.2. Antiarthritic Activity

The leaf extract of *G. sylvestre* was examined for antiarthritic activity on albino rats. The water soluble and petroleum ether (40–60°C) extract was found to be significantly effective in controlling arthritis. It was also assumed that the most potent antiarthritic activity of the leaves may be due to the nature of triterpenoids, steroids, and saponin glycosides [48].

3.1.5.3. Treatment of Dental Caries

Dental caries can be defined as infection of tooth, occurring due to various kinds of gram-positive cariogenic bacteria like *S. aureus, S. mitis,* and *S. mutans,* and fungus-like *Candida albicans* which attaches to the tooth surface through release of extracellular polysaccharides from sucrose and metabolize sugar to organic acid mainly lactic acid resulting in demineralization of the tooth enamel [49]. The good potential of the hydroalcoholic extract of the plant leads to the development and manufacture of gurmar tooth powdered marketed as “Gurmar

Herbal tooth paste" and "Gurmar Herbal Tooth powder." These herbal formulations offer new prospects in the treatment of dental caries once clinically approved by the scientific community [50].

3.1.5.4. Antibiotic and Antimicrobial Activity

The antibiotic and antimicrobial activity of different extracts of *G. sylvestre* was determined against a number of pathogens, namely, *S. aureus, E. coli,* and *B. subtilis* while no activity was observed against gram-negative bacteria. *G. sylvestre* leaf extracts showed good prospects as an antibiotic herbal remedy was effective as herbal formulation for the treatment of microbe's related infections. The antibacterial activity of *G. sylvestre* and gymnemic acid was also studied against *E. coli* and *B. cereus* and the antimicrobial effect was significant against the microbes [51].

3.1.5.5. Anti-Inflammatory Activity

In the Ayurvedic system of medicine, the leaf of *G. sylvestre* has been widely used and is considered as bitter, acrid, thermogenic, digestive, liver tonic, anodyne, and anti-inflammatory. The bioactive constituents in *G. sylvestre* known as tannins and saponins are responsible for the anti-inflammatory activity of the plant [52].

3.1.5.6. Anticancer and Cytotoxic Activity

Many plant-derived saponins, namely, ginsenosides, soyasaponins, and saikosaponins have been found to exhibit significant anticancer activity. Anticancer potential of gymnemagenol on HeLa cancer cell lines in in vitro conditions was determined. The cytotoxic activity of the saponins was tested by

MTT cell proliferation assay. Different concentrations of gymnemagenol (5, 15, 25, and 50 µg/mL) were taken and plates were incubated for 48 hours. The IC50 value was found to be 37 µg/mL for gymnemagenol and after 96 hours, the extract at a concentration of 50 µg/mL showed good cytotoxic activity on 73% on HeLa cells. The isolated bioactive constituent, gymnemagenol, was found to show a high degree of inhibition to the proliferation of HeLa cancer cell line [53]. With the rising percentage of cancer in people, the herbal formulation is a prospective medication in cancer therapy.

3.1.5.7. Antihyperlipidemic Activity

The prevalence of coronary artery disease is the cause of higher incidence of mortality than other causes combined. The major factor contributing to atherosclerosis and related disorders like coronary artery diseases is hyperlipidemia. Reduction in serum cholesterol levels may significantly reduce the chances of coronary heart disease. Due to the limitations of synthetic drugs in having adverse effects, plant-based formulations offer a good prospect for the treatment of heart disease. Gymnemic acids preparations have been found to be effective against obesity [54].

3.1.5.8. Immunostimulatory Activity

Immunomodulation is referred to as the regulation or control of the immunity which involves the enhancement or reduction in the immune responses. The body response to a particular condition might be regulated by agent that enhances or suppresses its action [55]. *G. sylvestre* is reported to be an immunostimulatory plant and the leaves possess immunostimulatory effect [53] the aqueous leaf extract was tested for immunostimulatory activities by detecting the

movement of neutrophils, chemotaxis tests, phagocytosis of killed *C. albicans,* and nitroblue tetrazolium assays. Aqueous leaf extract of *G. sylvestre* showed remarkable immunostimulatory activity on human neutrophils under in vitro conditions [56].

3.1.5.9. Hepatoprotective Activity

The hepatoprotective effect of hydro-alcoholic extract of *G. sylvestre* was evaluated. The rat hepatocytes (freshly prepared) were subject to treatment with different concentration of hydroalcoholic extract prepared by the hot maceration process. The *G. sylvestre* showed significant antihepatotoxicity against the D-galactosamine-induced hepatotoxicity, and the concentration was found to be cytotoxic. The cells exhibited a significant restoration of the altered biochemical parameters towards the normal when compared to D-galactosamine treated groups in a dose-dependent manner, when treated with the hydroalcoholic extract different extracts of *G. sylvestre* [57].

3.1.5.10 Wound Healing Activity

The alcoholic extract of leaves of *G. sylvestre* was found to exhibit significant wound healing activity in rats. *G. sylvestre* has good wound healing property. TLC analysis, wound contraction, and qualitative tests supported the synergistic wound healing effect of the plant. The increased wound healing activity of hydroalcoholic extracts may be attributed to the free radical scavenging action and the presence of phytoconstituents (flavonoids) which may act individually or have additive effect [58].

3.2 *PERGULARIA DAEMIA* [59]

Botanical name	:	Pergularia daemia
Family	:	Asclepiadaceae

3.2.1 Vernacular names

Tamil	:	Uttamani, Veliparuthi, Beliparti, Nandamani,
English	:	Hariknot plant
Hindi	:	Utaran, sagovani, Aakasan, Gadaria Ki bel,Jutak
Sanskrit	:	Uttamarani, Kurutakah, Visanika, Kakajangha
Malayalam	:	Veliparatti
Telugu	:	Dustapuchettu, Jittupaku, Gurtichettu
Kannada	:	Halokoratige, Juttuve, Talavaranaballi
Marathi	:	Utarn
Gujarati	:	Chamardudhi
Bengali	:	Chagalbati, Ajashringi

3.2.2 Taxonomical name

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Subdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Asteridae
Order	:	Gentianales
Family	:	Asclepiadaceae

Genus : Pergularia

Species : *P. daemia* (Forsk) Chiv

3.2.3 Distribution of *Pergularia daemia*

Figure No.9: Aerial Parts of *Pergularia daemia*

Pergularia daemia is a perennial twining herb, foul-smelling when bruised; Stems bears milky juice and covered with longer stiff erect hairs 1mm; Leaves are thin, broadly ovate and heart-shaped 2-12 cm long, covered with soft hairs; Greenish yellow or dull white, sweet-scented flowers born in axillary, double white corona at the base of a stamina column, long-peduncled, umbellate or corymbose clusters tinged with purple; Fruits paired with follicles 5.8 cm long and 1 cm in diameter, reflexed, beak long, covered with soft spinous outgrowth and release many seeds with long white hairs when they split open. Seeds are densely velvety on both sides. The entire plant constitutes the drug and is used as a medicine [60].

3.2.4 Phytoconstituents of *Pergularia daemia*

Most commonly found phytochemicals from the leaves of *P. daemia* are flavonoids alkaloids, terpenoids, tannins, steroids and carbohydrates. Phytochemical studies have shown the presence of cardenolides, alkaloids, triterpenes (lupeol), saponins, steroidal compounds. The seeds of *P. daemia*

contain uzarigenin, coroglaucigenin, calactin, calotropin, other cardenolides and a bitter resin, Pergularin and have a cardiotoxic action. It has been suggested that the plant seed action on the uterus is similar to that of pituitrin and is not inhibited by progesterone [61].

3.2.5. Pharmacological action of *Pergularia daemia*

Pharmacological activity of the *Pergularia daemia* such as Anti-inflammatory, analgesic and antipyretic activity, Anti diabetic activity, Anti fungal activity, Anti bacterial activity, Central nervous system depressant activity, Hepatoprotective activity, Anti oxidant activity, Anticancer activity [62].

CHAPTER-4

AIM AND OBJECTIVE

CHAPTER- 4

AIM AND OBJECTIVES

Polycystic ovary syndrome is one of the most common endocrine metabolic disorders characterised by polycystic ovaries, chronic anovulation and hyperandrogenism leading to symptoms of menstrual irregularity, hyperinsulinimia and obesity.

In current allopathy medication such as clomiphene citrate (CC), tamoxifen, aromatase inhibitors, metformin, glucocorticoids and surgically by laparoscopic ovarian drilling are may less effective for the polycystic ovarian syndrome and also it will produce the adverse effect and high cost. But, now a day's Phytomedicines are effective for the Potential treatment of polycystic ovary syndrome.

Many plants like *Asparagus Racemosus, Grifola frondosa, Lepidium meyenii, Tinospora Cordifolia, Curcuma longa* etc., have been highly esteemed sources which have the advantages to reduce PCOS and also having hypoglycaemic and anti-obesity effect. Nowadays some of the familiar medicinal plants and their bioactive extract which plays a crucial role in treatment of PCOS. The easily available beneficial herbs along with the lifestyle management are much effective in the prevention of PCOS than allopathic treatments.

The treatment of herbal plants *Gymnema sylvestre* to increase the insulin sensitivity and decrease excess of insulin level, it increase the SHBG synthesis in liver cells leads to decreases in the level of androgen production. *Pergularia daemia* regulate the estrus cycle and hormonal levels and also it decreases the

follicular cyst development. It is well known treatment of Polycystic Ovarian Syndrome in regularized pattern of estrus cycle.

Hence objective of this study is to evaluate the *Gymnema sylvestre* and *Pergularia daemia* plants individual and combination effect on

- Hyperinsulinemia
- Irregular mensutural cycle and
- Follicular cyst developments in estrodiol valerate induced PCOS in rats.

