PROTECTIVE EFFECT OF *COSCINIUM FENESTRATUM* BARK EXTRACT ON LETROZOLE INDUCED POLYCYSTIC OVARY SYNDROME (PCOS) IN SPRAGUE DAWLEY RATS

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MASTER OF PHARMACY
IN
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Submitted by

HARI PRIYA K
Register No: 261925003

Under the Guidance of
Dr. P. Muralidharan, M.Pharm., Ph.D.,
Professor and Head of the Department
Department of Pharmacology

C.L. BAID METHA COLLEGE OF PHARMACY

(AN ISO 9001-2008 CERTIFIED INSTITUTION)

THORAIPAKKAM, CHENNAI – 600 097.

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Phone : 24960151, 24962592, 24960425
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

C.L. Baid Metha College of Pharmacy
An ISO 9001:2008 approved institution
Jyothi Nagar, Old Mahabalipuram Road,
Thorapakkam, Chennai - 600 097.

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education, New Delhi

Dr. P. Muralidharan, M.Pharm., Ph.D.,

Professor and Head,

Department of Pharmacology.

CERTIFICATE

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Date: **Guide:**

Place: Chennai **Dr.P.Muralidharan**

Phone : 24960151, 24962592, 24960425
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

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Dr.Grace Rathnam, M.pharm., Ph.D,

Principal

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Date: **Principal:**

Place: Chennai **Dr. Grace Rathnam**

DECLARATION

The thesis entitled "**PROTECTIVE EFFECT OF *COSCINIUM FENESTRATUM* BARK EXTRACT ON LETROZOLE INDUCED POLY-CYSTIC OVARY SYNDROME (PCOS) IN SPRAGUE-DAWLEY RATS"** was carried out in Department of Pharmacology, C.L. Baid Metha College of Pharmacy, Chennai-600097 during the academic year 2019-2021. The work embodied in this thesis is original and is not submitted in part or full for any other degree of this or any other university.

Date: HARI PRIYA.K

Place: Chennai Reg.no: 261925003

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ABBREVIATIONS

1.	ASRM	Embryology/American Society for Reproductive Medicine.
2.	BECF	Bark Extract of Coscinium fenestratum
3.	C. fenestratum	Coscinium fenestratum
4.	DHEA	Dehydroepiandrosterone
5.	DHT	Dihydrotestosterone
6	ESHRE	European Society for Human Reproduction
7	FSH	Follicle Stimulating Hormone
8	G	Gram
9	GDM	Gestational Diabetes Mellitus
10	GnRH	Gonadotropin Releasing Hormone
11.	HA	Hyperandrogenism
12.	HDL-C	High Density Lipoprotein Cholesterol
13.	IU	International Unit
14.	Kg	Kilogram
15.	LDL	Low Density Lipoprotein
16.	LH	Luteinizing Hormone
17	Mg	Milligram
18.	Ml	Millilitre
19.	Mm	Millimetre
20.	Ng	Nanogram
21.	NICH	Health/National Institute of Child Health and Human Disease

22.	NIH	National Institutes of Health
23.	OD	Ovulatory dysfunction
24.	p.o.	Per os
25.	PCO	Polycystic ovaries
26.	PCOS	Polycystic ovary syndrome
27.	PI3K	Phosphoinositide 3-kinases
28	POA	Premature Ovarian Aging
29	POF	Premature Ovarian Failure
30	SHBG	Sex Hormone Binding Globulin
31.	w/w	Weight/weight
32	WHO	World Health Organisation
33.	%	Percentage

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INTRODUCTION

1. INTRODUCTION

Plant-derived products have an imperative biological role against certain pathogenic organisms and were considered to be a major source of modern drugs. Rural people residing in developing countries are relying on traditional herbal medical system due to their strong belief and minimum access to allopathic medicines. Hence, ethnomedicinal knowledge is useful for the maintenance of community - based approaches under this medical system.

Traditional medicine also known as indigenous or folk medicine which comprises medical knowledge systems that developed over generations within various societies before the era of modern medicine. Practices known as traditional medicines include, Ayurveda, herbal, siddha medicine, unani, Chinese medicine, Islamic medicine, ancient Iranian medicine, traditional acupuncture and other medical knowledge and practices all over the globe

Traditional medicine as defined by the world health organization is the sum total of knowledge skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. [1]

The traditional uses attributed to each medicinal plant depend on where the plant is consumed and the social group that use them. Traditional medicine is an important healthcare component in low-income countries. [2]

Traditional medication involves the use of herbal remedies, animal parts and minerals. Herbal medicines most widely used are herbs, herbal materials herbal preparations and finished herbal product that contain as active ingredient parts of plants or other plant materials or combinations [3]

The quantity and quality of the safety and efficacy data on traditional medicines to meet the criteria needed to support its use worldwide. For this reason, WHO has been increasingly involved in developing international standard and technical guidelines for their medicines and also in increasing communication and cooperation between countries. [4]

WHO has defined four categories for Traditional Medicines:[5]

Category 1: These are traditional medicines that have been prepared by traditional health practitioners for treatment of their patients. These are medicines that are prepared extemporaneously using traditional methods.

Category 2: These are traditional medicines widely available in the community and that have commercial value. They are traditionally used in a given locality and well known in that setting in terms of composition and treatment; the formulation is well known, preparation is according to traditional methods, and safety and efficacy are justified by a long history of use.

Category 3: These are traditional medicines developed through scientific research based on ethnomedical use; their formulation, dosage, and therapeutic use are based on research data, and their safety and efficacy information is based on standard scientific and clinical investigation.

Category 4: This category belongs to imported traditional medicines that originate from other countries, including WHO African countries. These are required to meet the definition of traditional medicines which should be registered in the source country, and should meet the regulatory requirements of the country into which they are being imported. In some countries, such as Tanzania, this category has fairly stringent regulatory requirements, similar to conventional medicines.

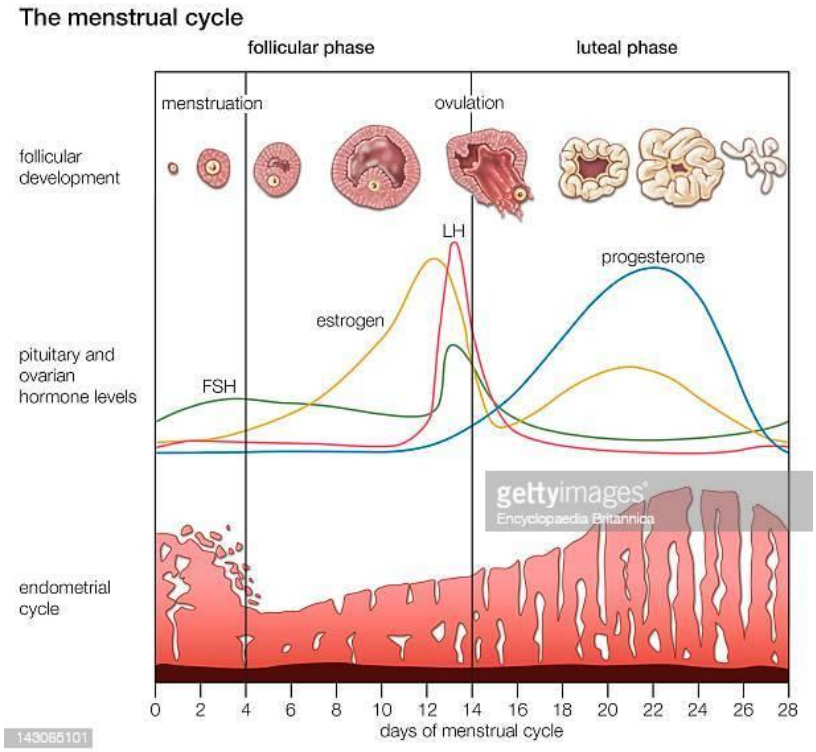
The increase in the use of herbal products is due to their cultural acceptability, availability, affordability, efficacy and safety chain. So this study is planned for the evaluation using a natural herbal.

The female reproductive system is composed of time-regulated, complex sub systems and multiple functional processes. The female reproductive tract is composed of the uterus fallopian tubes and ovaries, which interconnects by the broad and ovarian ligaments. [6]

The menstrual cycle describes the changes in female reproductive system which occurs during a typical 28-day long the menstrual cycle but there is significant variation in the length of the menstrual cycle in normal women often ranging from 21 days up to 40 days or sometimes more. In such cases, there is variation in the duration of both the follicular and the luteal phases of the menstrual cycle, but the luteal phase seems to be of more fixed duration and typically lasts around 14 days, with more of the difference in cycle length being attributable to variation in the duration of the follicular part of the cycle.[7]

The length of the menstrual cycle is 21 to 35 days, with most averaging 26 to 28 days. The duration of the secretory phase is relatively constant, averaging 14 days [8]. The phases in menstrual cycle includes:

- **Follicular phase [development of the egg]**
- **Ovulatory phase [release of the egg]**
- **Luteal phase [hormone levels decrease if the egg does not implant]**



Follicular phase

This phase starts on the first day of your period. During the follicular phase of the menstrual cycle, the following events occur:

- Two hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH) are released from the brain and travel in the blood to the ovaries.
- The hormones stimulate the growth of about 15 to 20 eggs in the ovaries, each in its own "shell," called a follicle.
- These hormones (FSH and LH) also trigger an increase in the production of the female hormone estrogen.
- As estrogen levels rise, like a switch, it turns off the production of follicle-stimulating hormone. This careful balance of hormones allows the body to limit the number of follicles that will prepare eggs to be released.
- As the follicular phase progresses, one follicle in one ovary becomes dominant and continues to mature. This dominant follicle suppresses all of the other follicles in the

group. As a result, they stop growing and die. The dominant follicle continues to produce estrogen.

Ovulatory phase

The ovulatory phase (ovulation) usually starts about 14 days after the follicular phase started, but this can vary. The ovulatory phase falls between the follicular phase and luteal phase. Most women will have a menstrual period 10 to 16 days after ovulation. During this phase, the following events occur:

- The rise in estrogen from the dominant follicle triggers a surge in the amount of luteinizing hormone that is produced by the brain.
- This causes the dominant follicle to release its egg from the ovary.
- As the egg is released (a process called ovulation) it is captured by finger-like projections on the end of the fallopian tubes (fimbriae). The fimbriae sweep the egg into the tube.
- For one to five days prior to ovulation, many women will notice an increase in egg white cervical mucus. This mucus is the vaginal discharge that helps to capture and nourish sperm on its way to meet the egg for fertilization.

Luteal phase

The luteal phase begins right after ovulation and involves the following processes:

- Once it releases its egg, the empty ovarian follicle develops into a new structure called the corpus luteum.
- The corpus luteum secretes the hormones estrogen and progesterone. Progesterone prepares the uterus for a fertilized egg to implant.
- If intercourse has taken place and sperm has fertilized the egg (a process called conception), the fertilized egg (embryo) will travel through the fallopian tube to implant in the uterus. The woman is now considered pregnant.
- If the egg is not fertilized, it passes through the uterus. Not needed to support a pregnancy, the lining of the uterus breaks down and sheds, and the next menstrual period begins.

REVIEW OF LITERATURE

1. POLYCYSTIC OVARY SYNDROME:

Polycystic ovary syndrome (PCOS) also referred as hyper-androgenic and common endocrine disorder in women reproductive age [9]. The complex condition is characterized by elevated androgen levels. In India about 10% of women are affected by PCOS [10]. Basically, a woman with PCOS have overweight or obese this affects the metabolism and reproductive function of the body [11]. The manifestation of PCOS affects mostly in women of childbearing age (from 18-30) because the ovaries develop into numerous small collection follicles and failed to regulatory release [12]. PCOS increase the risk of various fields like dermatologic, oncologic, metabolic, reproductive and psychological aberrations. [13]

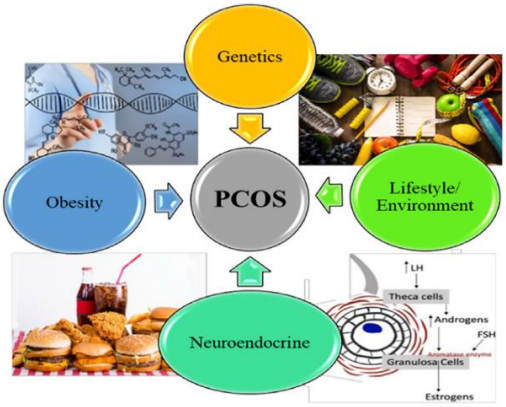


Figure 1

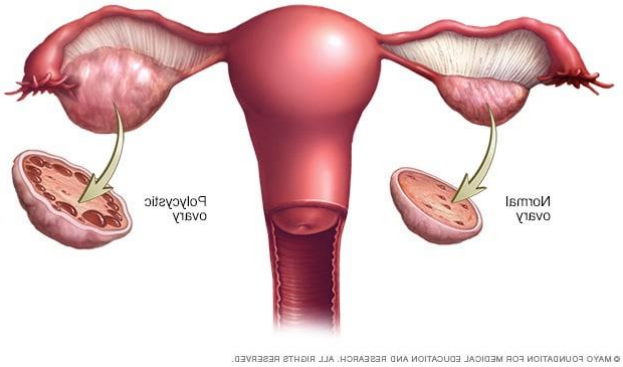


Figure 2

2.1 Types of PCOS:

Insulin resistant PCOS

Type 1 PCOS is one of the major symptoms of PCOS. PCOS additionally show cases capacity for growing diabetes and elevated testosterone levels each of which can be virtually resulting from the underlying insulin and leptin resistance

Non-insulin resistant PCOS

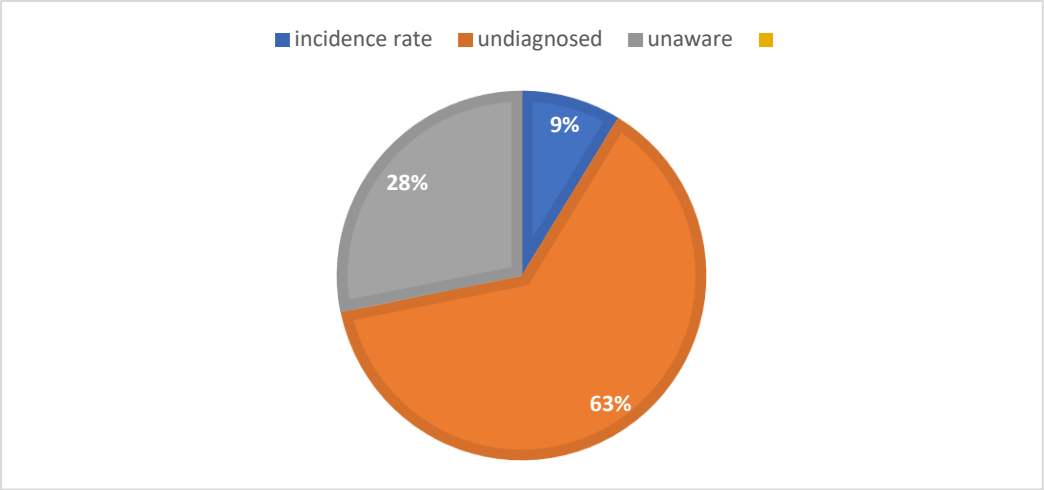
There can be a variety of causes for this type of PCOS, including vitamin D or iodine deficiency, hormone-disrupting toxins, thyroid disease, and adrenal stress. For women experiencing. Non-insulin resistant PCOS, anti-diabetic drugs will have no effect on the condition and neither will help in reducing the weight which is gained due to hormonal.