CHAPTER- 5

PLAN OF STUDY

CHAPTER-5

PLAN OF STUDY

1. Collection of Plant materials
2. Pharmacological study design
    a) Selection of Animals
    b) Animal Grouping
3. Induction of Polycystic Ovary Syndrome in rats
4. Physical Evaluation
    a) Body weight
    b) Feed intake
5. Examination of Vaginal smear
6. Biochemical Estimation
    a) Estimation of Blood glucose
    b) Estimation of Total Cholesterol
    c) Estimation of Triglycerides
    d) Estimation of serum glutamic-oxaloacetic transaminase (SGOT)
    e) Estimation of serum glutamic pyruvic transaminase (SGPT)
    f) Estimation of Alkaline phosphatase (ALP)
    g) Estimation of Urea
    h) Estimation of Creatinine
7. Hormonal Determination
    a) Determination of LH
    b) Determination of FSH
    c) Determination of Total Testosterone
    d) Determination of Estrogens

e) Determination of Progesterone

8. Ultrasound Scan Analysis
9. Measurement of Organs Weight
10. Histopathological Examination
11. Statistical Analysis

CHAPTER 6

MATERIALS AND METHODS

CHAPTER - 6

MATERIALS AND METHODS

6.1. Drugs and chemicals

All the chemicals and drugs used in this study were of analytical grade. The following chemicals were used for the experimental study.

S.No.	Material	Source
1.	Estrodiol valerate	Bayer Zydus Pharm Pvt. Ltd., Theane
2.	*Gymnema sylvestre* powder	Genius Nature Herbs Pvt. Ltd. Coimbatore
3.	*Pergularia daemia* powder	Genius Nature Herbs Pvt. Ltd. Coimbatore
4.	Methylen blue	S.d fine chemicals Ltd. Mumbai
5.	Formic acid	NICE chemicals Pvt. Ltd. India
6.	Diethyl ether	LOBA Chemie Pvt. Ltd. Mumbai
7.	Choloroform	LOBA Chemie Pvt. Ltd. Mumbai
8.	Biochemical diagnostic kit	Thermo Fisher Scientific. India
9.	Enzyme immunoassay kit	Thermo Fisher Scientific. India

6.2. Pharmacological study Design

6.2.1. Selection of Animals

The colony inbred adult Female Albino Wistar Rats, weighing 180-230gm were obtained from Central Animal house of Swamy Vivekanandha College of Pharmacy, Elayampalayam, Namakkal – 637 205. The animals were kept under standard environmental conditions of 12/12 light/dark rhythm, maintained under controlled room temperature (23±2ºC) and a relative humanity of 60%±10% in polypropylene cages. They were fed with standard pellet diet and water *ad libitum.* Each cage contained 3 rats of the sex with a bedding of husk. The

immature animals were acclimatized under laboratory conditions three days prior to initiation of the experiment. The cages were cleaned daily by changing the husk bedding.

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Swamy Vivekanandha College of Pharmacy, Elayampalayam, Namakkal – 637 205. Care and use of laboratory animals were confirmed to CPCSEA guidelines.

IAEC Reference No: SVCP/IAEC/PG/1/03/2017 dated 09.12.2016

6.2.2. Animal grouping

12 weeks aged thirty wistar female rats weighing 180-230 gm were randomly divided into five groups of six per each. The groups and treatment are designed as follows

Group I: Control receives 0.4ml in Sesam oil i.p. + Distilled water p.o.

Group II: PCOS control receives Estradiol valerate (4mg/kg in 0.4ml Sesam oil i.p.)

Group III: *Gymnema Sylvestre* (400mg/kg/p.o.) + Estradiol valerate (4mg/kg in 0.4ml Sesam oil i.p.).

Group IV: *Pergularia daemia* (200mg/kg/p.o) + Estradiol valerate (4mg/kg in 0.4ml Sesam oil i.p.)

Group V: Estradiol valerate (4mg/kg in 0.4ml Sesam oil i.p.) +Gymnema *Sylvestre* (400mg/kg/p.o) + *Pergularia daemia* (200mg/kg/p.o) (EV+GS+PD)

Estradiol Valerate (4mg/kg) was administered in all group of animal except Normal control. Normal control was receives o.4 ml of sesam oil on first day. After inducing at 60th days the vaginal smear were taken and conform the menstrual irregularity of the animals and serum blood glucose level was measured to monitor the insulin resistance. After conformation of PCOS the treatment was started with *Gymnema sylvestre* (400mg/kg), *Pergularia daemia* (200mg/kg) and combination [*G.D* (400mg/kg) +*P.D* (200 mg/kg)] for 14th day.

6.3. Induction of Polycystic Ovary Syndrome in rats

Induction of PCOS in the rats was carried out by administration of Estradiol Valerate Intraperitonial route at the dose of 4mg/kg for single dose [63].

6.4. Physical Evaluation

6.4.1. Measurement of Body weight

Body weight of each rats in all groups were measured weekly till end of the treatment using a weighing balance and the changes were recorded.

6.4.2. Measurement of Feed Intake

Daily Feed consumption was measured in individual treatment groups by using standard weighing balance.

6.4.3. Examination Vaginal Smear

During a study period the smear was taken from the entire rat. The regularity of period was observed the hence under the microscope and photographed [64].

6.5. Biochemical Determination

On 74th day of the study, the animals were anesthetized with diethyl ether. The blood was drawn through retro orbital plexus and the serum was separated after centrifugation of total blood without anticoagulants, at 3000rp, for 10 min. The analysis of Blood glucose [65] Total cholesterol, [66]. Triglycerides, [67] serum

gluctamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), [68] Alanine aminotransferase (ALT), [69] Urea, Creatinine [70] were estimated in serum by standard laboratory technique.

6.6. Hormonal Determination

The Serum Luteinising hormone (LH), Follicule stimulating hormone (FSH), Testosterone Estrogen and Progesterone were measured using an enzyme immunoassay kits by standard laboratory techniques [71].

6.7. Ultrasound Scan Analysis

At end of the study animal were anesthetizedand placed in ultrasound scanner for the scan analysis of the ovary. Scan was taken at the ovary for the development of cyst in estradiol induced rats [72].

6.8. Measurement of Organs Weight

At the end of study all the animals were sacrificed Liver, kidney, heart, uterus and ovary were removed and subjected to gross examination and later weighted.

6.9. Histopathological Evaluation

Histopathological evaluation was performed the ovaries of control and experimental groups were excised immediately after treatment, cleaned from fats and weighed. The excised ovaries were fixed in 10% neutral formal saline, embedded in paraffin wax, and then sectioned serially at 5-µm thickness. Sections were mounted and stained by the haematoxylin and eosin procedure. Also some sections were stained by Masson's trichrome (M.T.) and under light microscope with 100x magnification for histopathological changes [73].

6.10. Statistical analysis

The data represents as mean ± SEM to determine significance and when animals were compared over time or within multiple groups, we used one-way analysis of variance (ANOVA) followed by post hoc Dunnet's test by using Graph Pad Statistical Package software. The values were considered significant when $P<0.05$.

CHAPTER-7

RESULTS

CHAPTER-7

RESULT

7.1. CHANGES IN BODY WEIGHT

The changes in body weight during the trial period was measured, initial and final body weight changes in treatment group was compared with control group (Group-I) initially there were no significant changes in body weight between treatment groups when compare to the control group. On day 74th final body weight of Estradiol Valerate (4mg/kg) treated Group II showed significant increase in body weight when compare to other treatment groups **(Table 4 & Graph 1)**.

7.2. Effect of *Gymnema sylvestre, Pergularia daemia* and *G.sylvestre* + *P.daemia* on Feed intake in EV induced PCOS rats.

Table 5 & Graph 2 illustrated that effect of *G.sylvestre, P.daemia and G.sylvestre + P.daemia* on feed intake in EV induced rats, there is no significant different in feed intake between the treatment groups when compared to the Vehicle control group (Group-I). Only in Group-II shows little decrease in feed intake.

7.3. Effect of *Gymnema sylvestre, Pergularia daemia* and *G.sylvestre* + *P.daemia* on Menstural changes in EV induced PCOS rats.

On 60th day irregularity of menstrual cycle observed in PCOS induced rats as respect from first day. After the 14 days of treatment of *P.daemia* and combination (*G.sylvestre+P.daemia)* regulate the irregularity of menstrual cycle.

Fig No. 10 Represent pictures from methylene blue stained samples from vaginal smear at different phase of the estrous cycle. At diestrus, only leukocytes

can be observed, accompanied by few epithelial nucleated cells. At proestrus, mostly nucleated and few cornified cells are present along with some leukocytes. In estrus (at receptivity), only cornified cells are present. At metestrus, cornified epithelial cells and leukocytes are present.

7.4. Effect of *Gymnema sylvestre, Pergularia daemia* and *G.sylvestre+P.daemia* on Blood glucose in EV induced PCOS rats.

The blood glucose level was no changes on first day in all groups. At day 60 significantly (P<0.001) increase in blood glucose EV induce PCOS group compare to the Normal control. At 74th the blood glucose level was significant (P<0.001) increase in Group-II compare the Normal control and significantly (P<0.001) decrease in treatment groups as compare to the Normal control. As compare to individual treatment *G.Sylvestre, P.daemia* combination possesses more significant decrease in Blood glucose level. **(Table 6 &Graph 3)**

7.5. Effect of *Gymnema sylvestre, Pergularia daemia* and *G.sylvestre+P.daemia* on Biochemical Parameters in EV induced PCOS rats.

Total cholesterol & triglycerides was significantly (P<0.001) increase in PCOS control when compare to the Normal control & *G.sylvestre, P.daemia &* combination *(GS+PD)* treated group shows significant (P<0.001) decrease in Total cholesterol & Triglycerides level. **(Table.7) Graph.4&5)**

The SGOT level was significantly (P<0.001) increased in Group-II, more significant (P<0.001) decrease in Group-III, Group-IV and Group-V as compare to the Normal control. SGPT level was significantly (P<0.05) increase in Group-II as compare to the Normal control. *G.sylvestre, P.daemia* & *G.sylvestre + P.daemia*

combination were siginificantly (P<0.05) increase as compare to the Normal control. EV group have more significant (P<0.001) as compare to the Normal control. *G.sylvestre, P.daemia* and Combinations shows significant (P<0.001)) increased in ALP as compare to the Normal control. Serum Urea, Creatinine level was significantly (P<0.001) & (P<0.05) increased as compare to the control group. **(Table.8&9) Graph.6-10)**

7.6. Effect of *Gymnema sylvestre, Pergularia daemia* and *G.sylvestre+P.daemia* on Hormonal changes in EV induced PCOS rats.

Table 10 represent the hormonal changes level in PCOS induced rats .LH, Testosterone, Estrogen were increased significantly (P<0.001) in PCOS control with concominant decreases (P<0.001) in FSH, Progesterone level. These changes were reverted to near normal level in all treatment groups. **(Graph 11-15)**

7.7. Ultrasound Scan Analysis

Fig No: 11 a) shows no changes in ovary in Normal control. **Fig No.11 b)** shows PCOS control with increases number of follicles and cysts. **Fig No.11 c)** Treatment of *G.sylvestre* shows decrease amount of cyst formation with normal growth of follicles. **Fig No.11 d)** *P.daemia* treatment shows the normal follicular developments **Fig No: 11 e)** Combination treatments shows no evidence of PCOS.

7.8. Measurement of Organs Weight

The weight of liver & Ovary significantly (P<0.05) increase in the PCOS control as compare to the normal control. Group-III, IV&V showed significantly

decrease in liver & Ovary weight. The group III, IV & V showed greater reduction in the ovary and liver weight as compare to the PCOS control. But no significant changes in kidney, Uterus and heart in the entire treatment group as compare to the normal control.

7.9. Histopathological Examinations

Histopathological examination of ovary section of normal control **(Fig No.12 a)** showed no histopathological structure alteration of ovary. Micrograph of ovary section of Estradiol induced PCOS group showed number of polycyst and follicular development with theca lutein cells **(Fig No.12 b).** The *G.Sylvestre* treatment group showed the minimum number of follicule with saccular dilation increased **(Fig No.12 c)**. The *P.daemia* treatment group showed the granulose cells proliferated with follicles **(Fig No.12 d)**. The micrograph of combination treatment with Combination shows no evidence of histological alteration in ovary **(Fig No.11 e)**

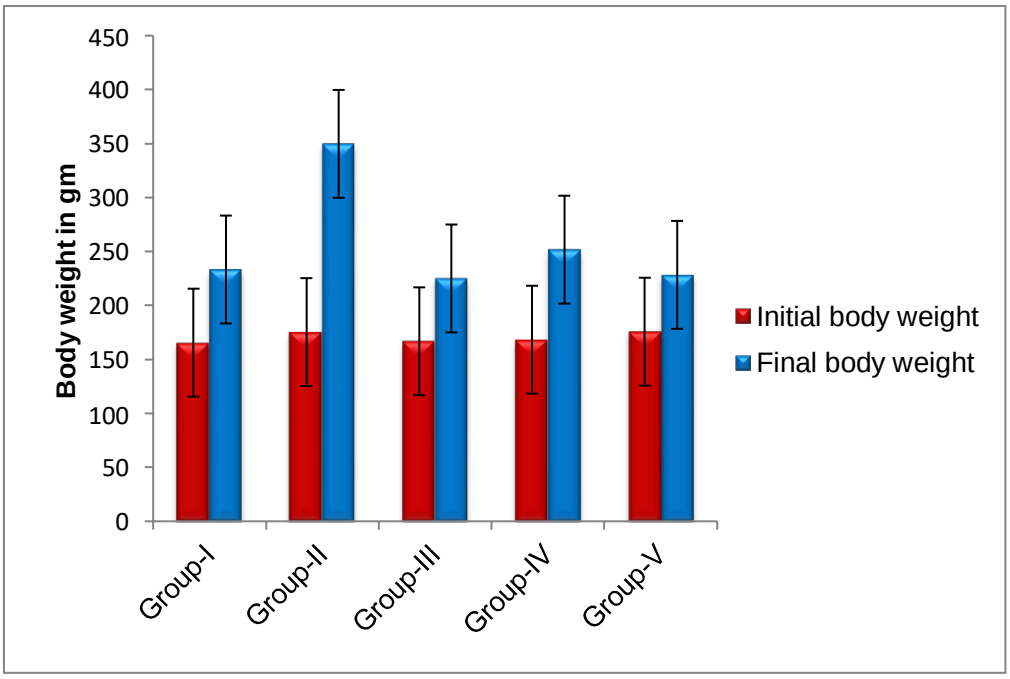
Table No.3: Changes in Body weight

Treatment	Initial body weight (g)	Final body weight (g)
Group I (Vehicle Control)	165.5±11.03	233.3±16.26
Group II (Estradiol Valerate)	175.3±12.07	349.7±14.73 a***
Group III (EV+*G.sylvestre*)	166.8±11.16	225.0±10.57 b***
Group IV (EV+*P.daemia*)	168.3±8.72	251.7±6.54 b***
Group V (EV+G.S+P.D)	175.7±10.07	228.3±15.79 b***

Values are expressed as mean ± SEM, n-6. Comparisons were made between:

a- Group I vs II, III and. b- Group II vs I, III, IV, V.

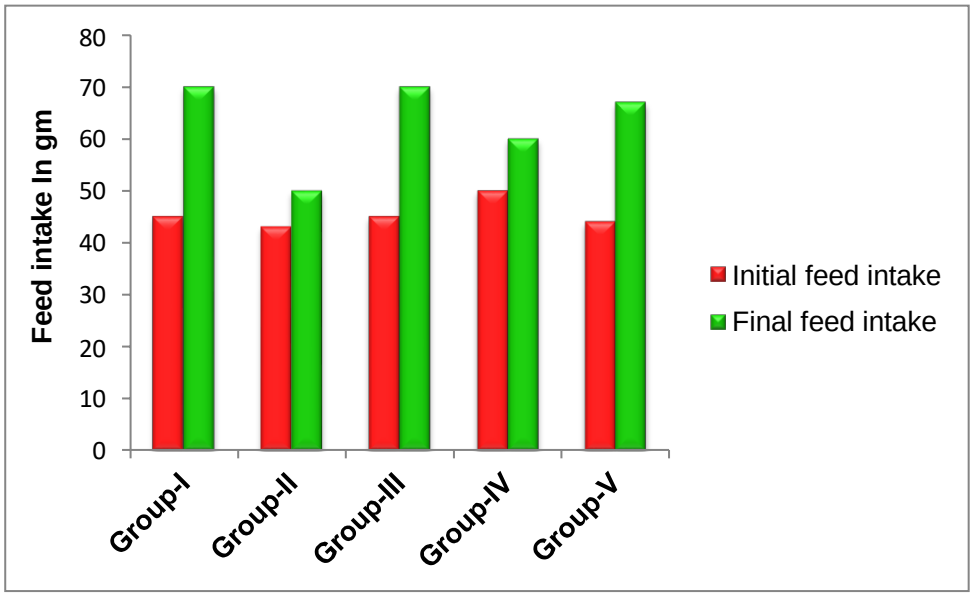
Symbols represent statistical significance: ***P<0.001, **-P<0.01, *-P<0.05



Graph.1: Effect of *Gymenma sylvestre* and *Pergularia daemia* changes on bodyweight in EV induced PCOS rat

Table.4: Effect of *Gymenma sylvestre* and *Pergularia daemia* on feed intake in EV induced PCOS rat

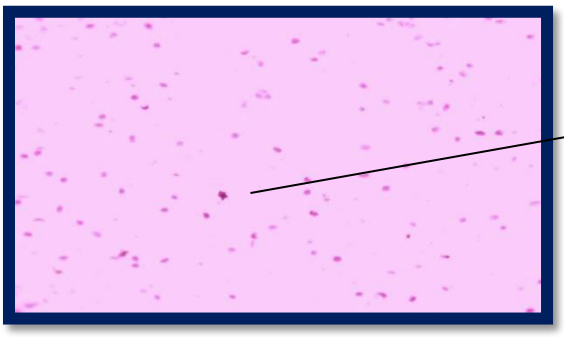
Treatment	Initial feed intake (g)	Final feed intake (g)
Group I (Vehicle Control)	45	70
Group II (Estradiol Valerate)	43	50
Group III (EV+*G.sylvestre*)	45	70
Group IV (EV+*P.daemia*)	50	60
Group V (EV+G.S+P.D)	44	67



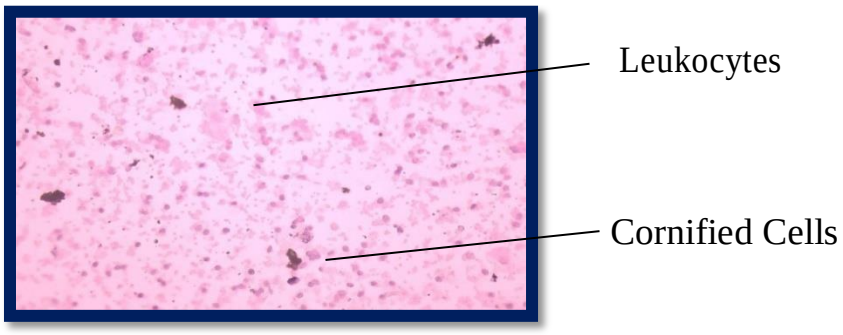
Graph 2: Effect of *Gymenma sylvestre* and *Pergularia daemia* on feed intake in EV induced PCOS rat