The treatment options in this ease 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.

2.2 Prevalence of PCOS:

Global [14-17]



India

Prevalence of PCOS in India ranges from 3.7 to 22.5 % depending on the population studied and the criteria used for diagnosis [18]. 18% of PCOS prevalence were found to be in Tamil Nadu. In Mumbai, found the prevalence of PCOS was 22.5% by the Rotterdam criteria and 10.7% by the androgen excess society criteria. In Andhra 9.13% of them satisfied the Rotterdam criteria. [19]

2.3 Pathophysiology of PCOS:

PCOS is a phenotype reflecting which involves neuroendocrine, metabolic and ovarian dysfunction [20]. Approximately in 60-80% of patients the main key feature in PCOS are Excess androgen and hirsutism. The elevated androgen concentration suppresses sex hormone binding globulin (SHBG) concentrations contributing to higher free testosterone concentrations [21]. Hyperinsulinemia results in increased androgen level by suppressing the hepatic sex hormone binding globulin. The primary defects of PCOS are hypothalamic pituitary axis, ovarian function and insulin secretion and action.

Excessive androgen production results in the genetic defects (cryptic-21-hydroxylase functions) and then suppressed by LH by gonadotropin releasing hormone (GnRH) super agonist. The biochemical features of PCOS is hyperinsulinemia and it may lead to

hyperandrogenmia and hyperandrogenism and both are acts in a different mechanism i.e., it influencing androgen synthesis, as well as increasing the circulating pool of bio-available androgen. [22]

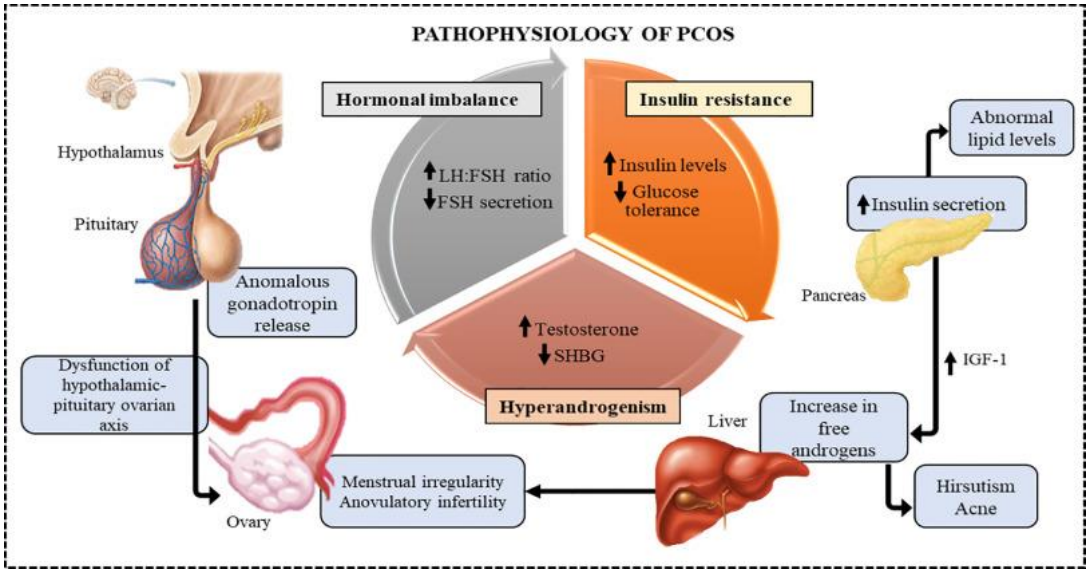


Figure 3

2.4 Mechanism of action:

The mechanism of menstrual cycle is in two phase's follicular phase and luteal phase. The total duration of the phases is 28 days. In that the follicular phases- 0 to 14 days and luteal phases- 14 to 28 days [23]. Upon GnRH stimulation the Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) are produced. In follicular phase, the estrogen is generated. The primordial follicle is converted into primary follicle and induce the secondary follicle in the presence of FSH receptors and then increase the amount of follicle begot by lowering the amount of LH the androgen. Then the androgen is converted into estrogen. When high amount of estrogen is formed, they act as negative feedback, and results in production of Luteinizing hormone. When luteal phase are started and its leads to ovulation during this process the ovary are released from the follicle and corpus luteum to produce progesterone [24]. In PCOS, high level of LH secretion contributes to high level of androgen and low level FSH and it may leads to poor egg development, inability to ovulate and also lead to deficiencies of progesterone production and most often leads to absence of menstrual cycle period.

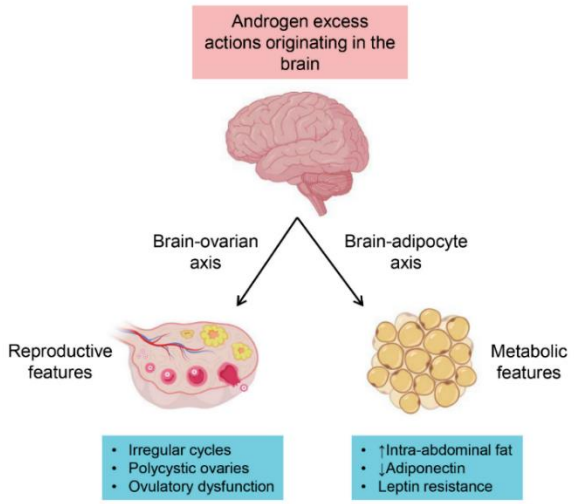
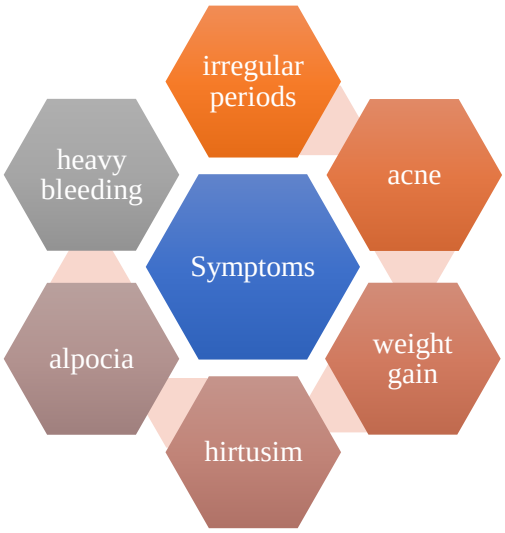


Figure 4

2.5 Characteristic of PCOS:

- PCOS may present with amenorrhea, infertility, hyperandrogenmia with the signs of metabolic disturbances.
- Anovulation (irregular periods)
- Overweight [25]
- Insulin resistance [26]
- Inflammation – which may directly affect the ovarian dysfunction. Pro-inflammatory markers are responsible for the PCOS or early developed obesity. [27]

2.6 Symptoms: [28]



2.7 Complications:

Complications of PCOS may include:

- Infertility
- Gestational diabetes or pregnancy – induced high blood pressure
- Miscarriage or premature birth
- Non- alcoholic steatohepatitis - a severe liver inflammation caused by fat accumulation in the liver
- Metabolic syndrome

2.8 Hormone Factors:

The hormone is involved in PCOS is follicle stimulating hormone (FSH) and luteinizing hormone (LH) which is responsible for reproduction as testis in male and ovarian in female.

2.8.1 Follicle stimulating hormone:

FSH glycoprotein polypeptide hormone is situated in the anterior pituitary. FSH is released by stimulation of GnRH secretion from the hypothalamic pituitary hormone [29], which is synthesized and secreted by the gonadotropin cells from the anterior pituitary gland and they regulate the development, growth, pubertal maturation and reproductive process of the body.

Functions:

- In both males and females – stimulation of primordial germ cells.
- In male- FSH induced sertoli cells to secrete androgen-binding proteins
- In females- FSH initiates follicular growth, specifically affecting granulosa cells

Effects in females:

Steriodogenesis

- FSH stimulates various functions such as;
    i. Stimulated progesterone secretion by the granulosa of intermediate-stage follicle
    ii. Stimulate granulosa cells of small follicles
    iii. Increase progesterone, androstenedione and estradiol production by theca cells form small follicles.
- FSH involve in the development of oocytes and yolk deposition

- It also increases the production of growth factors inside the ovary [30]

Normal value for FSH

The value is differing from age and sex. Usually, the value is low in childhood and high in adult

Tab (1.1)

	Before Puberty	During Puberty	Adult
Male	0 to 5.0 mlU/mL	0.3 to 10.0 mlU/mL	1.5 to 12.4 mlU/mL

Tab (1.2)

	Before Puberty	During Puberty	After Menopause
Female	0 to 4.0 mlU/mL	0.3 to 10.0 mlU/mL	25.8 to 134.8 mlU/mL

2.8.2 Luteinizing Hormone:

LH is a hormone produced by gonadotropic cells from the anterior pituitary gland [31]. LH is synthesized from the androstenedione by the ovarian theca cells for ovulation and involved in the formation in corpus luteum [32]. The action is differing in both sexes, in male it stimulates the testosterone in the testis. In females, it releases the progesterone by the stimulation of ovaries.

Function:

In males, LH is used for production for testosterone by acting against testicular leydig cells. In female LH is responsible for releasing progesterone after ovulation from the corpus luteum [33]. The ovarian follicle is made up of three cells called theca cells, granulosa cells and oocyte. Theca cells contains LH receptors- LH binds and stimulate to produce estrogen. [34]

Effect in female:

In female, LH responsible for maturation of ovary [35]. It acts on the theca cells for production and formation of androgen during folliculogenesis. Androgen are converted into estradiol by transferring theca cells to granulosa cells [36]. Then LH acts as steroid release from the ovaries and release progesterone by the corpus luteum.

Normal value for LH

Men: 1.24 – 7.8 IU/L

Female:

Follicular phase	Mid-cycle peak	Luteal phase	During menopause
1.68- 15 IU/L	21.9 - 56.6 IU/L	0.61-16.3 IU/L	14.2 – 52.3 IU/L

Disease state:

When high level of FSH and LH occur, following syndrome occur:

- Premature ovarian failure (POF):

  It is defined as ovarian which does not perform normal process thus results in unable to produce estrogen hormone and release egg. It also called hypergonadotropic ovarian failure. [37]

Causes:

i. Latrogenic effects
ii. Infections (e.g. herpes zoster)
iii. Chromosome defects
   1. Turner syndrome – Its neurogenic disorder and loss of sex chromosome [38]
   2. Fragile X syndrome- inherited intellectual disability which linked neurologic and psychotic disorder. [39]
      i. Monogenic defects
      ii. Syndromic defects

      1. Congenital disorders of glycosylation

      2. Galactosemia

iii. Isolated defects

1 Follicle stimulating hormone receptor (FSH) mutations

2. Luteinizing hormone receptor mutations (LHR)

iv. Idiopathic

Diagnosis:

The diagnosis method of POF is emotional health, hormone replacement therapy, maintaining bone health and family planning. [40]

- Premature ovarian aging (POA)

POA is occur in when low number of ovaries are produced in young aged thus results in female infertility. The main risk factors of POA is genetic, autoimmune disorder and modified factors like chemotherapy, radiotherapy and pelvic synergy. [41]

- Gonadal dysgenesis- impaired function of gonads [42]
- Klinefelter syndrome -it congenital chromosomes in which extra X chromosome in male cause infertility, hypogonadism. [43]
- Swyer syndrome- absence of sex glands. [44]
- Testicular failure
- Lupus

When low amount of FSH and LH is present, the following syndrome are seen, such as,

- Polycystic ovary syndrome
- Kallmann syndrome- genetic disorder delay or absence of puberty and delay or absence of smell
- Hypothalamic suppression
- Hypopituitarism
- Hyperprolactinemia
- Gonadotropin deficiency

2.9 Role of PCOS:

2.9.1 Role of obesity in PCOS

Obesity with PCOS causes hyperandrogenism, hirsutism, insulin resistance, and infertility and pregnancy complications and associated with genes [45]. It is the major key component for metabolic syndrome and associated with level of insulin which subsequently increased in ovarian androgen production. The excessive adipose tissue is responsible for ovulatory dysfunction and menstrual abnormalities [46]. Obesity and insulin resistance increase the risk of type 2 diabetes and cardiovascular disease [47]. The upper body obese mainly causes the excessive production of androgen and results in decrease insulin sensitivity and increase the risk of cardiovascular disease [48]. About 38-88% of obese women has PCOS. [49]

Figure 5

2.9.2 Role of insulin in PCOS:

About 50 – 70% women with PCOS have insulin resistance [50]. Hyperinsulinemia and insulin resistance play major role in PCOS. It elevates the GnRH and LH pulse secretion continuous elevation resulting in ovarian steroid hormone particularly androgen [51]. Sex hormone binding globulin (SNBG) is associated with the insulin resistance. SNBG has strong affinity towards sex steroid. The research shows that, women with insulin resistance should have low sex hormone binding globulin concentrations thus results in PCOS [52]. Insulin acts

on steroidogenesis by activating LH by inducing secondary messengers cAMP in which it turns to activate PI3K and produce excessive androgen [53]. Metformin reduces insulin resistance in women with PCOS and it improves the spontaneous ovulation. [54]