Figure No.10: Vaginal smear of Normal estrus stages

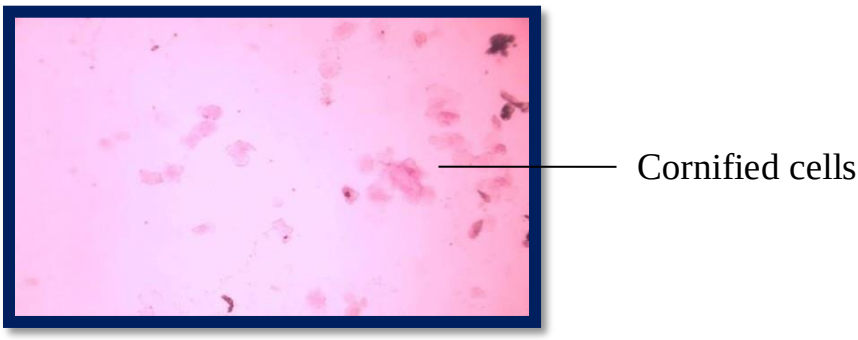
Stage-1 Diestrus



Stage -2 Proestrus



Stage – 3 Estrus



Stage – 4 Metestrus

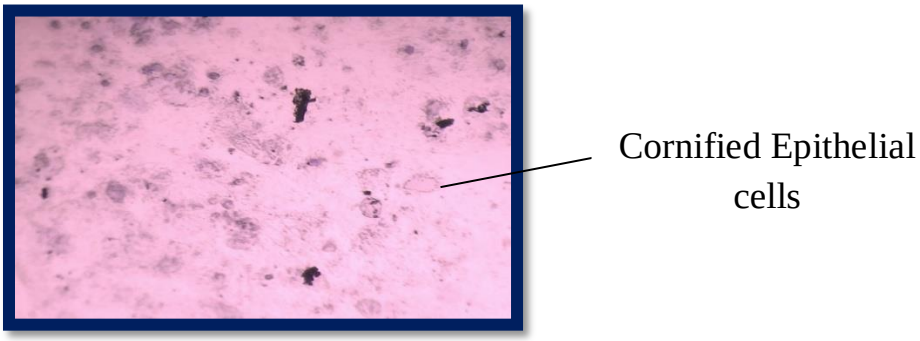
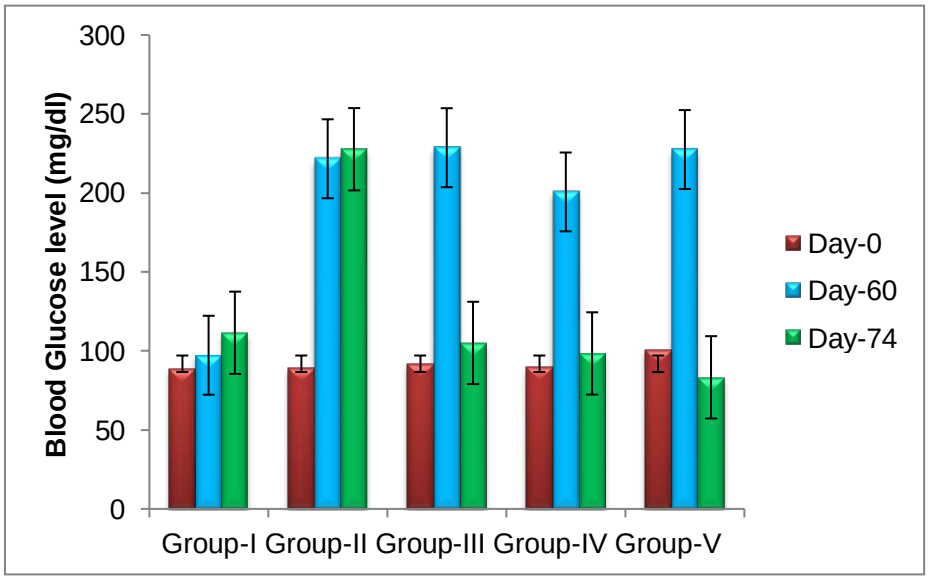


Table No.5: Effect of *Gymenma sylvestre* and *Pergularia daemia* on Blood glucose level in EV induced PCOS rat

Treatment	Day-0	Day-60	Day-74
Group I (Vehicle Control)	88.17±10.12	97.31±11.15	111.5±11.86
Group II (Estradiol Valerate)	88.94±11.71	221.7±36.81^{a**}	227.7±23.05 ^{a***}
Group III (EV+*G.sylvestre*)	91.82±10.07	228.7±28.42^{a**}	105.1±13.87 ^{b***}
Group IV (EV+*P.daemia*)	89.49±13.19	200.7±15.55^{a*}	98.42±22.63 ^{b***}
Group V (EV+G.S+P.D)	100.9±14.85	227.5±32.29^{a**}	83.35±11.44^{b***}

Values are expressed as mean ± SEM, n-6. Comparisons were made between: a-Group I vs II, III,IV,Vand. b- Group II vs III, IV, V.

Symbols represent statistical significance: ***P<0.001, **-P<0.01, *-P<0.05



Graph.3: Effect of *Gymenma sylvestre* and *Pergularia daemia* on Blood glucose level in EV induced PCOS rat

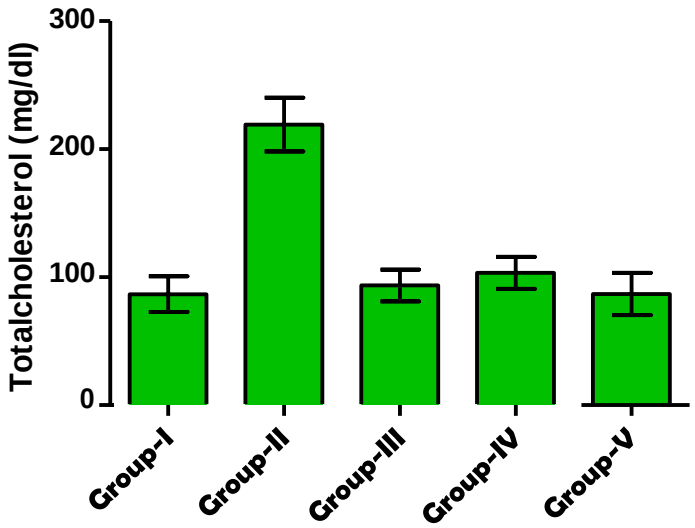
Table No.6: Effect of *Gymenma sylvestre* and *Pergularia daemia* on Total cholesterol and Triglycerides in EV induced PCOS rat

Treatment	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
Group I (Vehicle Control)	86.67±13.88	75.18±11.99
Group II (Estradiol Valerate)	219.2±21.03 a***	163.8±14.00 a***
Group III (EV+*G.sylvestre*)	93.50±12.34b***	71.35±11.22 b***
Group IV (EV+*P.daemia*)	103.3±12.56 b***	76.67±13.08 b***
Group V (EV+G.S+P.D)	86.83±16.49 b***	69.33±9.25 b***

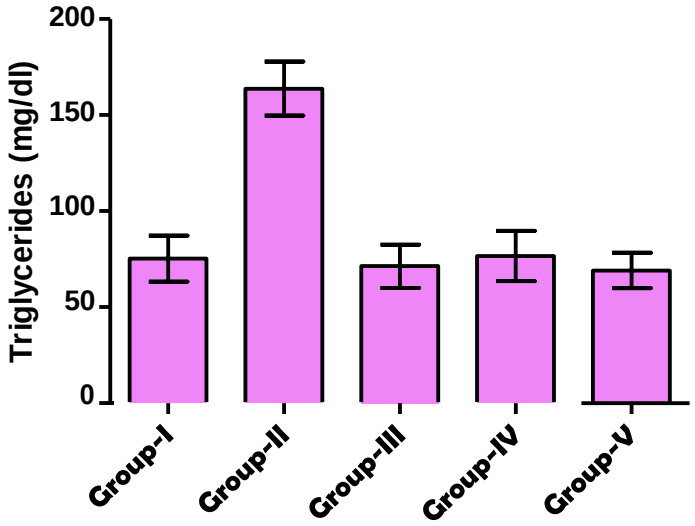
Values are expressed as mean ± SEM, n-6. Comparisons were made between:

a- Group I vs II, III, IV, V and. b- Group II vs III, IV, V

Symbols represent statistical significance: ***P<0.001, **-P<0.01, *-P<0.05



Graph.4: Effect of *Gymenma sylvestre* and *Pergularia daemia* on Total cholesterol in EV induced PCOS rat



Graph.5: Effect of *Gymenma sylvestre* and *Pergularia daemia* on Triglycerides in EV induced PCOS rat

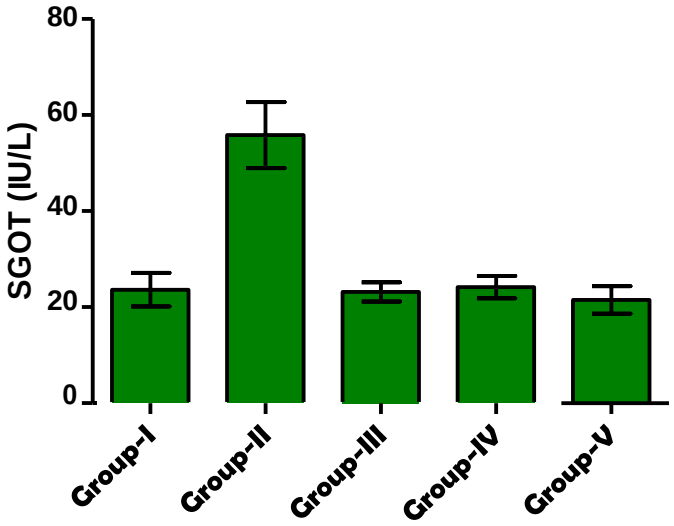
Table No.7: Effect of *Gymenma sylvestre* and *Pergularia daemia* on SGOT, SGPT and ALP in EV induced PCOS rat

Treatment	SGOT	SGPT	ALP
Group I (Vehicle Control)	23.6±3.57	34.80±5.67	70.00±9.66
Group II (Estradiol Valerate)	55.83±6.88 ^{a***}	55.83±5.21 ^{a*}	143.8±16.0 a***
Group III (EV+*G.sylvestre*)	23.17±2.02 ^{b***}	34.67±4.61 ^{b*}	70.67±16.02 b***
Group IV (EV+*P.daemia*)	24.17±2.30 ^{b***}	34.50±2.18 ^{b*}	71.83±12.02 b***
Group V (EV+G.S+P.D)	21.50±2.89 ^{b***}	32.17±2.24 ^{b*}	67.50±7.39 b***

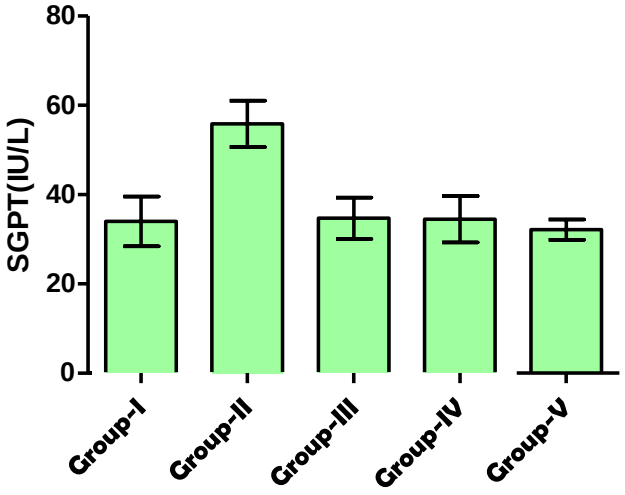
Values are expressed as mean ± SEM, n-6. Comparisons were made between:

a-Group I vs II, III, IV, V and. b- Group II vs III, IV, V.