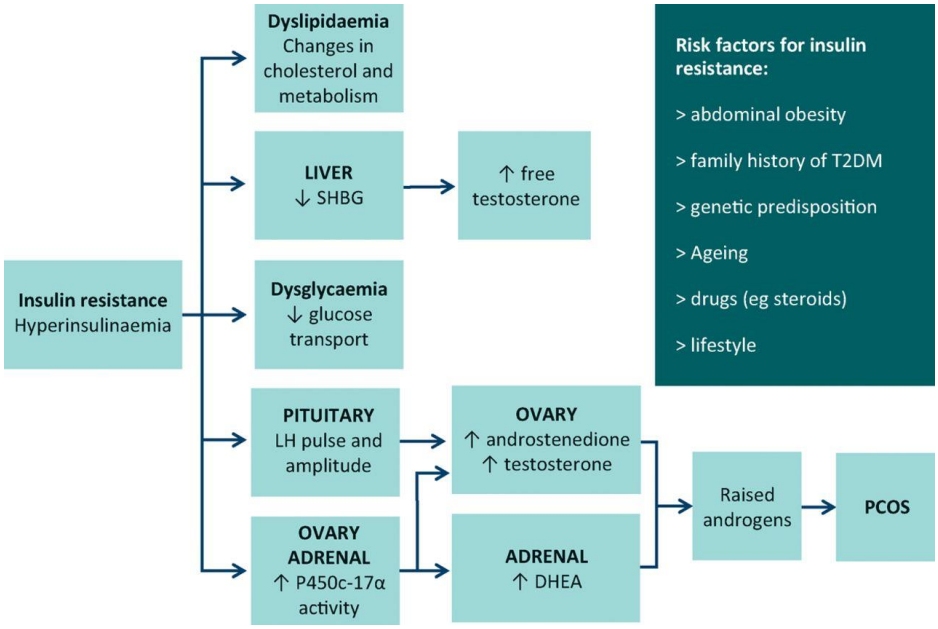


Figure 6

2.9.3 Role of cholesterol in PCOS:

Dyslipidaemia play major role in PCOS about 70% women are diagnosed with dyslipidaemia [55]. The Decrease in HDL - C and increase in triglycerides and LDL level can lead to PCOS [56-57]. Abnormal lipid profile is common pattern in women with PCOS [58]. The women with PCOS and Dyslipidaemia are more prone to cardiovascular disease. [59]

2.9.4 Role of diabetes in PCOS:

In the Prevalence of PCOS, 6-8% of women are developing the risk of diabetes mellitus[60]. Gestational diabetes mellitus (GDM) are more common in women with PCOS, the main risk are obesity and also family history and age. [61]

2.10 Phenotype:

The phenotype limits of PCOS is defined by 1) identified the specific phenotype and 2) determine the long term morbidity [62]. The different PCOS phenotype have varying degree of adiposity and may differ in metabolic and reproductive profile [63]. Division of phenotype can be help to understand the pathophysiology of PCOS and to predict the adverse metabolic

and cardiovascular disease. In these studies, the PCOS phenotype can classified into the four types.

Phenotype A (HA+OD+PCO)

Phenotype B (HA+OD)

Phenotype C (HA+PCO)

Phenotype D (OD+PCO)

Hyperandrogenism (HA), ovulatory dysfunction (OD), polycystic ovaries (PCO).

In phenotype A most common phenotype among B and C. Phenotype D is least common one [64]

2.11 Factors Influencing PCOS:

The influencing factors of PCOS are genetic factors, environmental factors and endocrine factors.

2.11.1 Genetic factors:

Family members who have PCOS also have higher risk for developing the same metabolic abnormalities. They are wide variety of genes and mechanism is involved in PCOS. Several studies show that, PCOS gene affect the hormone level and insulin resistance. Monozygotic twins are more prone PCOS than the dizygotic twins [65]. The genes are involved in PCOS is CYP11a, CYP21, CYP17 and CYP19. Each gene has own responsible activity; For example: CYP11a is involved in conversion of cholesterol to Progesterone, CYP21is responsible for synthesis of steroid hormones, CYP17 is involved in conversion pregnenolone and progesterone into 17- hydroxy pregnenolone and 17- hydroxyprogesterone and CYP19 – aromatase p450 formation of estragon1. [66]

Two possible approaches are used to identify a genetic locus

1. Association studies – where a predisposing allele is expected to be found more frequently in the affecting population than the normal individual.

2. Linkage studies- investigated to determine if particular genomic landmark are distributed independently or in linkage with phenotype. [67]

2.11.2 Environmental factors:

Environmental factors play an important role in genetic variants and they involved in etiology, prevalence and modulation of the PCOS phenotype including environmental toxins, diet and nutrients, socioeconomic status and geography. Environmental toxins in which it includes the chemical pollutants such as tobacco smoke, lead, pesticide and mercury are harmful for endocrine disrupting chemical and reproductive health [68]. In women the high stress level [69], Lack of physical activity and exercise which lead to PCOS [70]. Lifestyle and dietary fibres are most prominent to PCOS. The excess weight loss, moderate diet with carbohydrate, fats and high content of fibre lead alteration in menstrual regulations. [71]

2.11.3 Endocrine factors:

Endocrine disrupting compounds are chemical agents affecting synthesis, transport, metabolism are attacking through hormone receptors like endocrine receptors and thyroid receptors. [72]

2.12 Diagnosis of PCOS:

The Rotterdam consensus include the diagnostic criteria National institute of Health (NH) proposed set any two from the three must present to diagnosis PCOS: hyperandrogenism, ovulatory dysfunction and polycystic ovaries. In adolescents, the diagnosis is based on the hyperandrogenism in the presence oligomenorrhea. In pre-menopause and menopausal stage it is diagnosed based on the post medical history. [73]

Criteria for the diagnosis of Polycystic ovary syndrome [74 - 75]

NIH/NICHD 1992	**ESHRE/ASRM (Rotterdam criteria) 2004**	**Androgen Excess Society 2006**
Exclusion of other androgen excess or related disorders	Exclusion of other androgen excess or related disorders	Exclusion of other androgen excess or related disorders
Includes all of the following:	Includes two of the following:	Includes all of the following:
Clinical and/or biochemical hyperandrogenism	Clinical and/or biochemical hyperandrogenism	Clinical and/or biochemical hyperandrogenism

Menstrual dysfunction	Oligo-ovulation or anovulation Polycystic ovaries	Ovarian dysfunction and/or polycystic ovaries

2.13 Treatment Used Traditional Medicine For PCOS: [76-77]

Herbal medicine	**Physiological effects in menstrual irregularity (oligo/amenorrhoea), hyperandrogenism and/or PCOS**
Vitex agnus-castus	Binds to β estrogen receptors
Cimicifuga racemose	Binds with α estrogen receptors in the pituitary and reduces LH secretion
Cinnamon cassia	Equivalence for metformin for reduced testosterone in PCOS
Tribulus terrestris	Ovulation induction in polycystic ovaries
Glycyrrhiza glabra	Increased aromatization of testosterone of 17 beta estradiol shows significant dose dependent reduced testosterone and increased estradiol
Allium cepa L.	Antioxidants level compensation to modulate apoptosis/PCO -induced
Anethum graveolens L. þ	Increase in estrous cycle duration and diestrus phase and progesterone concentration/ Rat
Asparagus officinalis L.	increase in GnRH, FSH, LH, estrogen, progestin hormones levels and number of ovarian follicles/ rats

Citrus medica L	estrogenic effects/Immature ovariectomized rats

2.14 Management of PCOS:

Treatment of PCOS should be proposed not only to alleviate symptoms but also to prevent the occurrence of long- term complications, combined oral contraceptives and antiandrogens are the standard care to reduce androgen level and treat symptoms while providing endometrial protection. [78]

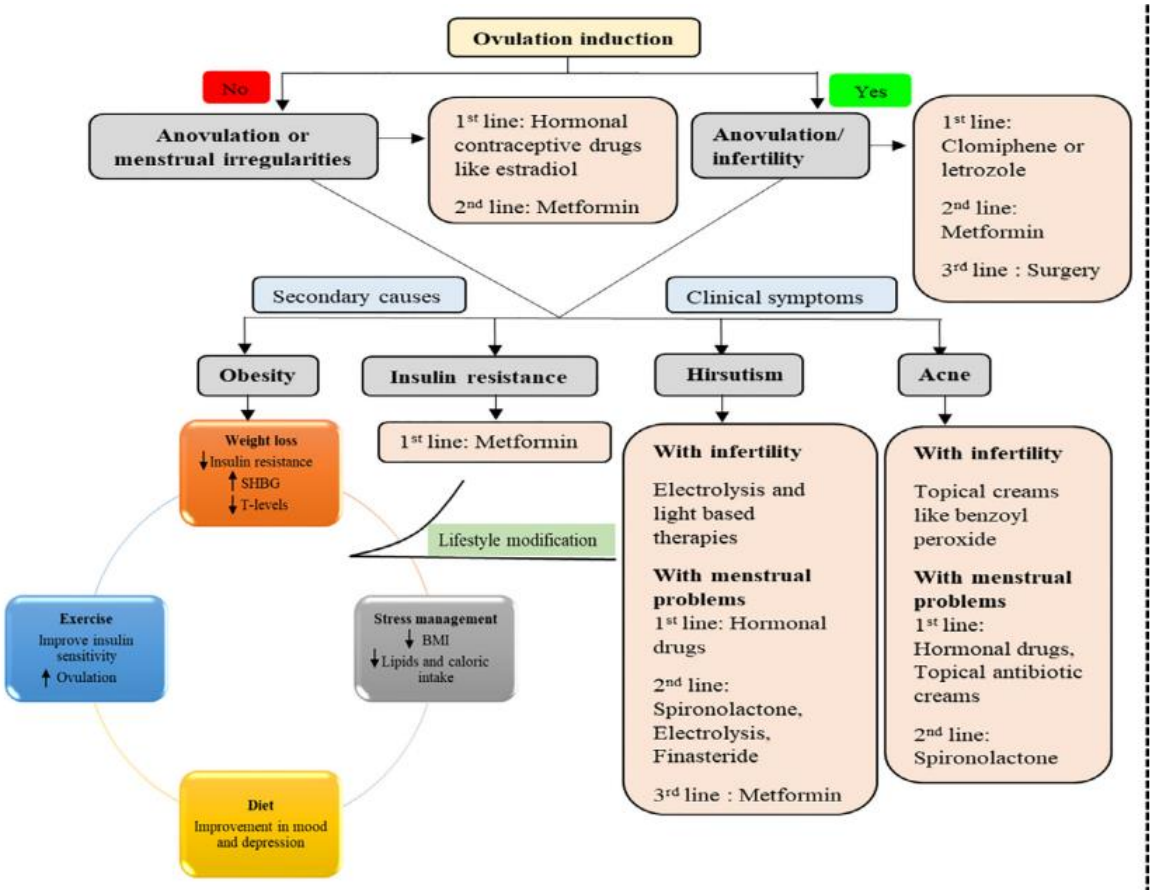


Figure 7

2.14.1 Metabolism:

The first line of treatment in patients with PCOS should be the improvement. In overweight and obese patients, weight loss due to change in diet and physical activity decreases serum insulin and androgen levels and reduces the risk of developing glucose intolerance and type- 2 diabetes. [79]

Metformin is the most commonly used drug for the metabolic control of PCOS patients. The therapeutic effects of metformin as insulin-sensitizing and hypoglycemic agent have been well confirmed in women with PCOS. Metformin does not seem to decrease body adiposity as it has little if any effect on reducing waist circumference and serum triglyceride levels in women with PCOS. [80]

Liraglutide is glucagon- like peptide 1 receptor 1 agonist approved for treating type 2 diabetes and obesity. In obese women with PCOS, liraglutide was effective to induce significant weight loss and reduce waist circumference. [81]

Orlistat is a lipase inhibitor labeled for treatment of obesity. In overweight or obese women with PCOS, orlistat is effective to induce weight loss and improve clinical and bio-chemical markers of hyperandrogenism and insulin resistance. [82]

2.14.2 Quality of life

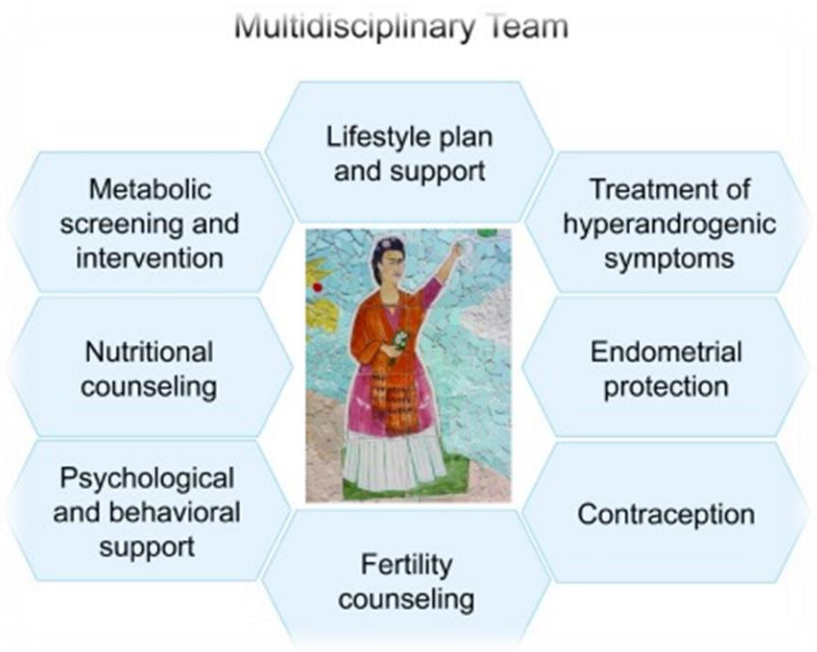


Figure 8

Depression and anxiety are highly prevalent in women with PCOS found to be fourfold increase in the prevalence of depressive symptoms [83]. The psychological impact of PCOS may even surpass chronic diseases such as asthma, diabetes, arthritis and coronary artery heart disease. [84]

Daily fatigue and sleep disorders, changes in appetite and loss of interest in everyday activities are the most common symptoms. The evaluation of quality of life in women with PCOS is essential for better case and clinical management of those patients. [85]

2.14.3 Infertility: [86]

The second line treatment is ovulation induction. This step must be preceded by careful evaluation of other causes of infertility, such as male factor or tubal obstruction, which demand IVF and may coexist with PCOS.

Clomiphene citrate is the reference therapy for ovulation induction in anovulatory women with PCOS. In the absence of ovulation for three cycles of clomiphene citrate at the highest dose (150mg/kg). The women can be considered non-responsive and another drug should be introduced as an adjuvant or substitute of clomiphene citrate.

2.15. Review On Methods Available For Inducing Pcos In Animals:

Following methods used for the induction of PCOS in animals:

1. **Testosterone - induced PCOS**: [87]

   Pre- or postnatal administration of Testosterone can induce hyperandrogenemia in rats. In addition, prenatal exposure to Testosterone during the critical period of fetal development was shown to cause developmental and morphological abnormalities in the reproductive system. For prenatal administration, pregnant rats were given a single-dose injection of 5 mg free Testosterone on gestational day 20 or Testosterone propionate (TP) from day 16 to 19 (3 mg T daily) of pregnancy. Postnatally, rats were administered TP at 1.25 mg/100 g body weight at 5 days of age or were injected daily with TP at 1 mg/100 g body weight from 21 to 56 days of age.