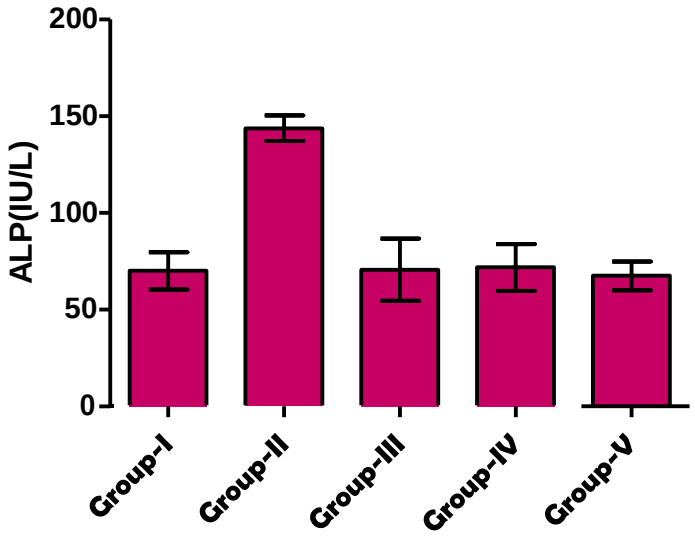
Symbols represent statistical significance: ***P<0.001, **-P<0.01, *-P<0.05



Graph.6: Effect of *Gymenma sylvestre* and *Pergularia daemia* on SGOT in EV induced PCOS rat



Graph.7: Effect of *Gymenma sylvestre* and *Pergularia daemia* on SGPT in EV induced PCOS rat



Graph.8: Effect of *Gymenma sylvestre* and *Pergularia daemia* on ALP in EV induced PCOS rat

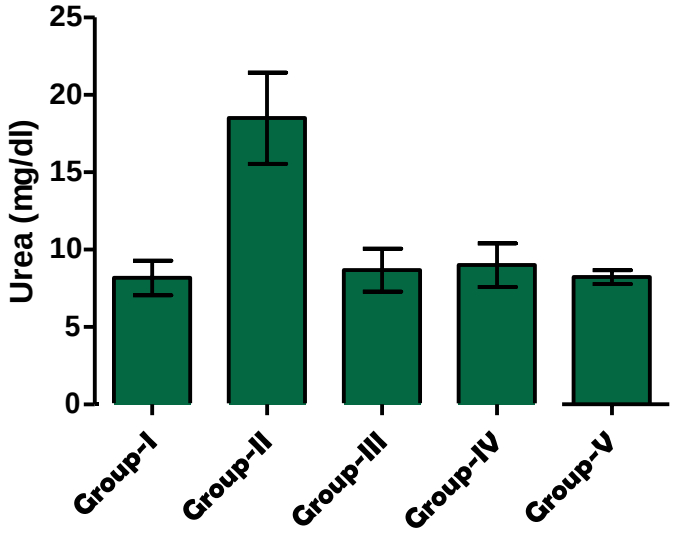
Table No.8: Effect of *Gymenma sylvestre* and *Pergularia daemia* on Urea and Creatinine in EV induced PCOS rat

Treatment	Urea (mg/dl)	Creatinine (mg/dl)
Group I (Vehicle Control)	8.16±1.10	0.45±0.15
Group II (Estradiol Valerate)	18.50± 2.74 ^{a***}	1.30±0.09 ^{a***}
Group III (EV+*G.sylvestre*)	8.66±1.38 ^{a**}	0.46±0.13 ^{b***}
Group IV (EV+*P.daemia*)	9.00±1.41 ^{a**}	0.48±0.08 ^{b***}
Group V (EV+G.S+P.D)	8.23±0.44 ^{a**}	0.43±0.01 ^{b***}

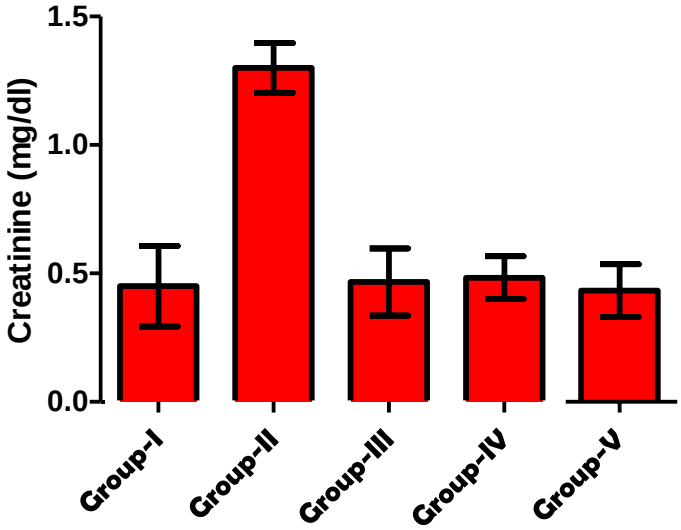
Values are expressed as mean ± SEM, n-6. Comparisons were made between:

a-Group I vs II, III ,IV, V and. b- Group II vs III, IV, V.

Symbols represent statistical significance: ***P<0.001, **-P<0.01, *-P<0.05



Graph.9: Effect of *Gymenma sylvestre* and *Pergularia daemia* on Urea in EV induced PCOS rat



Graph.10: Effect of *Gymenma sylvestre* and *Pergularia daemia* on Creatinine in EV induced PCOS rat

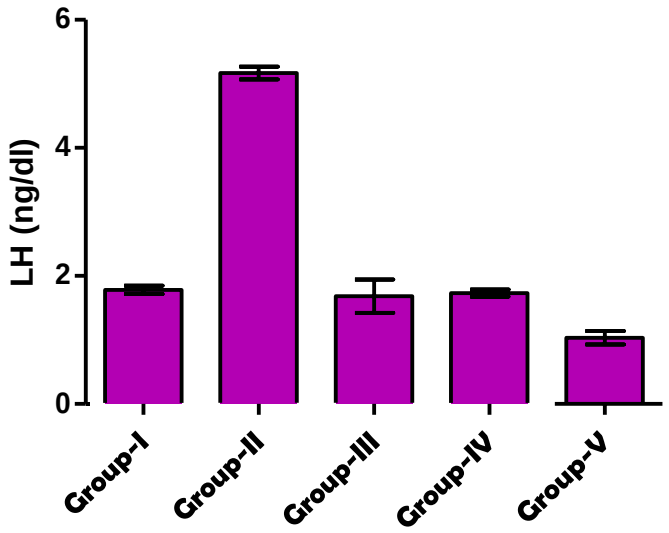
Table No.9: Effect of Gymnme sylvestre and pergularia daemia on Hormonal determination in EV induced PCOS rat

Treatment	LH (ng/dl)	FSH (ng/dl)	Estrogen (ng/dl)	Testosterone (ng/dl)	Progesterone (ng/dl)
Group I (Vehicle Control)	1.78±0.06	5.65±1.78	7.50±0.56	36.67±2.10	0.66±0.04
Group II Estradiol Valerate	5.16±0.09 a ***	2.66±0.20 a***	23.83±1.70 a***	103.3±13.08 a***	0.18± 0.03 a***
Group III (EV+*G.sylvestre*)	1.68±0.02 b ***	7.98±0.14 a** b***	4.90±0.28 b***	52.83±1.701 b***	0.66±0.08 b***
Group IV (EV+*P.daemia*)	1.73±0.05 b***	8.85±1.01 a*** b***	6.70±0.88 b***	42.67±2.04 b***	0.73±0.07 b***
Group V (EV+G.S+P.D)	1.03±1.30 a** b***	7.43±0.13 a* b***	8.31±0.37 b***	22.17±1.42 b***	0.71±0.05 b***

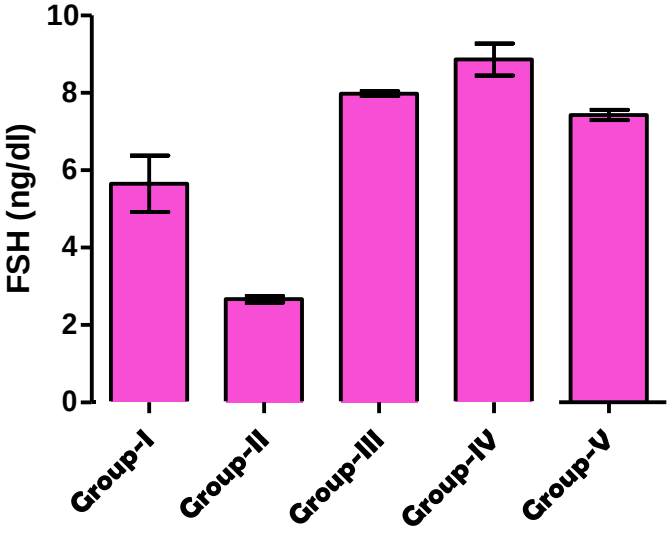
Values are expressed as mean ± SEM, n-6. Comparisons were made between:

a-Group I vs II, III,IV,V and. b- Group II vs III, IV, V.