2. **DHEA induced PCOS [88]**

   Dehydroepiandrosterone sulphate **(**DHEA) is an androgen that is primarily produced in the adrenal gland. Women with PCOS have high levels of DHEA. Therefore, DHEA is administered to rodents to generate PCOS models. A typical protocol is 6 mg/100 g body weight/day starting from postnatal day 21 to 23 for about 20-40 consecutive days. DHEA has been administered by implantation of 7.5-mg 90-day continuous-release pellets in mouse models.

3. **DHT-induced model PCOS: [89-90]**

Dihydrotestosterone (DHT) is not converted into E2 by aromatase; therefore, the PCOS phenotype can be analysed in DHT-treated animals without considering the effects of estragon converted from androgens.

For Prenatal DHT-treated animals, mice were injected with 250 µg of DHT on days 16, 17, and 18 of gestation whereas rats were administered 3 mg of DHT daily from gestational day 16 to 19. The offspring served as prenatal DHT-treated PCOS models.

For Postnatal DHT treatment, rats were subcutaneously administered DHT pellets (7.5 mg/pellet, 90-day release, daily dose = 83 µg) on postnatal day 21, whereas in mice a tube containing 10 mg DHT was implanted subcutaneously at this time point.

4. Aromatase induced PCOS; [91]

Aromatase is an enzyme that converts testosterone and androstenedione into E2 and estrone, respectively. Letrozole, a nonsteroidal aromatase inhibitor, blocks the conversion of androgens to estrogen and thus increases androgen level. As such, letrozole has been used to generate animal models of PCOS mostly by postnatal administration; in some cases, it was continuously administered to immature or adult rats (3-8 weeks of age) from about day 21 to 90. In rat models, letrozole doses vary from 1-3 mg daily by oral administration to 100-400 µg/d/100 g body weight by implantation of a subcutaneous pellet. For mouse models, 9 mg letrozole were delivered via 90-day continuous-release pellets starting from postnatal day 21.

5. Progesterone Receptor Antagonist-Induced Models [92]

RU486 (mifepristone), a progesterone receptor antagonist, is one the most common drugs used for emergency contraception. The binding affinity of RU486 to progesterone receptor is five times greater than that of P4. Thus, RU486 can potently block the functions of progesterone. Evidence from clinical studies indicates that RU486 suppresses follicle development, ovulation, and CL formation by disrupting the negative feedback of P4 to the hypothalamus. Accordingly, RU486 has been used to generate rat models of PCOS by administering 2 mg RU486/100 g body weight to adult rats for 1-2 weeks.

6. Estrogen-Induced Models: EV (E2 valerate) [93]

EV is a long-acting estrogen. Rat models of EV-induced PCOS have been established by injecting young adult female rats in estrus with a single dose of 2-4 mg EV.

DRUG PROFILE

2. DRUG PROFILE

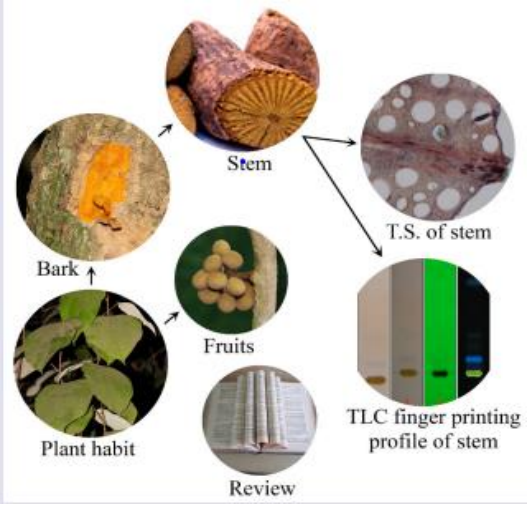


Figure 9

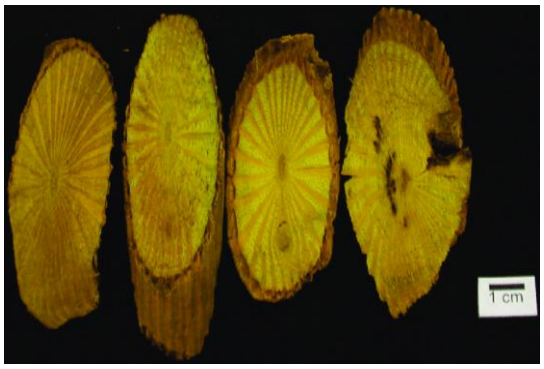


Figure 10

One of the most beneficial plants in the world whose root and stem serve as excellent antibiotic and antiseptic properties, 'Tree turmeric" it is called in English, Maramanjal in Malayalam, Daruharidra in Sanskrit, Daruhaldi in Hindi. Belongs to Menispermaceae family and is botanically known as Coscinium fenestratum.

Native to India the natural evergreen forests of south India, 900-1000 m highlands with high relative humidity about 15-20°C and shade are all the favourable conditions for its growth. Its root and stem are widely used in various Ayurvedic, Unani, Siddha as well as traditional medicinal preparations for the treatment of diabetics, skin diseases, Jaundice, wounds and ulcers. Its stem is used for snake bites.

Berberin is the active ingredient that gives the medicinal properties. Many ayurvedic preparations like Aswagandha Arishtam, Khadira Arishtam, Anuthailam, Ilaneerkuzhambu, Mahapanchagavyam contain maramanjal. Many cosmetic preparations also utilize maramanjal preparations.

Roots and stems are the useful parts. Mature stems are cut at about 50 cm above the base and should be done preferably once in three years. The stem is peeled and dried. The skin could be used as natural dye preparations.

Over harvesting and difficulty in nurturing the vine has endangered. Though the exports are banned, due to the great demand for berberine in the international market there is always the danger of unscrupulous harvesting looming high. So alternate methods of maramanjal

cultivation and preservation should be given preference. It can be grown in homesteads and should be included as a component in social forestry. Also, medicinal plant gardens and selective plantations can be utilized for its propagation and commercial cultivation.

Taxonomical Classification:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Family: Menispermaceae

Genus: Coscinium Colebr.

Species: Coscinium fenestratum (Gaertn.) Colebr.

Vernacular Names:

English: Tree turmeric, false calumba, Columbo weed

Kannada: Maradashina, Maramanjali

Malayalam: Maramanjal, Manjavalli

Sanskrit: Darvi, Daruharidra, Pitadaru

Tamil: Maramanjal, Atturam, Kadari, Udaravi, Pasamantram, Imalam

Telugu: Manupasupu

Chemical composition:[94]

The major alkaloids are yellow crystalline berberine, protoberberine and jatrorrhizine. Many other alkaloids, mainly of the protoberberine type, isolated from stem and root are magnoflorine, berberrubine, thalifendine, palmitine and oxyberberin. The stem and root also contain ceryl-alcohol, saponin, hentriacontane, sitosterol, palmitic acid, oleic acid and sitosterol glucoside. The total extractives are: petroleum ether 2.1%, ether 2.5%, alcohol 6.9%, chloroform 3.4%, acetone 4.1%, benzene 2.7% and water 5.1%. Other compounds reported from the stem and root are N,N-dimethyllindacarpine, oxypalmitine, (-)-8-oxotetrahydrothalifendine, (-)-8-oxoisocorypalmine, (-)-8-oxothaicanine, (-)-8-oxo-3-hydroxy-2,4,9,10-

tetramethoxyberberine, (-)-8-oxocanadine,12,13-dihydro-8-oxoberberine,5,6,13,13a-tetrahydro-9,10,dimethoxydibenzo(a,g)1,3-benzodioxolo(5,6a) quinalizine-8-one, stigmasterol, berlambine, dihydroberlambine and noroxyhydrastinine

Plant Description: [95]

Coscinium fenestratum is large dioecious liana with 10m long. The stem and root slices are hard and woody. Wood is yellowish-brown in color externally and yellow internally. The leaves are oblong deltoid, obscurely peltate, acuminate, glabrous, and hairy and reticulate beneath and petiole is long. The flowers are green, borne in dense globose heads. Sepals and petals in the ratio of 6:3. The seeds are globose.

Geographical Distribution:

The habitat for Coscinium fenestratum spans south Asia and parts of South-east Asia i.e. India, Sri Lanka, Thailand, Cambodia, Vietnam, peninsular Malaysia, Sumatra, Bangka, Western Java and Borneo. In India, states of Kerala, Karnataka and Tamil Nadu.

Medicinal Uses [96]

The drug is useful in vitiated conditions of *kapha* and *vata*, inflammations, wounds, ulcers, jaundice, burns, skin diseases, abdominal disorders, diabetes, fever and general debility. An infusion of tincture is prepared to wash wounds and skin rashes. Stem pieces are most common used against tetanus. The root bark is used for dressing wounds, ulcers and in cutaneous leishmaniasis. It is known to treat influenza and eye diseases.

Coscinium is also used to treat bleeding piles and excessive bleeding during menstruation. For snakebite poisoning, paste of *Coscinium* and turmeric is applied. Bark is also used in the treatment of leucorrhoea and other gynaecological troubles.

LITERATURE REVIEW

1. Anti-diabetic activity: [97]

Annie Shirwaikar et.al. 2005 study shows that the C.fenestratum alcoholic stem was used for the treatment of Diabetes mellitus evaluated against streptozotocin – nicotamide induced Type 2 diabetes model. The C.fenestratum extract is nontoxic substance from the selected dose of 250mg/kg and 500mg/kg. The alcoholic extract of the stem have the ability of lowering blood glucose levels. The anti-diabetic activity of Coscinium fenestratum is not related to the insulin secretion and the post prandial glucose radiation mainly it is due to the effect of extra pancreatic mechanism and it also increase the serum triglycerides and cholesterol levels in diabetic rats. The administration of this extract, shows hypolipidemic effect by decreasing the cholesterogenesis and fatty acid synthesis.

2. Anti-gonococcal activity: [98]

Chomnawang MT et.al, 2009 depicts that, the medicinal Plant plumbago, Coscinium fenestratum, Alpinia conchigera and Caesalpinia sappan are studied for the Anti-gonococcal activity by disc diffusion method against Nesseria gonorrhaea.The study concluded that Coscinium fenestratum had high anti-gonococcal activity by inhibition of Nesseria gonorrhaea.

3. Anti-oxidant and Anti-microbial activity: [99]

Santhosh W. Govas et.al, 2013 The Antioxidant and Antimicrobial activity were estimated in stem and leaf extract of Coscinium fenestratum. Antioxidant activity is evaluated against 1, 1 diphenyl – 2 – picrylhydroxy (DPPH) and 2, 12’ azino – bis (3-ethyl benzothiazoline – 6- sulphonic acid) (ABTS). The Coscinium fenestratum extract have the ability to reduce DPPH, a stable free compounds whereas ABTS have maximum scavenging effect. The anti-oxidant activity mainly due to phenolic compounds.

Antibacterial activity of aqueous and methanolic extract of C.fenestratum examined against four different strains in that the methanolic extract shows greater antibiotic activity when compared to the aqueous extract.

4. Anti-hepatotoxic activity: [100]

B.K Manjunatha et.al. 2013 reported that, the hepatoprotective activity against CCI4 to induced liver damage upon treatment with ethanolic extract of the C.fenestratum showed

reduction in the serum markers and normal hepatocytes and significant protection against CCI4 induced hepatocellular injury.

5. Immunomodulatory activity: [101]

Shashika Dinethri Kothalawal et.al 2020 this study determines the immunomodulatory activity of concoction C.fenestratum and Coriandum satiumn ,30g of the each seeds were boiled with water and made as concoction according to the traditional Srilankan medicinal practice. Three doses of freeze-dried concoction were orally administered to the rats which shows significant effects against anti-inflammatory activity and immunomodulatory activity by enhancing the effect of specific immune response as when administered the concoction. Further findings of the study validated that the concoction is used for the treatment of cold and reduce the inflammation.

6. Anti-Proliferative Activity: [102-103]

Rudeewan tungpradit et.al 2011, the C.fenestratum of berberine have the main component of cytotoxic activity against NCI_H838 cells in various concentrations by using MTT method [3, (4, 5 dimethyl thiazol – 2- yl) 2, 5 – diphenyl tetrazolium bromide]. This study concluded that the phyto-constituents of berberine induced G2/M arrest in the cell line and results in apoptosis of NCI – H838 cells.

S. Justin Packia Jacob et al 2012, states that Synthesis of silver nanorods using C.fenestratum extract as a reducing agent and cytotoxic activity against Hep-2 cell line. The sliver nanoparticles are formed from the sliver nitrate which reduces into sliver ions. The berberine present in C.fenestratum and other alkaloids responsible for the synthesis of sliver nanorods and it exhibit the cytotoxic activity in HEP – 2 cell lines and it also used for anti-cancer agents.

7. Anti-acne activity: [104]

GS Kumar et.al 2007 done a study on antimicrobial effect of Indian medicinal plants against acne-inducing bacteria in 12 medicinal plant were examined of Propionibacterium acnes and Staphylococcous epidermis. Among 12 extracts, 07 extracts could effectively inhibit the growth Propionibacterium acnes. In the 07 extracts C.fenestratum is one of the plant material and have anti-microbial activity used for alternative medicine for acne.