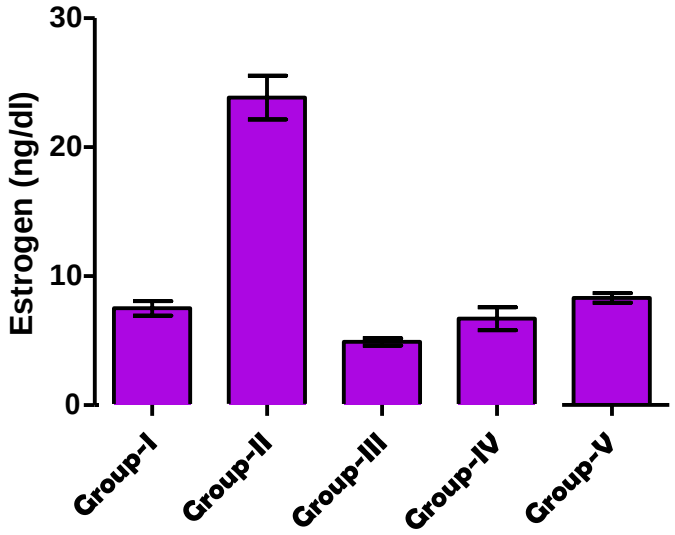
Symbols represent statistical significance: ***P<0.001, **-P<0.01, *-P<0.05



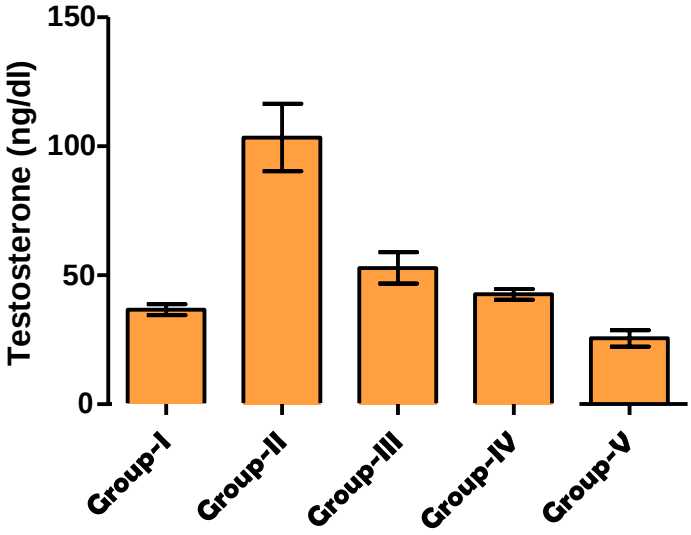
Graph.11: Effect of *Gymenma sylvestre* and *Pergularia daemia* on Leutinizing Hormone (LH) in EV induced PCOS rat



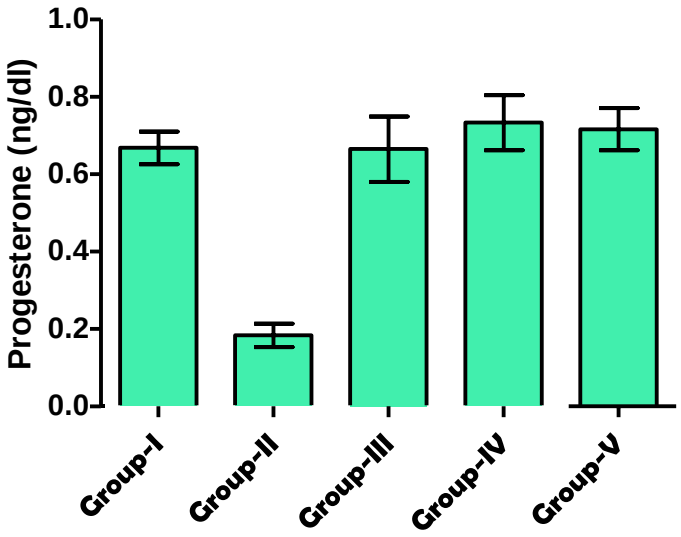
Graph.12: Effect of *Gymenma sylvestre* and *Pergularia daemia* on Follicular stimulating Hormone (FSH) in EV induced PCOS rat



Graph.13: Effect of *Gymenma sylvestre* and *Pergularia daemia* on Estrogen in EV induced PCOS rat



Graph.14: Effect of *Gymenma sylvestre* and *Pergularia daemia* on Testosterone in EV induced PCOS rat



Graph.15: Effect of *Gymenma sylvestre* and *Pergularia daemia* on Progesterone in EV induced PCOS rat

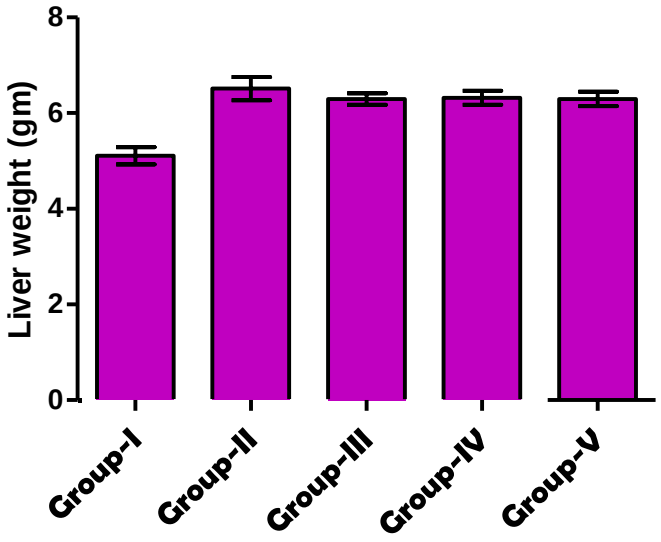
Table No.10: Changes in Liver & Ovary weight

Treatment	Liver weight (g/100g body weight	Ovary weight (g/100g body weight)
Group I (Vehicle Control)	5.11±0.18	0.34±0.14
Group II (Estradiol Valerate)	6.51±0.24	1.06±0.33 [a*]
Group III (EV+*G.sylvestre*)	6.29±0.12	0.45±0.16
Group IV (EV+*P.daemia*)	6.32±0.14	0.37±0.05
Group V (EV+G.S+P.D)	6.29±0.14	0.35±0.08

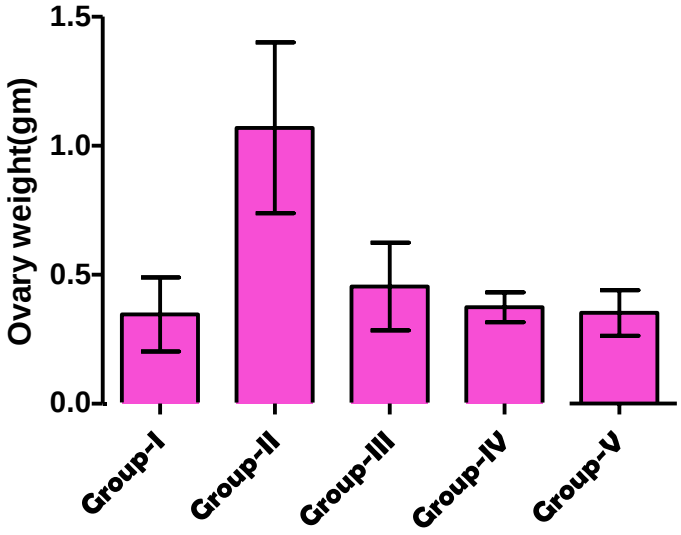
Values are expressed as mean ± SEM, n-6. Comparisons were made between:

a-Group I vs II, III,IV,V and. b- Group II vs III, IV, V.

Symbols represent statistical significance: ***P<0.001, **-P<0.01, *-P<0.05



Graph.16: Changes in Liver Weight



Graph.17: Changes in Ovary Weight

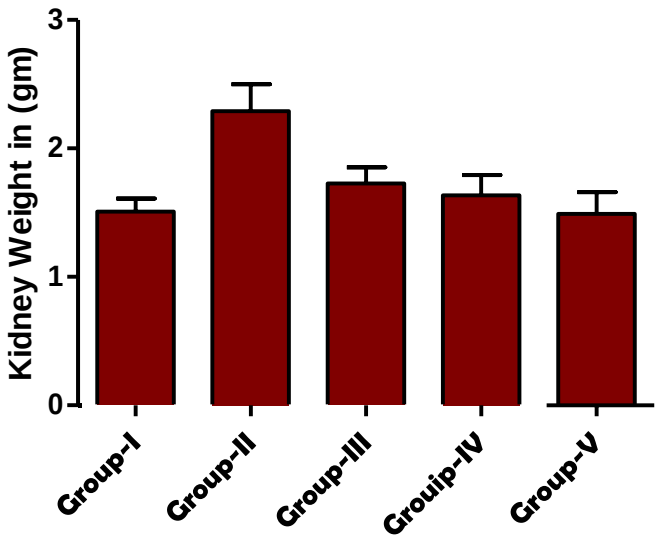
Table.11: Changes in kidney, Uterus and Heart weight

Treatment	Kidney weight (g/100g body weight	Uterus weight (g/100g body weight)	Heart weight (g/100g body weight
Group I (Vehicle Control)	1.50±0.01	1.36±0.09	0.59±0.20
Group II (Estradiol Valerate)	1.61±0.18 [a*]	1.46±0.15	0.66±0.18
Group III (EV+*G.sylvestre*)	1.64±0.17	1.39±0.11	0.67±0.08
Group IV (EV+*P.daemia*)	1.58±0.17	1.40±0.04	0.64±0.13
Group V (EV+G.S+P.D)	1.49±0.10 [b*]	1.32±0.01	0.62±0.11

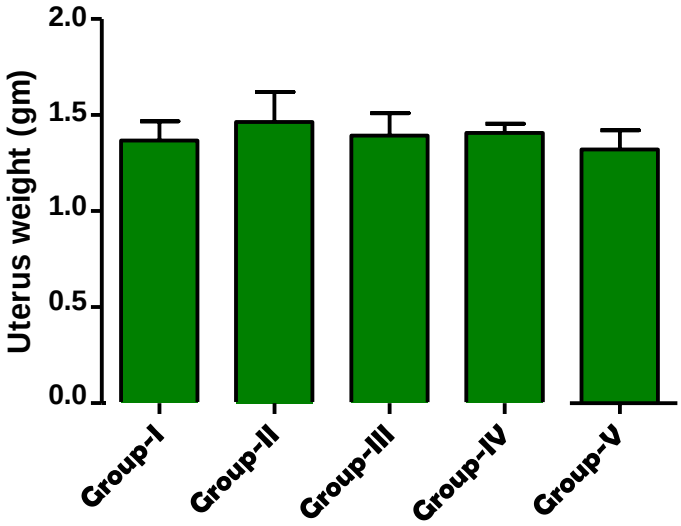
Values are expressed as mean ± SEM, n-6. Comparisons were made between:

a-Group I vs II, III,IV,V and. b- Group II vs III, IV, V.