8. Hypotensive activity: [105]

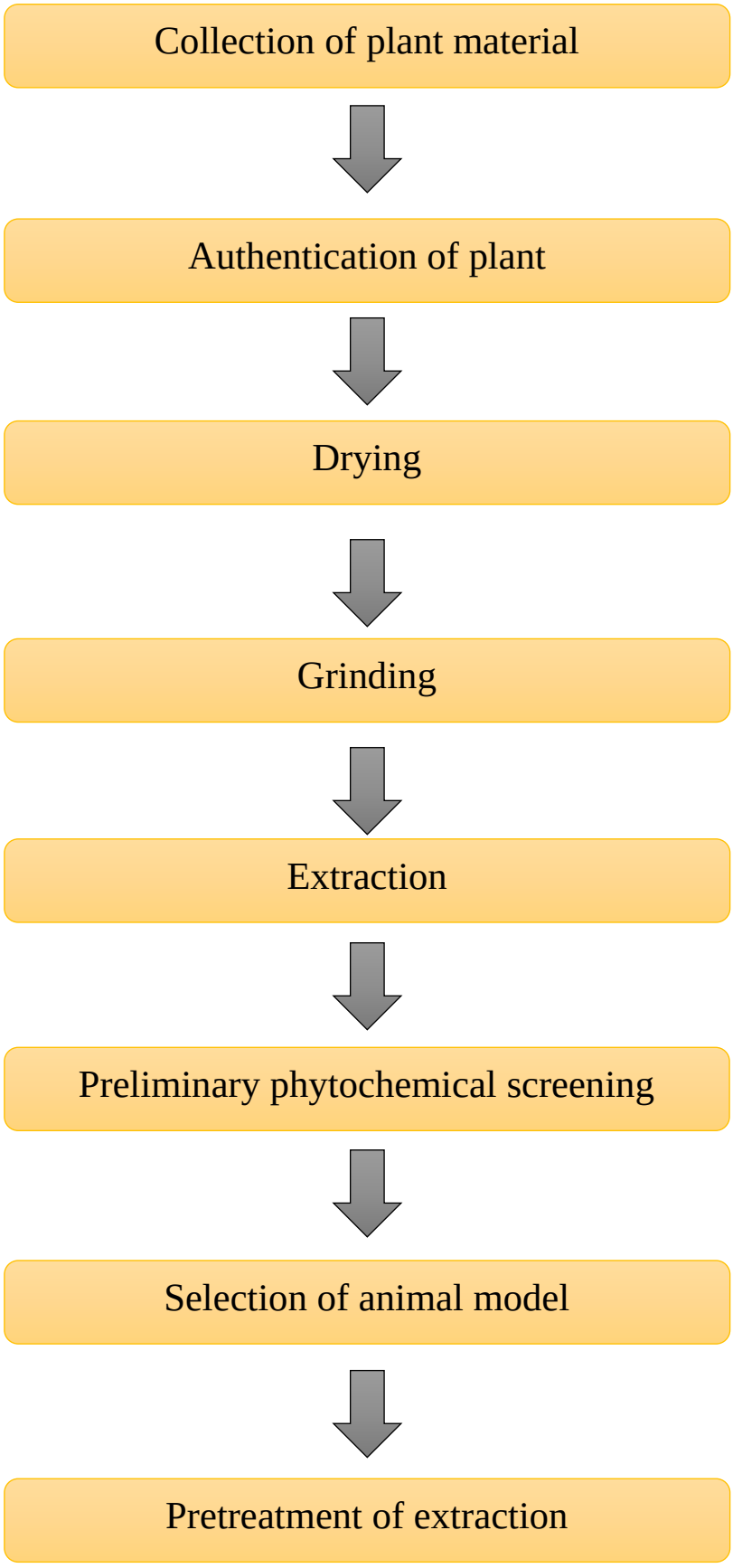
Singh GB et al 1990 study shows that 50% of ethanolic extract of C.fenestratum stem material has found to possess hypotensive action in animal model. The drug was administered orally to mice, the ethanol extract of C.fenestratum (AECF) did not exhibit and also failed when administered via cannula into the lateral cerebral ventricle.

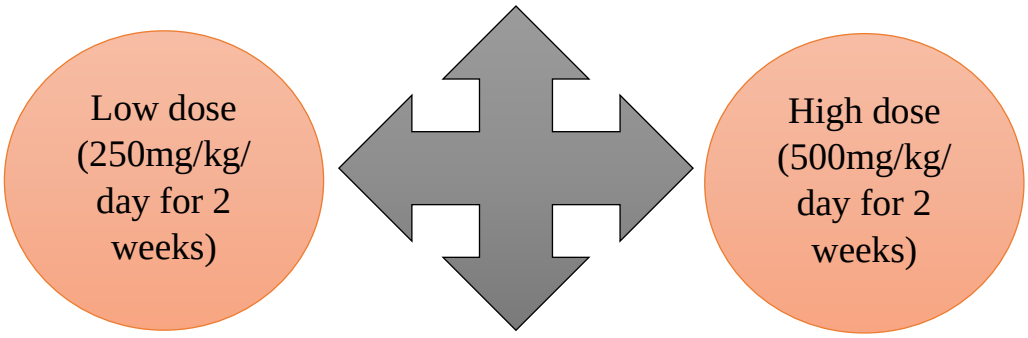
9. CNS depressant and Analgesic activity: [106]

Sudharshan SJ et.al 2009, proven that the methanol extracts of Nardostachys jatamansi and C.fenestratum has CNS depressant were evaluated by actophotometer and the analgesic activity was evaluated by tail flick method

PLAN OF WORK

3. PLAN OF WORK





In-vivo studies

Ex-vivo studies

Data collection

Statistical evaluation

Results

SCOPE OF WORK

4. SCOPE OF WORK:

Polycystic ovary syndrome is a very common endocrine disorder characterized by chronic anovulation, clinical biochemical hyperandrogenism and/ or polycystic ovaries. But most experts consider that hyperandrogenism is the main characteristics of PCOS. Several theories propose different mechanisms to explain PCOS manifestations: 1) a primary enzymatic default in the ovarian and/or adrenal steroidogenesis; 2) an impairment in gonadotropin releasing hormone (GnRH) secretion that promotes luteal hormone (LH) secretion; or 3) alteration in insulin actions that lead to insulin resistance with compensatory hyperinsulinemia.

The bark of Coscinium fenestratum has been used in different system of traditional medication for the treatment of diseases and ailment of human beings. The major constituents of C.Fenestratum is Berberine which is yellow crystalline alkaloid and other constituents are protoberberine alkaloids, oxypalmatine berrubine, (-) – 8 oxotetrahydrothalifendine, (-) – 8 – oxoisocorypalmine and either (-) – 8 – oxothaicanine and (-) – 8 – oxocanadine. Coscinium fenestratum reported to have anti-diabetic, anti-oxidant, anti-microbial, anti-hepatotoxic, immunomodulatory, anti-proliferative and CNS depressant and analgesic activity.

Objective of the study is to disrupts the conversion of androstenedione to 17β estradiol by inducing aromatase inhibitor – letrozole for the induction of polycystic ovarian syndrome. The bark extract of Coscinium fenestratum is given to the PCOS induced rats for the treatment in case of expected to have degeneration and significant decrease in counts and size of follicular ovarian cysts. The study evaluates by various parameters like vaginal smear, body weight and in vitro parameters like antioxidant, lipid profile and oral glucose tolerance test.

MATERIALS AND METHODS

5. MATERIALS AND METHODS:

Collection and Authentication:

The bark of Coscinium fenestratum were collected from local source, Tamilnadu. The bark material was identified and authenticated by Dr.P.Jayaraman, Retd. Professor. Presidency college, Chennai-600005, Tamilnadu. **REFERENCE NO: PARC/2021/4607.**

Method of Extraction: [107]

The bark of Coscinium fenestratum was collected and ground into coarse powder. The powered bark was extracted with ethanol: water (3:1) in Soxhlet's apparatus. 630g powder of Coscinium fenestratum is extracted with 1500ml of hydroalcoholic solvent using a Soxhlet apparatus at 80°C for 72h. The extract is filtered and vacuum dried. The dried residue of extract was cooled in desiccator for 30 min and then accurately weighed for analysis.

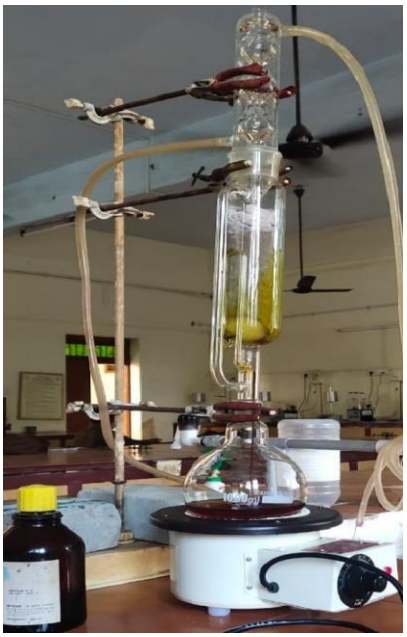


Figure 11

Percentage Yield:

The percentage yield of hydroalcoholic extract was 3.26% w/w and it was preserved in refrigeration for further use.

Experimental animals:

The female Sprague – Dawley rats 3 weeks old were used for this study. The inbreed animals were procured from the animal house of C.L. Baid Metha College of Pharmacy, Chennai-97. They were housed five per cage under standard laboratory conditions at a room temperature at 22±2°C with 12hr light / dark cycle. The animals were acclimated to laboratory conditions one week and provided with standard pellet chow and water ad libitum. Ethical committee clearance was obtained from IAEC of CPSCEA.

IAEC REFERENCE NO: 02/321/PO/Re/S/01/CPSCEA

Disease model:

Letrozole Induced PCOS: [108]

Letrozole is a non-steroidal inhibitor of estrogen synthesis with anti-neoplastic activity. As a third generation aromatase inhibitor, Letrozole selectively and reversible inhibits aromatase which may result in growth inhibition of estrogen dependent breast cancer cells. Letrozole is rapidly and completely absorbed from the GI tract following oral administration.

Letrozole is a non-steroidal type II aromatase inhibits. It blocks the active site and therefore the electron transfer chain of CYP19A1. This competitive inhibition prevents the conversion of androgens to estrogen. This action leads to a reduction in uterine weight and elevated LH. In post-menopausal women the action of aromatase is responsible for the majority of estrogen production with reduced availability of estrogen.

Figure 12

Upon administration of Letrozole in rat both testosterone and LH levels are elevated. High testosterone are due to accumulation of androgens because conversion androgen to estrogens are inhibited. Letrozole are aromatase inhibition in which reduction of estrogen production in hypothalamus and pituitary presumably enhanced LH secretion by releasing feedback of estrogen. Decreased progesterone production, which may result anovulation.

Experimental design:

Duration of study: 36 days

Grouping:

Animal required: Sprague Dawley rats

Age: 3 weeks old female rats

Animals are divided into 5 groups; each group consists of 6 rats (n=6) as follows

S.No	Groups	Treatment
1.	Group I	Control (untreated)
2.	Group II	Letrozole (1 mg/kg) induced PCOS/P.O
3.	Group III	Letrozole induced PCOS + Clomiphene citrate (standard)/P.O
4.	Group IV	Letrozole induced PCOS + Coscinium fenestratum extract (Low Dose)/P.O
5.	Group V	Letrozole induced PCOS + Coscinium fenestratum extract (High Dose)/P.O

Procedure:

Group II, III, IV, V are treated with letrozole (l mg/kg/day) orally for 21 days

On 22nd day group III and IV are treated with BECF and group V are treated with standard drug (clomiphene citrate) orally for 15 days

On 36th day animals are anesthetized and blood is collected from each animal through tail vain for hormonal estimation the uterus ovaries are removed for histopathological examination.

PHYTOCHEMICAL ANALYSIS:[109]

1. Test for phenolics:

   Vanillin Test: To a few drops of extract, add few drops of 10% vanillin in ethanol and concentrated HCl. Pink colour indicates the presence of Phenolic acid. Red-pink colour indicates the presence of Phenyl propene. Red or Purple colour indicates the presence of Flavonols and Flavones.

2. Test for flavonoids:

   To 5ml of dilute Ammonia solution, 3ml of aqueous filtrate was added followed by the addition of concentrated H_2SO_4. Yellow colour formed, disappears on standing indicates the presence of Flavonoids.

3. Test for tannins:

   Lead Acetate Test: To a few drops of the extract few drops of lead acetate was added. Yellow or florescent yellow precipitate indicated the presence of tannins.

4. **Test for Alkaloids:**

   Mayer's test: 200 mg of plant material was dissolved in 10ml of Methanol and filtered. To 2ml of filtrate, 1% HCl and 6 drops of Mayer's reagent was added. Creamish, brownish red or orange precipitate indicated the presence of Alkaloids.

5. **Test for Saponins:**

   Frothing Test: To 0.5ml of filtrate, add 5ml of water. Shake vigorously. Persistence of froth for at least 10minutes indicated the presence of saponins

6. **Test for Steroids:**

   2ml of Acetic Anhydride was added to 0.5grams of Ethanolic extract. 2ml of H_2SO_4 was added. A colour change from violate to blue or green indicated the presence of steroids

7. **Test for Terpenoids:**

   Salkowski Test: 5ml of extract was mixed with 2 ml of chloroform. Add 3ml of concentrated H_2SO_4 along the sides to form a layer. A reddish-brown colour indicated the presence of Terpenoids.

8. **Test for Cardiac Glycosides:**

   To 5ml of extract add 2ml of glacial Acetic acid and 1 drop of $FeCl_3$. This was under layered with concentrated H_2SO_4. A brown ring of the interface indicates deoxy sugar

IN- VIVO PARAMETER:

Estimation of the morphology of estrus cycle: [110 – 111]

Vaginal lavage method:

Vaginal smears must be taken at the same time each day, preferably in the morning for a minimum of 7 consecutive days.

1. Moistened the cotton swab with saline prior to use
2. The saline moistened cotton swab is inserted into the vagina orifice of rats, they were carefully rotated against the vagina wall, which allow the cotton tip to pick up an adequate load of cells. The swab can be gently withdrawn. The recollected sample of vaginal smears was placed on glass slide, dried at 37°c and fixed in a ethanol solutions for one minute.
3. The slide is air-dried and stained with 0.1% crystal violet stain (0.1g of crystal violet powder in 100 ml of double distilled water) and viewed under the 100X microscope
4. Record the cells density and numbers of leukocytes, cornified epithelial and nucleated epithelial cells.

Examine smear under a microscopic at low power:

Proestrus: lasts approximately 12hrs and has abundant nucleated non-cornified epithelial cells.

Estrus: lasts up to 12 hours and is indicated by the presence of large cornified cells in the vaginal smear.

Metestrus: lasts approximately 21 hours and usually has many neutrophils and in the smear and scattered.

Diestrus: lasts up to 57 hours and there are abundant neutrophils and a few nucleated non-cornified epithelial cells.

Visual method

Vagina of each animal was examined carefully to avoid misinterpretations. The investigation room was provided with adequate illumination because light source is extremely important for visual examination. The tail of the rat was lifted gently and the vulva was observed.

Proestrus: the vaginal opening was wide, moist and the tissues appeared pink. Striations were seen in both the dorsal and the ventral lips of the vulva.

Estrus: less pink, less moist but striations were more pronounced at this stage.

Metestrus: pale dry opening which was sloughed with white cellular debris.

Diestrus: vaginal opening was very moist, too small and closed in some mice with no tissue.

IN VITRO PARAMETER:

Hormonal studies:

On 36^{th} day grouped rats were sacrificed by overdose anesthesia was collected and serum was separated to study the hormonal parameters. For serum, separation the blood was allowed to stand for 20 minutes at room temperature to clot and then centrifuged at 3000 rpm for 10 min. The supernatant (serum) was then tipped off into a sperate vial and subsequently subjected to as assessment of hormonal levels such as testosterone, FSH and LH.

Preparation of serum in blood:

Blood was collected and centrifuged. The supernatant liquid was used for hormonal analysis such as FSH, LH and Testosterone.