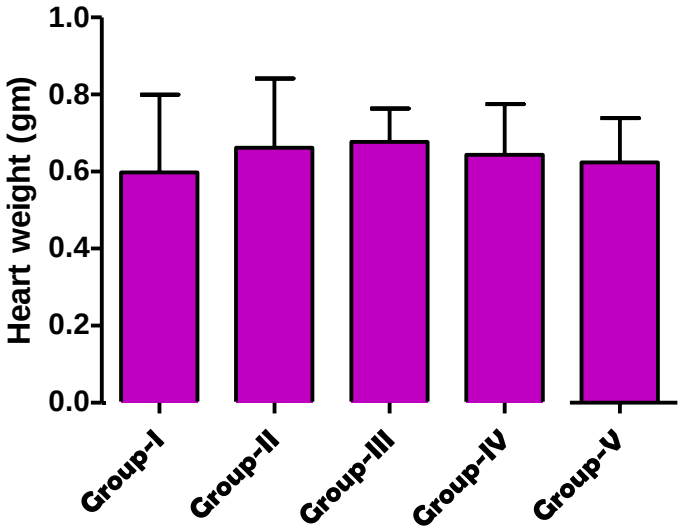
Symbols represent statistical significance: ***P<0.001, **-P<0.01, *-P<0.05.



Graph No.18: Changes in Kidney weight

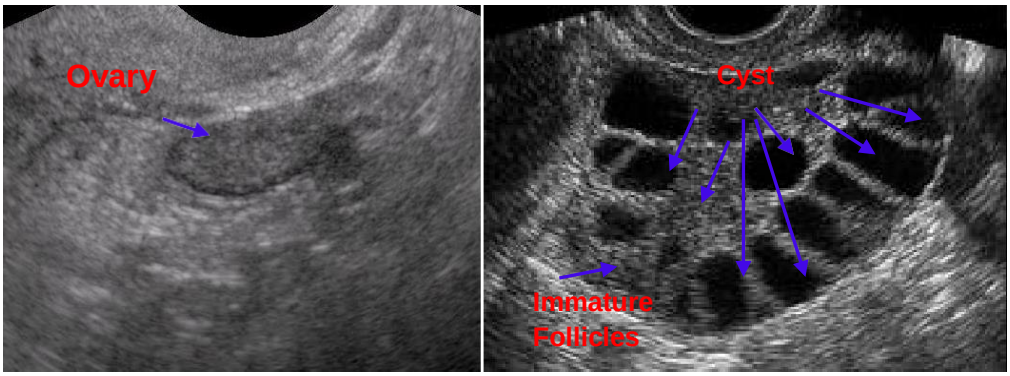


Graph No.19: Changes in Uterus weight



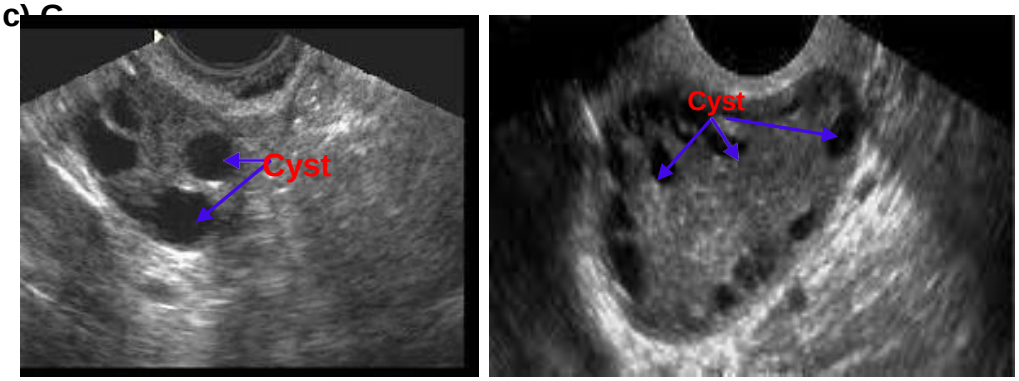
Graph No.20: Changes in Heart weight

Fig no: 11 Ultrasonic scan analysis for EV induce PCOS rat.

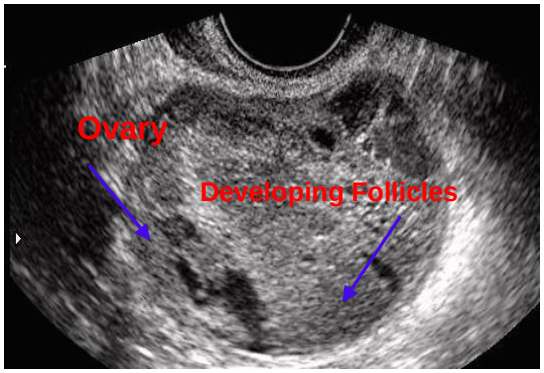


a) Group-I Normal Control **b) Group-II PCOS control**

c) C

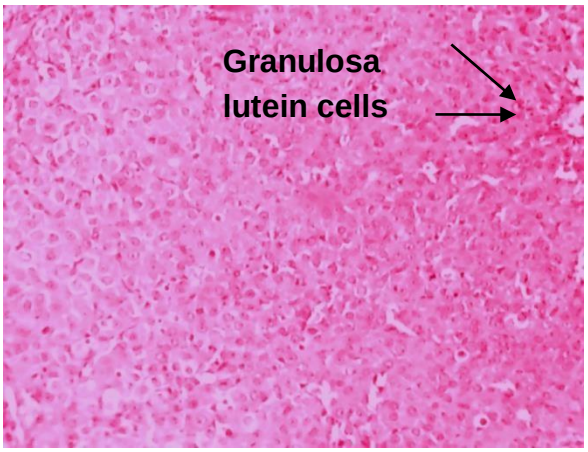


c) Group-III *G. sylvestre* **d) Group-IV *P. daemia***

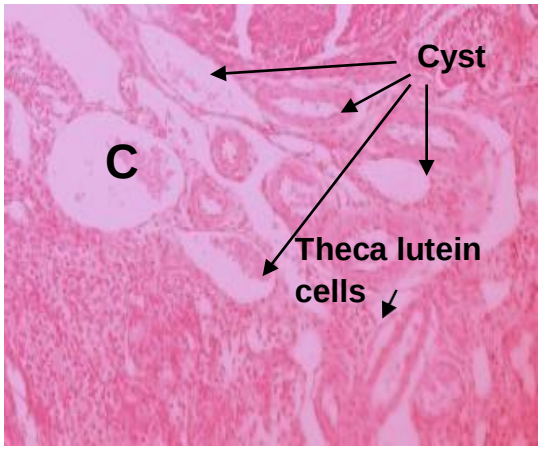


e) Group-V *G.Sylvestre+P.daemia*

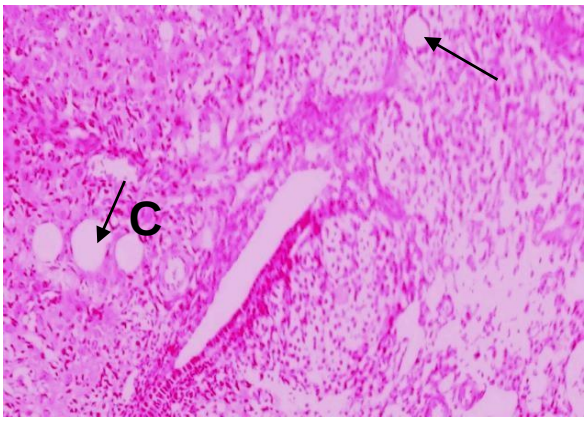
Fig No: 11 Histopathology changes for estradiol valerate induced PCOS in rat Ovary.



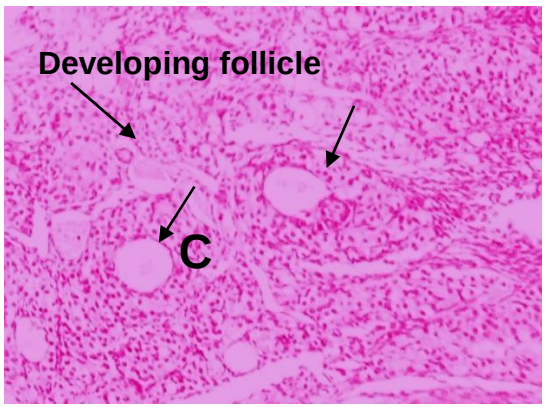
Group-I Normal Control



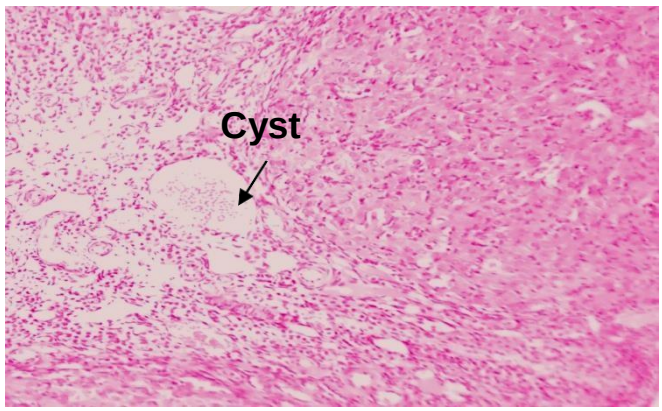
Group-II PCOS Control



Group-III *Gymnema* treatment



Group-IV *Pergularia* treatment



Group-V *G.sylvestre* +*P.daemia*

CHAPTER-8

DISCUSSION

CHAPTER - 8

DISCUSSION

Polycystic ovary syndrome is a common endocrine disorder affecting 4-12% of women in their reproductive age. [74] PCOS is frequently associated with anovulation and infertility. Due to anovulation, 75% of women are affected by infertility. In that among PCOS women 47% are affecting by oligomenorrh.[84] In PCOS women, clinically characterized by hyperinsulinaemia, hyper androgenism, obesity and enlarged ovaries with small multiple cysts leads to anovulation and pregnancy complications.

In this study polycystic ovary syndrome was induced by the administration of Estradiol Valerate 4mg/kg at a single dose. Exposure to a single dose of EV in adult rat cause irregular estrus cycles, lack of ovulation and excessive amount of follicular development and irregular estrus cycle[75]. Results of this study conform the administration of EV 4 mg/kg produce the PCOS in rats.