Estimation of total testosterone: [112]

Testosterone, is a vital androgen in mammals. It is mainly secreted by ovarian and adrenal reticular cells in women. Testosterone is the principal androgen hormone essential for the development of secondary sexual characteristics. It promotes protein synthesis, accelerates bone growth, promotes growth and development, and maintains endocrine stability.

Aim:

To estimate the total amount of testosterone in the given sample using chemiluminescence immunoassay (CLIA).

Material and reagents:

- Blood sample
- Automatic biochemical analyzer CLIA kit

Procedure

- 100 microlitre of either serum or whole blood is applied to a reagent cartridge for the Testosterone assay.
- The reagent cartridge was into CLEIA analyzer to run assays.
- All reagent were contained in the cartridge, and all of procedures of the assay were performed inside it.
- The CLEIA system is based on a one-step competitive immunoassay system method. The samples were first mixed with an alkaline phosphatase (ALP) - labeled antibody and a magnetic latex reagent.
- Magtration was the used to remove excess reagent and residual materials not bound to the magnetic latex. A chemiluminescent substrate was added and luminescence was emitted upon binding to the ALP.
- Luminescence was then measured and hormonal concentrations were determined.

Estimation of Follicle-Stimulating Hormone (FSH):

Aim:

To estimate the Chemiluminescence enzyme immunoassay for the quantitative determination of Follicle-Stimulating Hormone (FSH) in human serum.

Principle:

The FSH CLIA Kit is based on the principle of a solid phase enzyme- linked immunosorbent assay. The assay system utilizes a polyclonal anti-FSH antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-FSH antibody in the antibody – enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in FSH molecules being sandwiched between the

solid phase and enzyme-linked antibodies. After 60 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. a solution of chemiluminescent substrate is the added. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of FSH in the sample. By reference to a series of FSH standards assayed in the same way, the concentration of FSH in the unknown sample is quantified.

Materials required:

- Anti-FSH antibody coated microtiter wells – 96 wells
- Enzyme conjugate reagent – 12ml
- Reference standard
- 50X wash buffer – 15ml
- Chemiluminescence reagent A and B – 0.0 ml

Procedure:

- Secure the desired number of coated well in the holder. Make data sheet with sample identified.
- Dispense 50 µl of FSH standard, samples and controls into appropriate wells.
- Dispense 100 µl of enzyme conjugate reagent into each well.
- Thoroughly mix for 30 seconds. It is important to have completely mixing in this step.
- Incubate at room temperature (18-25°C) for 60 minutes.
- Remove the incubation mixture by flicking plate contents into a waste container.
- Rinse and flick the microtiter wells 5 times with washing buffer.
- Strike the wells sharply onto absorbent paper to remove residual water droplets.
- Dispense 100 µl chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
- Read wells with a chemiluminescence microwell reader 5 minutes later.

Estimation of Luteinizing Hormone (LH):

Aim:

To estimate the Chemiluminescence enzyme immunoassay for the quantitative determination of Luteinizing Hormone (LH) in human serum.

Principle:

The LH CLIA Kit is based on the principle of a solid phase enzyme- linked immunosorbent assay. The assay system utilizes a polyclonal anti-LH antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-LH antibody in the antibody – enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 60 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of chemiluminescent substrate is the added. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of LH in the sample. By reference to a series of LH standards assayed in the same way, the concentration of LH in the unknown sample is quantified.

Materials required:

- Anti-LH antibody coated microtiter wells – 96 wells
- Enzyme conjugate reagent – 12ml
- Reference standard
- 50X wash buffer – 15ml
- Chemiluminescence reagent A and B – 6.0 ml

Procedure:

- Secure the desired number of coated well in the holder. Make data sheet with sample identified.
- Dispense 50 µl of LH standard, samples and controls into appropriate wells.
- Dispense 100 µl of enzyme conjugate reagent into each well.
- Thoroughly mix for 30 seconds. It is important to have completely mixing in this step.
- Incubate at room temperature (18-25°C) for 60 minutes.
- Remove the incubation mixture by flicking plate contents into a waste container.
- Rinse and flick the microtiter wells 5 times with washing buffer.
- Strike the wells sharply onto absorbent paper to remove residual water droplets.
- Dispense 100 µl chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
- Read wells with a chemiluminescence microwell reader 5 minutes later.

Estimation of superoxide [113]

SOD enzyme activity based on Xanthine and xanthine oxidase were used to generate superoxide anion radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride quantitatively to form a red formazan dye. SOD inhibits the reaction by converting the superoxide radical to oxygen. Homogenized liver tissues, *Fasciola* spp. parasites and standard solutions were used for the assay of SOD. Absorbance was measured at 505 nm on a UV/visible spectrophotometer for 30s after the addition of xanthine oxidase as start reagent and 3 minute after reaction as duplicate samples

Solutions	Uninhibited tubes	Inhibited tubes	Standards (St_{1-5}) tubes
Substrates	850 µl	850 (µl)	850 (µl)
Phosphate buffer 7.0	25 µl	0	0
Xanthine oxidase	125 µl	125 µl	125 µl
Samples	0	25 µl	0
Standard solution	0	0	25 µl 0.2, 0.5, 1.0, 2, 4 (U/mg)

Inhibited percentage of standards and samples were calculated by following formula.

100-(▵ A Standard /min×100)/ (▵ AS1/min) = % inhibition

(Where S1 is ▵ A2-A1/3 uninhibited tube and ▵ Standard equal A2-A1/3 of inhibited tubes).

Estimation of Catalase Enzyme: [114]

The current method is based on the concept of establishing a simple assay of catalase enzyme activity for biological tissues, which depends on the conversion of the oxidation state of cobalt (II) to cobalt (III) by hydrogen peroxide in the presence of bicarbonate solution. This process ends with the formation of a carbonato-cobaltate (III) complex ([Co $(CO_3)_3$] Co). This end product has two clear absorption peaks at 440 and 640 nm. The 440-nm band has been used for the assessment of catalase activity. Dissociation of hydrogen peroxide is proportional to the activity of catalase enzyme in the used sample. The method has been developed for the measurement of catalase activity in biological samples (bacteria, red blood cells, and liver and kidney tissue homogenates).

Procedure

Reagents	Test	Standard	Blank
Catalase source sample	500 µl	–	–
Distilled water	–	500 µl	1500 µl
Hydrogen peroxide	1000 µl	1000 µl	–
The tubes were mixed with a vortex and incubated at 37 °C for 2 min, after which the following substance was added:			
Working solution	6000 µl	6000 µl	6000 µl
Next, the tubes were vortexes for 5 s and then kept at room temperature for 10 min in the dark. The changes in absorbance were recorded at 440 nm against the reagent blank			

Estimation of lipid peroxidase enzyme: [115]

The oxidative degradation of unsaturated fatty acids can be followed by determining the amount of a product of lipid peroxidation or by determining the total consumption of oxygen. The colorimetric reaction with 2-thiobarbitutric acid (TBA) of an unknown substance formed during the aerobic incubation of tissue homogenates, this substance as a three-carbon oxidation product of fats recognized this as malonyl dialdehde (MDA), a secondary product of lipid peroxidation. The unsaturated fatty acids are measured in photometric.

Procedure:

- 1.5 ml of solution (containing 0.1-0.2 ml of homogenate, or mitochondrial or microsomal suspension, or emulsion of unsaturated fatty acids, plus inhibitors or activators, all in a 0.2 M tris-maleate buffer of pH 5.9 or 0.2 M tris-0.16 M KC1 buffer of pH 7.4, e.g. 0.1 ml of emulsion of 10 mM polyunsaturated fatty acid + 0.1 ml of activator or inhibitor + 1.3 ml of buffer) were incubated 30 min at 37”
- After which 1.5 ml of TBA reagent was added, the mixture was heated in a boiling water bath for 10 min using a marble as a condenser.
- After cooling, 3.0 ml pyridine ln-butanol (3/l, v/v) and 1.0 ml 1 N NaOH were added and mixed by shaking or by aeration.
- The photometric measurement was carried out at X = 548 mp.
- A non-incubated blank was used.

Histopathology:

The organ ovaries of rats from all groups were removed dissected out and washed out with ice-cold saline and preserved in 10% formalin solution for histopathological studies.

Statistical analysis

The data was analyzed in terms of Mean± standard error of Mean (SEM). For statistical analysis, multiple comparisons of data were made using Analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was used for analysis. Software program GraphPad prism 9 was used for all data analysis.

TABLES AND GRAPHS

5. TABLES AND GRAPHS

Phytochemical screening of test drug of Coscinium fenestratum:

TABLE 1

Phytochemicals	**BECF**
Phenolics	Present
Flavonoids	Present
Tannins	Absent
Alkaloids	Present
Saponins	Present
Steroids	Absent
Terpenoids	Absent
Cardiac glycosides	Present

Confirmation of estrus cycle in PCOS induced rats after the treatment the of BECF:

TABLE 2

Proestrus	vagina is gapped, striations are visible. Tissues were reddish pink very swollen and moist.
Estrus	tissues were lighter pink, less moist, less swollen
Metestrus	vaginal tissues are pale no striations and dry
Diestrus	vagina has a small opening, not pink, no striations and moist.

Effect of BECF on Follicle Stimulating Hormone:

TABLE 3

S.NO	Grouping	FSH (ng/ml)
1.	Group I	7.3 ± 1.40
2.	Group II	1.21 ± 0.27 **a******
3.	Group III	5.95 ± 0.29 **a^{ns} b******
4.	Group IV	2.50 ± 0.24 **a**** b^{ns} c******
5.	Group V	4.82 ± 0.28 **a^{ns} b*** c***

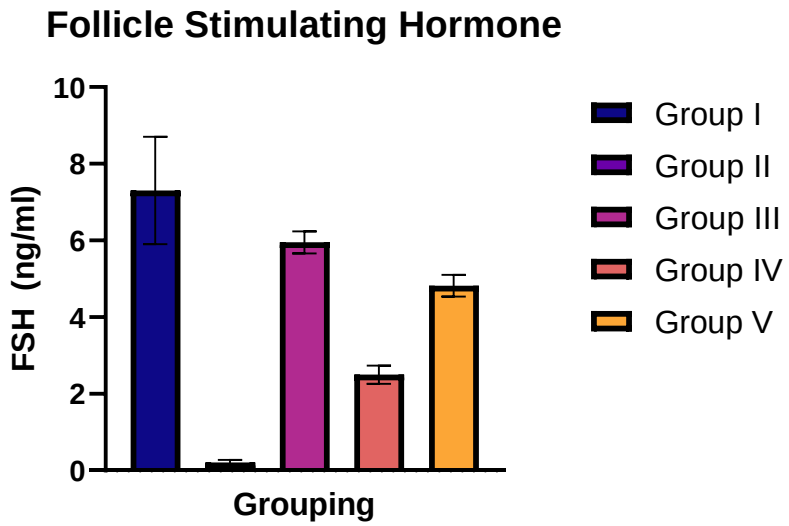
All the values are expressed on mean ± SEM n=6

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns – non significant

Group I vs Group II, Group III, Group IV, Group V is considered as a

Group II vs Group III, Group IV, Group V is considered as b

Group III vs Group IV, Group V is considered as c (one – way ANOVA followed by Dunnett's multiple comparisons) Graph pad prism 9.3.1 (471) version



Effect of BECF on Luteinizing Hormone:

TABLE 4

S.NO	Grouping	LH (ng/ml)
1.	Group I	4.53 ± 0.10
2.	Group II	17.15 ± 1.30 **a****
3.	Group III	5.29 ± 0.09 **a^{ns} b****
4.	Group IV	7.65 ± 0.19 **a** b**** c****
5.	Group V	6.07 ± 0.21 **a^{ns} b**** c***

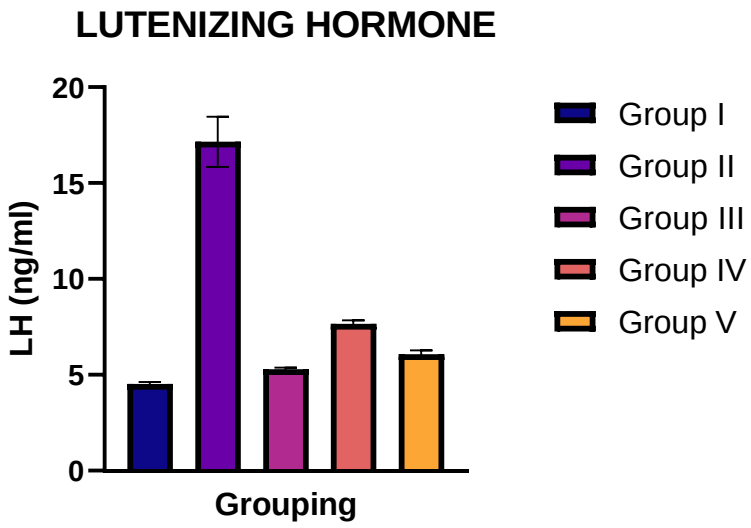
All the values are expressed on mean ± SEM n=6

*p<0.05, **p<0.01, ****p<0.0001, ns – non significant

Group I vs Group II, Group III, Group IV, Group V is considered as a

Group II vs Group III, Group IV, Group V is considered as b

Group III vs Group IV, Group V is considered as c (one – way ANOVA followed by Dunnett's multiple comparisons) Graph pad prism 9.3.1 (471) version



Effect of BECF on Total Testosterone:

TABLE 5

S.NO	Grouping	TT (ng/ml)
1.	Group I	0.4 ± 0.01
2.	Group II	2.25 ± 0.07 **a******
3.	Group III	0.36 ± 0.01 **a^{ns} b******
4.	Group IV	0.21 ± 0.02 **a^{ns} b**** c^{ns}**
5.	Group V	0.19 ± 0.05 **a^{ns} b**** c^{ns}**

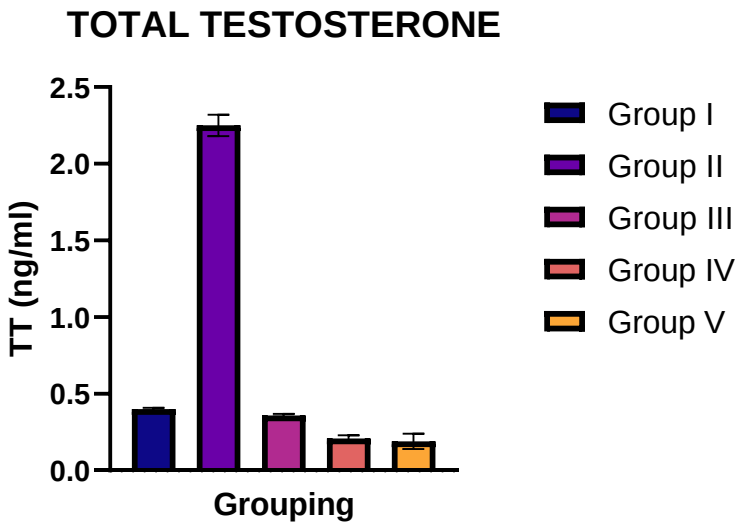
All the values are expressed on mean ± SEM n=6

*p<0.05, **p<0.01, ****p<0.0001, ns – non significant

Group I vs Group II, Group III, Group IV, Group V is considered as a

Group II vs Group III, Group IV, Group V is considered as b

Group III vs Group IV, Group V is considered as c (one – way ANOVA followed by Dunnett's multiple comparisons) Graph pad prism 9.3.1 (471) version



Effect of BECF on Superoxide Dimutase:

TABLE 6

S.NO	Grouping	SOD (U/L)
1.	Group I	135.10 ± 1.53
2.	Group II	65.76 ± 1.44 **a******
3.	Group III	136.60 ± 1.26 **a^{ns} b******
4.	Group IV	86.13 ± 1.41 **a**** b**** c******
5.	Group V	124.75 ± 1.02 **a*** b**** c******

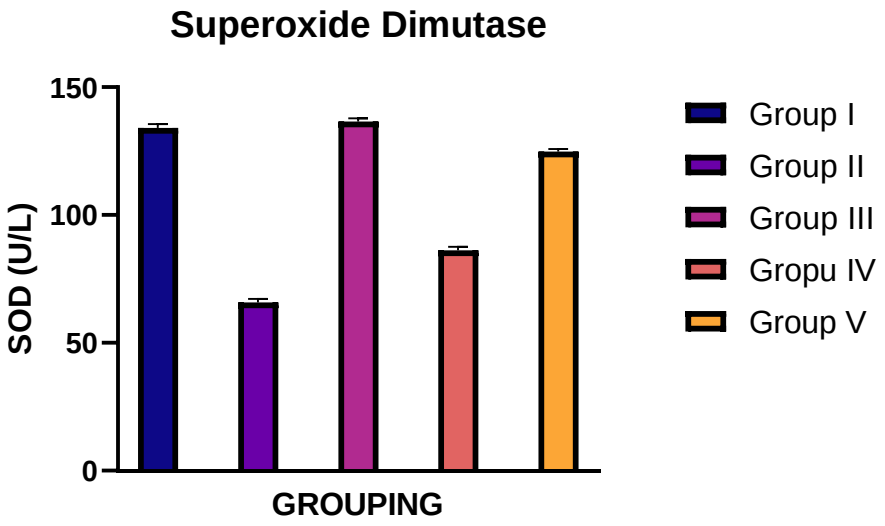
All the values are expressed on mean ± SEM n=6

*p<0.05, p<**0.01***p<0.001, ****p<0.0001, ns – non significant

Group I vs Group II, Group III, Group IV, Group V is considered as a

Group II vs Group III, Group IV, Group V is considered as b

Group III vs Group IV, Group V is considered as c (one – way ANOVA followed by Dunnett's multiple comparisons) Graph pad prism 9.3.1 (471) version



Effect of BECF on Catalases Enzymes:

TABLE 7

S.NO	Grouping	CAT (U/L)
1.	Group I	377.45 ± 2.93
2.	Group II	95.76 ± 1.30 **a******
3.	Group III	375.72 ± 1.67 **a^{ns} b******
4.	Group IV	217.51 ± 9.89 **a**** b**** c******
5.	Group V	352.99 ± 2.02 **a** b**** c***

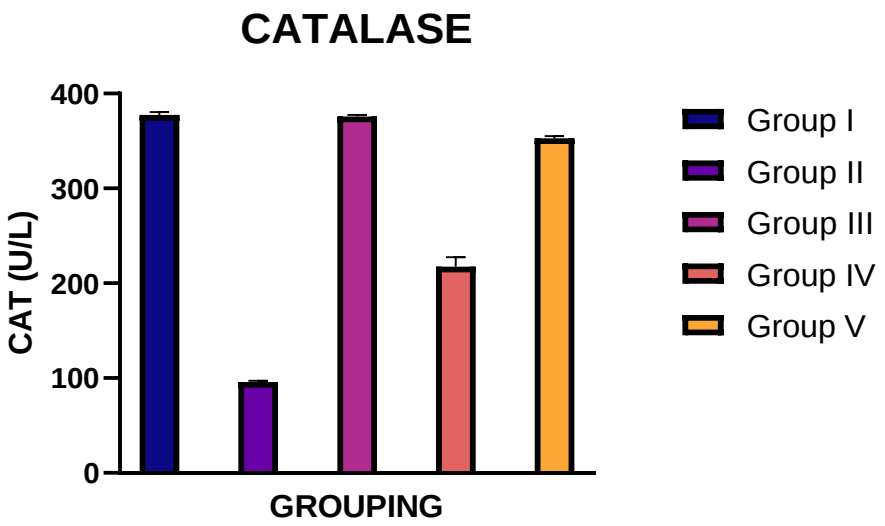
All the values are expressed on mean ± SEM n=6

*p<0.05, **p<0.01, ****p<0.0001, ns – non significant

Group I vs Group II, Group III, Group IV, Group V is considered as a

Group II vs Group III, Group IV, Group V is considered as b

Group III vs Group IV, Group V is considered as c (one – way ANOVA followed by Dunnett's multiple comparisons) Graph pad prism 9.3.1 (471) version



Effect of BECF on Lipid Peroxidation:

TABLE 8

S.NO	Grouping	LOP (nmoles/mg)
1.	Group I	171.62 ± 4.17
2.	Group II	375.16 ± 1.31 **a****
3.	Group III	165.96 ± 1.03 **a^{ns} b****
4.	Group IV	145.8 ± 1.18 **a**** b**** c****
5.	Group V	136.44 ± 0.88 **a**** b**** c****

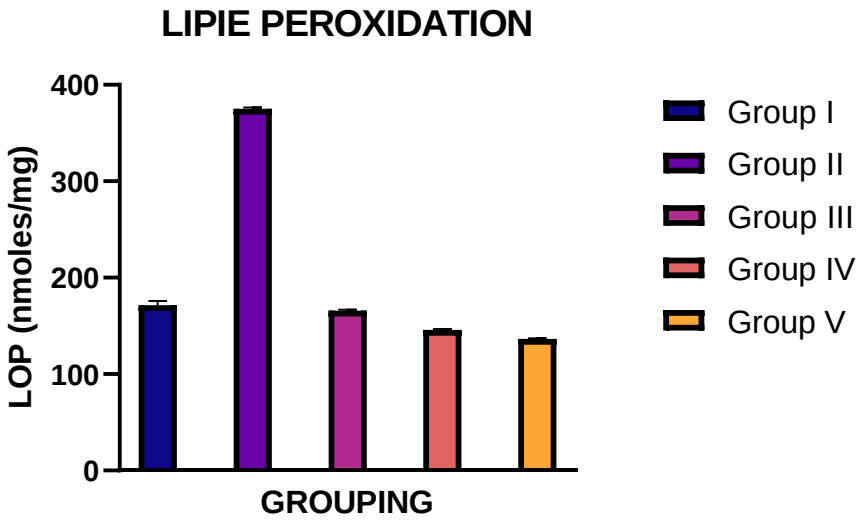
All the values are expressed on mean ± SEM n=6

*p<0.05, **p<0.01, ****p<0.0001, ns – non significant

Group I vs Group II, Group III, Group IV, Group V is considered as a

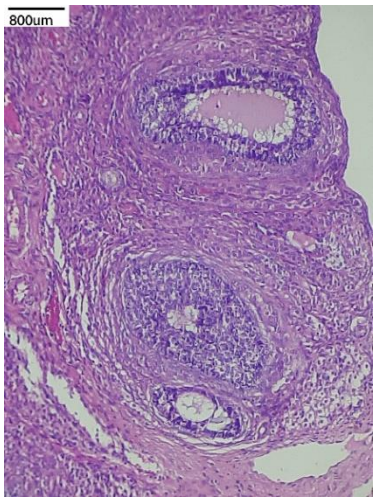
Group II vs Group III, Group IV, Group V is considered as b

Group III vs Group IV, Group V is considered as c (one – way ANOVA followed by Dunnett's multiple comparisons) Graph pad prism 9.3.1 (471) version

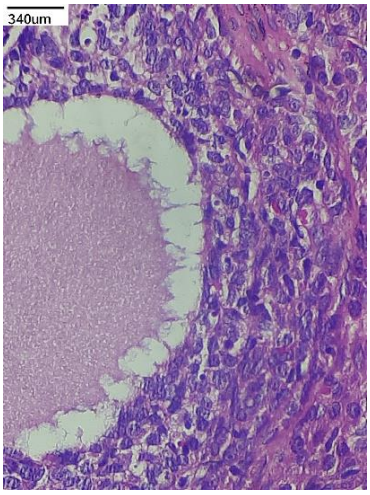


HISTOPATHOLOGICAL OF OVARY

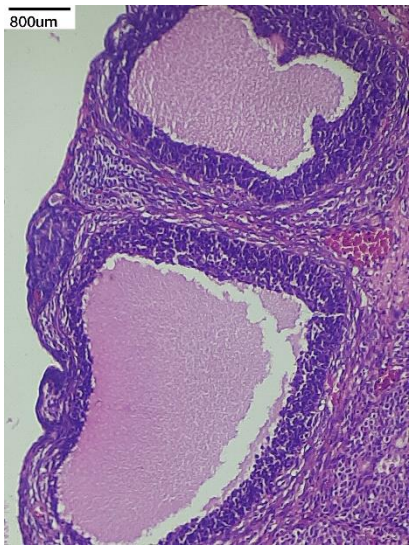
GROUP I



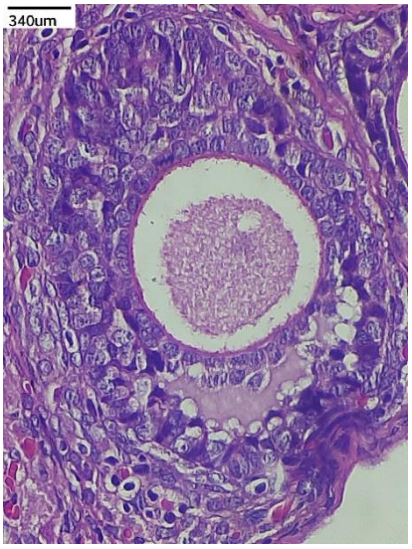
GROUP II



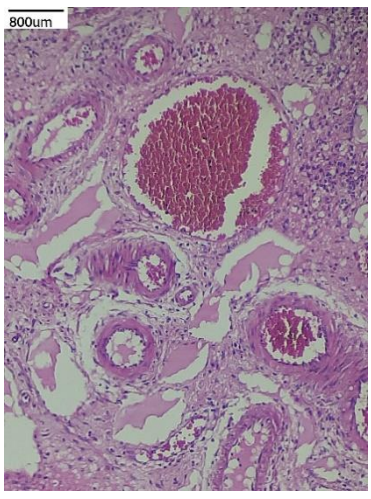
GROUP III



GROUP IV



GROUP V



RESULTS

6. RESULTS

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF BARK EXTRACT OF *COSCINIUM FENESTRATUM:* (TABLE 1)

The result of preliminary phytochemical analysis of bark extract of *Coscinium fenestratum* showed presence of phytochemical constituents such as phenols, flavonoids, alkaloids, saponins and cardiac glycosides.

EFFECT OF BECF ON FOLLICLE STIMULATING HORMONE IN PCOS INDUCED FEMALE RATS (TABLE 3)

Group I vs Group II, Group III, Group IV & Group V is considered as (a)

When compared to Group I, Group II ($p<0.0001$) there was a significant decrease FSH in serum

When compared to Group I, Group III non- significant decrease FSH in serum

When compared to Group I, Group IV non - significant decrease FSH in serum

When compared to Group I, Group V non - significant decrease FSH in serum

Group II Vs Group III, Group IV & Group V is considered as (b)

When compared to Group II, Group III ($p<0.0001$) there was a significant increase in FSH in serum

When compared to Group II, Group IV non - there was a significant increase in FSH in serum

When compared to Group II, Group IV ($p<0.001$) there was a significant increase in FSH in serum

Group III Vs Group IV & Group V is considered as (c)

When compared to Group III, Group IV ($p<0.0001$) there was a significant decrease FSH in serum

When compared to Group III, Group V ($p<0.05$) significant decrease FSH in serum

EFFECT OF BECF ON LUTEINIZING HORMONE IN PCOS INDUCED FEMALE RATS (TABLE 4)

Group I vs Group II, Group III, Group IV & Group V is considered as (a)

When compared to Group I, Group II ($p<0.0001$) there was a significant increase in LH in serum

When compared to Group I, Group III non - significant decrease LH in serum

When compared to Group I, Group IV ($p<0.01$) there was a significant decrease in LH in serum

When compared to Group I, Group V non - significant decrease LH in serum

Group II Vs Group III, Group IV & Group V is considered as (b)

When compared to Group II, Group III ($p<0.0001$) there was a significant decrease in LH in serum

When compared to Group II, Group IV ($p<0.0001$) there was a significant decrease in LH in serum

When compared to Group II, Group V ($p<0.0001$) there was a significant decrease in LH in serum

Group III Vs Group IV & Group V is considered as (c)

When compared to Group III, Group IV ($p<0.0001$) there was a significant increase in LH in serum

When compared to Group III, Group V ($p<0.05$) there was a significant increase in LH in serum

EFFECT OF BECF ON TOTAL TESTOSTERONE IN PCOS INDUCED FEMALE RATS (TABLE 5)

Group I vs Group II, Group III, Group IV & Group V is considered as (a)

When compared to Group I, Group II ($p<0.0001$) there was a significant increase in TT in serum

When compared to Group I, Group III non - significant decrease TT in serum

When compared to Group I, Group IV non - significant decrease TT in serum

When compared to Group I, Group V non - significant decrease LH in serum

Group II Vs Group III, Group IV & Group V is considered as (b)

When compared to Group II, Group III ($p<0.0001$) there was a significant decrease TT in blood

When compared to Group II, Group IV ($p<0.0001$) there was a significant in decrease TT in serum

When compared to Group II, Group V ($p<0.0001$) there was a significant decrease TT in serum

Group III Vs Group IV & Group V is considered as c

When compared to Group III, Group IV non - significant decrease TT in serum

When compared to Group III, Group V non - significant decrease TT in serum

EFFECT OF BECF ON SUPEROXIDE DIMUTASE IN PCOS INDUCED FEMALE RATS (TABLE 6)