Body weight was significant increased in EV induced PCOS group as compare to the control group. It might be due to a high incidence of atretic/degenerative secondary follicles and the ovaries in EV induced rats. The structural changes included an increase in the volume of the Ovary, Cortex, Cysts and Corpus luteum[76]. In treatment groups there were no significant change in body weight as compare to the normal control.

The effect of feed intake in EV induced PCOS rats, there is no significant different in feed intake between the treatment groups when compared to the Vehicle control group. Only Group-II shows little decrease in feed intake.

The irregularity of estrus cycle was examined in PCOS rats by examine the Vaginal smear. EV induces PCOS animal’s shows irregularity of estrus cycle due

to direct effect of hormonal secretions to increases the LH level and decrease the FSH level and also decrease production of progesterone. Progesterone is the main hormones of the menstrual cycle, Hyperandrogenimia, abnormal follicular development and anovulation. [77] Treatment groups of *Gymnema sylvestre, Pergularia daemia* and combination of *(G.D+P.D)* (III, IV&V) significantly restored the estrus cycle in PCOS induces rats as compare to PCOS control group. This revealed that treatment of Gymnema sylvestre and Pergularia daemia are more effective in correction of menstrual irregularity and abnormal follicular development.

Blood glucose level in EV induced PCOS rats showed significant increase the blood glucose. It conform the administration of EV increase the androgen production and insulin resistants leads to hyperinsulinamia. [78] *Gymnema sylvestre, Pergularia daemia* treatment groups were significantly decreased the blood glucose. The effect of *Gymnema sylvestre* plant powder alone shows glucose utilization in insulin sensitive tissue by reducing the hyperinsulinamia [79] and the *Pergularia daemia* plant powder treatment reduce the Blood Glucose level by stimulation of the residual pancreatic mechanism or to a probable increase in the peripheral utilization of glucose and the combination of *G.D+P.D* more significant decrease in blood glucose when compare to the PCOS control group. [80] In comparison between treatment groups combination possess profound reduced effect on the blood glucose level.

In present study, Total cholesterol and Triglycerides level in EV induce PCOS rats were significant increase as compare to the *Gymnema sylvestre, Pergularia daemia* and its combinations *(G.D+P.D)* treatment groups (III, IV&V). The EV induce PCOS rat has high lipid and cholesterol content. It might be

causes of obesity and atheroschelorsis. [81] The *Gymnema sylvestre* and *Pergularia daemia* treatment showed significant decrease the Total cholesterol and Triglycerides. In comparison between the treatment groups combination posses more reduction effect on Total cholesterol and Triglycerides.

In this study, SGOT, SGPT and ALP level was significantly increased in PCOS control group as compared to the normal control. It indicate that the impairment of hepatic function in PCOS group. The elevated level of SGOT, SGPT and ALP was significantly reverted in all the treatments. Improvement of hepatic function may raise the serum SHBG enzyme level which reduces the bioavailability of sex hormones[82].

The increased serum urea and creatinine level in PCOS rat it might be the causes of renal dysfunction the earliest stages of atherogenesis is endothelial cell dysfunction. [83] In additional increased serum creatinine level was found in EV induced PCOS rats, which shows the kidney dysfunction in PCOS rats. Alterations were observed in PCOS condition were normalised and greater effect in all the treatment groups. Result of this study suggests the *Gymnema sylvestre* and *Pergularia daemia* treatment prevent the impairment of renal functions evident by a decrease in serum urea and creatinine. [84]

According to the hormonal determination EV induce PCOS rats shown significant increase in serum LSH, Estrogen, Testosterone and decrease in FSH and Progesterone compare to control group.

Estrodiol Valerate administration increased sensitivity of the pituitary to GnRH result in increase in leutinizing hormone (LH) and increased insulin levels mainly amplify the intrinsic abnormality of their steroidogenesis. Excess androgen

activity leads to hyperandrogenism. By administration of *Gymnema sylvestre* reduce the insulin secretion [85] and degenerations of the follicular cells in ovaries as well as elevated LH level in *Pergularia daemia* treatment [86]. In comparison between the treatment groups combination treatment posses most decrease in LH level.

In present study there was significant decrease in FSH level in PCOS rats. EV treatment animals increased the sensitivity of the pituitary to GnRH results in FSH suppression it may responsible for the maintenance of the cystic development of the ovaries. In presence study individual and combination treatment of *Gymenema sylvestre* and *Pergularia daemia* allows the new follicular development, maturity of follicules and correction of irregularity of estrus cylce occur as compare to the PCOS control by significant increase in FSH [87]. The level of FSH was higher in *Pergularia daemia* treated group as compare to the other treatments.

In PCOS condition significantly increases in Estrogen level. Estradiol valerate administration causes alter the level of estrogens leads to the formation of cysts in the ovary and development of follicles[88]. In this study showed that EV induced PCOS rats are increases in serum estrogen. *Gymenema sylvestre* and *Pergularia daemia* treatment significantly decreases in estrogen level compared to the PCOS group. This implying caused to improvement in endocrine function and recovery of ovulatory functions.

In non steroidal aromatase inhibitors (EV) administration of PCOS rat shows excess amount of androgen and causes the anovulation. In this present study by administration of *Gymenema sylvestre* to drastically decreased testosterone and regulates the process of ovulation *Pergularia daemia* shows

more significant in the testosterone with regulate anovulation and combinations of *G.D+P.D* to significant decrease as compare to the Normal control. [89]

Progesterone was significant decrease in PCOS rats. Progesterone is important hormone for women menstrual cycle, absence or a decrease of progesterone ovulation does not occur leads to anovulation [90] Estradiol Valerate administration of PCOS rats decreased progesterone production, [91] in present study individual & combination treatment of *G.sylvestre* and *P.daemia* repair the luteal functions & anovulation in EV induce PCOS rats.

Ultra sound scan changes in EV induced Polycystic ovarian syndrome rats are the diagnostic ovary measures which indicating the follicular cyst development due to hyperandrogenism. In this study USS confirm the PCOS control animals with develop polycyst. *Gymnema sylvestre* and *Pergularia daemia* treated groups showed normal ovary with mild evidence of cyst development due to the number of granulose cell layers increased and follicular fluid was orderly arranged. Additionaly, theca cell layer become thinner while the number of corpora lutea increase and dialted follicles. [92] In comparison between treatment group *G.sylvestre+P.daemia* treated group showed more effective compare with very mild incidence of cyst as compare to the PCOS control.

The increase in liver and ovary weight are related to inhibit the hepatic synthesis effect of SHBG reduction, and increases the immature development of follicles in ovary and increase androgen secretions in EV induced PCOS rat. All the treatment groups indicate reduction in the weight of these organs, which are related to the endocrine functions. [93] It can be effective action for the *G.sylvestre* and *P.daemia.* When comparison between these treatments groups

G.sylvestre+P.daemia combination treated group have more effective action on these organs.

Histopathological study of PCOS control showed more number of developed cyst and theca lutein cells. *G.sylvestre and P.daemia* showed fewer developed cysts. In comparison combination *G.sylvestre+P.daemia* have no incidence of follicular cysts.

CHAPTER-9

SUMMARY AND CONCLUSION

CHAPTER-9

SUMMARY AND CONCLUSION

In this study PCOS was induced by administration of Estradiol Valerate. It conforms the elevated level of Blood glucose, total cholesterol, triglycerides and hormonal changes like increase in the level of LH, Testosterone, Estrogen, decreases the FSH and Progesterone with menstrual irregularity, confirmed by ultrasound analysis and histopathological changes in the ovary of PCOS control.

The treatment of *G.sylvestre* shows significant reduction effect in blood glucose level, Total cholesterol and Testosteone have a most prominant action it might be due to the presence of phytoconstituents like Gymnemic acid, glycosides, saponins.

P.daemia shows the most significant increased effect on the FSH and Progesterone it may be due to the presence of Pituitrin, alkalodids, triterpenes, saponins, and steroidal compounds.

It might be concluded the individual administraton of G.sylvestre reduce the insulin resistance, decrease androgen production and P.daemia have profound beneficial effect on anovulation, menstrual irregularity. But the combination *G.sylvestre+P.daemia* had shown the synergistic activity to correct the hyperinsulinemia, anovuation and hyperandrogenism.

Further Isolation of active constituents and scope full clinical studies data are needed to initiate these combination for the better treatment and management of PCOS.

CHAPTER-10

REFERENCE

CHAPTER-10

REFERENCE

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ANNEXURE

VIVEKANANDHA
EDUCATIONAL INSTITUTIONS

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

SPONSORED BY : ANGAMMAL EDUCATIONAL TRUST.
(NBA Accredited Institution, Affiliated to the TN. Dr. M.G.R. Medical University, Chennai, Approved by PCI, AICTE New Delhi.)
Elayampalayam - 637 205. Tiruchengode, Namakkal Dt., Tamil Nadu, India.
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INSTITUTIONAL ANIMAL ETHICAL COMMITTEE

(Reg.No.889/PO/ac/05/CPCSEA/dated 9th November 2011)

CERTIFICATE

This is certify that the project title "**Combination therapeutic potential of *Gymnema sylvestre* and *Pergularia daemia* on Estradiol valerate induced polycystic ovary syndrome in Wistar rats**" has been approved by the IAEC as follows:

1.	Date of Submission of Protocol	: 06.12.2016
2.	Date of Approval	: 09.12.2016
3,	Expiry Date	: 10.04.2017
4.	Animals	:

Species	Total Number of Animals Sanctioned
Albino Wistar rats	
(Female) -	**30**

5. Proposal number : SVCP/IAEC/PG/1/03/2017

Signature with Date
(P. Sudhakar)
IAEC - MEMBER SECRETARY,
Swamy Vivekanandha College of Pharmacy,
ELAYAMPALAYAM - 637 205,
Tiruchengode (Tk). Namakkal (Dt).
TAMIL NADU.

Signature with Date
(Dr. K. Sekar)
VETERINARIAN,
Swamy Vivekanandha College of Pharmacy,
ELAYAMPALAYAM - 637 205,
Tiruchengode (Tk), Namakkal (Dt).
TAMIL NADU.

Signature with Date
(Dr. G. Murugananthan)
IAEC - CHAIRMAN,
Swamy Vivekanandha College of Pharmacy,
ELAYAMPALAYAM - 637 205,
Tiruchengode (Tk), Namakkal (Dt).
TAMIL NADU.