Group I vs Group II, Group III, Group IV & Group V is considered as (a)

When compared to Group I, Group II ($p<0.0001$) there was a significant in decrease SOD in serum

When compared to Group I, Group III non-there was a significant increase in SOD in serum

When compared to Group I, Group IV ($p<0.0001$) significant decrease SOD in serum

When compared to Group I, Group V ($p<0.001$) significant decrease SOD in serum

Group II Vs Group III, Group IV & Group V is considered as (b)

When compared to Group II, Group III ($p<0.0001$) there was a significant increase in SOD in serum

When compared to Group II, Group IV ($p<0.0001$) there was a significant increase in SOD in serum

When compared to Group II, Group V ($p<0.0001$) there was a significant increase in SOD in serum

Group III Vs Group IV & Group V is considered as (c)

When compared to Group III, Group IV ($p<0.0001$) there was a significant decrease in SOD in serum

When compared to Group III, Group V ($p<0.0001$) there was a significant decrease in SOD in serum

EFFECT OF BECF ON CATALASE IN PCOS INDUCED FEMALE RATS (TABLE 7)

Group I vs Group II, Group III, Group IV & Group V is considered as (a)

When compared to Group I, Group II ($p<0.0001$) there was a significant decrease CAT in serum

When compared to Group I, Group III non- significant decrease CAT in serum

When compared to Group I, Group IV ($p<0.0001$) significant decrease CAT in serum

When compared to Group I, Group V ($p<0.01$) significant decrease CAT in serum

Group II Vs Group III, Group IV & Group V is considered as (b)

When compared to Group II, Group III non-there was a significant increase in CAT in serum

When compared to Group II, Group IV ($p<0.0001$) there was a significant increase in CAT in serum

When compared to Group II, Group V ($p<0.0001$) there was a significant increase in CAT in serum

Group III Vs Group IV & Group V is considered as (c)

When compared to Group III, Group IV ($p<0.0001$) there was a significant decrease in CAT in serum

When compared to Group III, Group V ($p<0.05$) there was a significant decrease in CAT in serum

EFFECT OF BECF ON LIPID PEROXIDATION IN PCOS INDUCED FEMALE RATS (TABLE 8)

Group I vs Group II, Group III, Group IV & Group V is considered as (a)

When compared to Group I, Group II ($p<0.0001$) there was a significant increase in LOP in serum

When compared to Group I, Group III non- significant decrease LOP in serum

When compared to Group I, Group IV ($p<0.0001$) there was a significant decrease in LOP in serum

When compared to Group I, Group V ($p<0.0001$) there was a significant decrease in LOP in serum

Group II Vs Group III, Group IV & Group V is considered as (b)

When compared to Group II, Group III ($p<0.0001$) there was a significant decrease in LOP in serum

When compared to Group II, Group IV ($p<0.0001$) there was a significant decrease in LOP in serum

When compared to Group II, Group V ($p<0.0001$) there was a significant decrease in LOP in serum

Group III Vs Group IV & Group V is considered as (c)

When compared to Group III, Group IV ($p<0.0001$) there was a significant decrease in LOP in serum

When compared to Group III, Group V ($p<0.0001$) there was a significant decrease in LOP in seerum

HISTOPATHOLOGY:

Group I - ovarian section of control group with healthy growing follicles and a corpus luteum is seen

Group II- ovarian section of disease group shows several cyst-like follicles

Group III – ovarian section of standard group shows decreases in cyst size

Group IV – ovarian section of treatment group (250mg/kg) shows decreases in cysts size

Group V – ovarian section of treatment group (500 mg/kg) shows decreases in cysts size and healthy follicles.

DISCUSSION AND CONCULSION

7. DISCUSSION

PCOS is the most common endocrine disorder and mainly affects 6-20% of women in reproductive age. It is a complex disease in which genetics, endocrine, environmental and behavioural factors intertwined and giving rise to heterogenous phenotype with a reproductive, metabolic and psychological characteristics.

Obesity increases hyperandrogenism, hirsutism, infertility and pregnancy complications both independently and by exacerbating PCOS. In PCOS, high circulating levels of androgens, estrogen and sex steroid precursors, follicle abnormalities are the most consistent feature in androgen hypersecretion. Hyperandrogenism, a clinical hallmark of PCOS, can cause inhibition of follicular development, microcysts in the ovaries, anovulation and menstrual changes. During the follicle development it reduces progesterone and estrogen levels due to regression of the corpus luteum.

The plant Coscinium fenestratum has been used in different system of traditional medication for the treatment of disease and ailments of human beings. The phytochemical screening of the bark of Coscinium fenestratum revealed the presence of bioactive compounds such phenolics, flavonoids, alkaloids, saponins and cardiac glycosides. C.fenestratum has been reported to have been some pharmacological activities.

Letrozole being a non-steroidal aromatase inhibitor blocks the conversion of testosterone to estradiol. This results in the reduction of estradiol production and elevated levels of testosterone. Letrozole an aromatase has increased the circulatory androgen levels. Clomiphene citrate a selective estrogen receptor modulator (SERM) has agonist and antagonist activity. Treatment of clomiphene citrate has been shown to improve ovulation induction in female rats.

In present study, we need induced aromatase inhibition in female rats in order to induce conditions similar to those PCOS in women it is then examined for hormonal concentration, antioxidant activity and histopathology changes in the ovaries on letrozole induced PCOS female rats.

Decreased or normal FSH and increased LH are due to (a) GnRH pulsatile secretion, at hypothalamic level (b) high estrogen environment at pituitary level. FSH is responsible for the development of eggs into mature follicles by acting on immature follicular cells of the ovary

in normal individual. In this study FSH level shows a significant increase in BECF treated female rats compared to letrozole induced female rats.

LH is responsible for the secretion of progesterone and estrogen which is elevated level in PCOS. The decrease in the sex hormone binding protein in the liver, and increase in insulin response in the ovary and the effect of high LH this results in increase androgen secretion in the ovary then, follicle growth and maturation are suppressed. In this study, LH level are significant decreased in BECF treated female rats than letrozole induced female rats.

Insulin resistance leads to hyperinsulinemia, which reduces SHBG and raises free circulating testosterone. Elevated levels of free or total testosterone are not converted to estradiol in PCOS. BECF treatment show significant decrease in total testosterone level than letrozole induced female rats.

The antioxidant activity like SOD and CAT level increased in BECF treated female rats than letrozole induced female rats and lipid peroxidation decreased in BECF treated female rats than letrozole induced female rats.

8. CONCLUSION:

The Estrus cycle is restored to regular pattern in the animals treated with BECF. Hence it can be concluded that this extract has a potential healing effect on PCOS rat model, bringing the reproductive cycle and other complications to normal. The finding of the study confirmed the BECF causes an increase in FSH level and decrease in LH and Testosterone level near to the control level. BECF shows remarkable antiandrogenic effect by degenerating the immature follicular cysts and decreasing the cysts size which can be assumed, to help in regulating normal ovulation. Therefore, this extract needs further study to establish the mechanism of action of BECF.

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INSTITUTE OF HERBAL SCIENCE
PLANT ANATOMY RESEARCH CENTRE

Prof. **P Jayaraman, Ph.D**
Director
Retd, Professor, Presidency College Chennai-5

AUTHENTICATION CERTIFICATE

Based upon the Organoleptic / Macroscopic / ~~Microscopic~~ Examination of Fresh / ~~Market~~ Sample, it is certified that the specimen given by K. Haripriya, M. Pharm., Dept. of Pharmacology, C.L. Baid Metha College of Pharmacy, is identified as below :

Binomial : Cascinium fenestratum (Graertn.) Coleb.

Family : Menispermaceae

Synonym(s) : Menispermum fenestratum Graertn.

Regional Names : Tamil :- Maramanjal

Reg. No of the Certificate : PARC/2021/4607

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Date : 16.12.2021.

(Prof. P.JAYARAMAN)
Prof. P. Jayaraman, M.Sc., Ph.D.,
Director
PLANT ANATOMY RESEARCH CENTER
(PARC)
West Tambaram, Chennai - 45.
Ph: 044-22263236, Cell: 9677237739
E-mail: herbalparc@yahoo.com

#4, 2nd Street, Sakthi Nagar,
West Tambaram, Chennai-600 045
Ph:044-22263236, +919444385098
Email- herbalparc@yahoo.com

INTERNATIONAL CONFERENCE ON DRUG DISCOVERY

Feb 29th - Mar 2nd, 2020, BITS-Pilani, Hyderabad

Certificate

This is to certify that Haripriya.K *has successfully participated in International Conference on Drug Discovery held at BITS-Pilani Hyderabad Campus from 29th Feb to 2nd Mar 2020*

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Vice President

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OVERVIEW OF POLYCYSTIC OVARY SYNDROME

HARI PRIYA. K AND MURALIDHARAN P*

Department of Pharmacology, C. L. Baid Metha College of Pharmacy, Chennai, Tamilnadu, India

***Corresponding Author: Dr. Muralidharan Palayyan: E Mail: pmuralidaran2020@gmail.com**

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ABSTRACT

The PCOS is anendocrine disorder characterized by elevated androgen level. The main defects in PCOS is ovarian function, insulin secretion and action. It manifests heavy bleeding, acne, weight gain, hirsutism and darkening of skin. The hormone involved in PCOS is FSH and LH and they are glycoprotein polypeptide hormone. FSH is responsible for androgen production and LH is responsible for progesterone production. Obesity plays are major role in PCOS and which results in metabolic disturbances. The factors involved in PCOS are genetic factors, environmental factors and endocrine factors. The drug used in treatment for PCOS is metformin, clomiphene and spironolactone.

Key words: follicle stimulating hormone, luteinizing hormone, hyperandrogenism, hyperinsulinemia

INTRODUCTION

Polycystic ovary syndrome (PCOS) is referred as hyper-androgenic and common endocrine disorder in women reproductive age [1]. The complex condition is characterized by elevated androgen levels. In India about 10% of women are affected by PCOS [2]. Basically, a woman with PCOS have overweight or obese this affects the metabolism and reproductive function of the body [3]. The manifestation of PCOS affects mostly in women of child bearing age (from 18-30) because the ovaries aredeveloping into numerous small collection follicles and failed to regulatory release [4]. PCOS increase the risk of various fields like dermatologic, oncologic, metabolic, reproductive and psychological aberrations [5].

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Review Article

Protective role of *Coscinium fenestratum* in health care – A review

K. Hari Priya*, Muralidharan Palayyan

Department of Pharmacology, C.L. Baid Metha College of Pharmacy, Chennai, 600 097, Tamil Nadu, India

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*Corresponding Author: K. Hari Priya (haridinesh523@gmail.com)

ABSTRACT

Coscinium fenestratum is generally known as tree turmeric and its major constituents is berberine and other constituents are protoberberine alkaloids, oxypalmatine berrubine, (-) – 8 – oxotetrahydrothalifendine, (-) – 8 – oxoisocorypalmine and either (-) – 8 – oxothaicanine and (-) – 8 – oxocanadine. All the parts of the plants has specific medicinal values. The alcoholic extract of the stem has the ability to reduce the glucose level and thus act as anti-diabetic agent. The stem and leaf extract contains anti-microbial and anti-oxidants properties and, methanolic extract in four different strains also results high anti-microbial activity. The *C. fenestratum* treats hepatic injury and reduce serum hepatic markers by conducting ethanolic extraction. The concoction of seeds improves the immune responses and it proven to relieve inflammation and cold related symptoms and anti-microbial activity used as alternative medicines. *Coscinium fenestratum* had high anti-gonococcal activity by inhibition of *Nesseria gonorrhaea* and also used in the treatment of cancer.

Keywords: *Coscinium fenestratum*, Berberine, Anti-microbial.

INTRODUCTION

Coscinium fenestratum (CF) belongs to Menispermaceae family and it is commonly known as tree turmeric (Figure 1). It is mainly distributed in South west India, chiefly in Western Ghats. The *Coscinium fenestratum* is yellow wood and sap with yellowish color bark and flowers and drupe fruits. The wood is considered to be toxic in some cases that may cause vomiting and diarrhea but it shows negative result in water extract by performing acute and sub-chronic toxicity test (Tushar *et al.*, 2008). The major constituents of CF is Berberine which is yellow crystalline alkaloid and other constituents are protoberberine alkaloids, oxypalmatine berrubine, (-) – 8 – oxotetrahydrothalifendine, (-) – 8 – oxoisocorypalmine and either (-) – 8 – oxothaicanine and (-) – 8 – oxocanadine (Rai *et al.*, 2013).

The Pharmacological action of *Coscinium fenestratum* as follow as:

Anti-diabetic activity

Shirwaikar *et al.* (2005) study shows that the *C.fenestratum* alcoholic stem was used for the treatment of Diabetes mellitus evaluated against streptozotocin – nicotamide induced Type 2 diabetes model. The *C.fenestratum* extract is non – toxic substance from the selected dose of 250mg/kg and 500mg/kg. The alcoholic extract of the stem have the ability of lowering blood glucose levels. The anti-diabetic activity of *Coscinium fenestratum* is not related to the insulin secretion and the post prandial glucose radiation mainly it is due to the effect of extra pancreatic mechanism and it also increase the serum triglycerides and cholesterol levels in diabetic rats. The administration of this extract, shows hypolipidemic effect by decreasing the cholesterogenesis and fatty acid synthesis.

Anti-gonococcal activity

Chomnawang *et al.* (2009) depicts that, the medicinal Plant plumbago, *Coscinium fenestratum*, *Alpinia conchigera* and *Caesalpinia sappan* are studied for the Anti-gonococcal activity by disc diffusion method against *Nesseria gonorrhaea*. The study concluded that Coscinium fenestratum had high anti-gonococcal activity by inhibition of *Nesseria gonorrhaea*.

Anti-oxidant and Anti-microbial activity

The Antioxidant and Antimicrobial activity were estimated in stem and leaf extract of Coscinium fenestratum. Antioxidant activity is evaluated against 1, 1 diphenyl – 2 – picrylhydroxy (DPPH) and 2, 12' azino – bis (3-ethyl benzothiazoline – 6- sulphonic acid) (ABTS). The Coscinium fenestratum extract have the ability to reduce DPPH, a stable free compounds whereas ABTS have maximum scavenging effect. The anti-oxidant activity mainly due to phenolic compounds (Goveas & Abraham, 2013).

Antibacterial activity of aqueous and methanolic extract of *C. fenestratum* examined against four different strains in that the methanolic extract shows greater antibiotic activity when compared to the aqueous extract.

Anti-hepatotoxic activity

Murthuza *et al.* (2013) reported that, the hepatoprotective activity against CCl4 to induced liver damage upon treatment