Research Article

Anti-Androgenic Potential of Ethanolic Bauhinia Variegata Stem Extract in A Letrozole-Induced PCOS Rat

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Abstract:

Introduction: Polycystic Ovary Syndrome (PCOS), an endocrine disorder affecting reproductive age women's, with symptoms including hormonal imbalances, irregular menstrual periods, anovulation, and appearance of numerous cysts in the ovaries. During androgen synthesis, testosterone and androstenedione is converted into E2 and estrone, respectively in the presence of an enzyme Aromatase. Letrozole is a nonsteroidal aromatase inhibitor used as induction model. Bauhinia variegata stem extract (BVSE) in ethanol as a solvent was used for the evaluation.

Objectives: The aim of this research was to evaluate anti-androgenic activity of BVSE in letrozole induced PCOS in female rats.

Methods: Bauhinia variegata stem was finely powdered and Soxhlet extraction was done. Sprague Dawley rats (n=30) were divided into 5 groups (n=6) named as Normal control (NC), Disease control (DC), Standard, BVSE low dose, and BVSE High dose. All groups excluding Normal control were administered Letrozole (1mg/kg) in 0.5% CMC for 21 days for induction of PCOS along with prophylactic treatment for 28 days; For Standard Group; Clomiphene Citrate(1mg/kg), BVSE low dose (200mg/kg) and BVSE high dose (400mg/kg). Body weight was measured weekly, and Oestrous cyclicity was monitored. After completion of Treatment, animals were sacrificed and, Hormone levels estimation (LH, FSH, Progesterone, Testosterone, Estrogen), morphology and histopathological study of ovaries was done.

Results: Higher dose of BVSE significantly decrease ($p<0.05$) the levels of Testosterone, LH and Progesterone; and significantly increase ($p<0.05$) the level of FSH and Estradiol which reduced the presence of Cysts in ovaries when compared to the DC group. Morphologically ovaries of DC showed cystic follicle bulging out. Histopathological examination showed increase in no. of developing follicles compared to DC group. Vaginal cytology showed regularity in Oestrous cycle.

Keywords: PCOS, Ovarian Cyst, Letrozole Induction Model, Anti-androgenic Activity, Bauhinia variegata stem.

1. Introduction

One of the most prevalent endocrine and metabolic disorders affecting women of reproductive age worldwide is polycystic ovarian syndrome (PCOS). PCOS, which is typified by hyperandrogenism, chronic anovulation, monthly irregularities, and polycystic ovarian morphology, was initially discovered by Stein and Leventhal in 1935. [1], [2]

Depending on the diagnostic criteria employed, the prevalence of PCOS varies from 6 to 20% globally, making it a serious public health issue.[3], [4] Women with PCOS frequently suffer infertility, obesity, insulin resistance, dyslipidaemia, and psychiatric disorders, all of which negatively impact their quality of life.[5]

The pathophysiology of PCOS is complicated and multifactorial, encompassing genetic susceptibility, hormone imbalance, metabolic dysfunction, and environmental factors.[6]

The hypothalamic-pituitary-ovarian axis is disrupted in this condition, resulting in relatively low levels of follicle-stimulating hormone (FSH) and increased secretion of luteinizing hormone (LH) and gonadotropin-releasing hormone (GnRH). Anovulation and cyst formation arise from this hormonal imbalance, which also impairs normal follicular development and leads to excess ovarian androgen production.[7], [8]

Additionally, insulin resistance and compensatory hyperinsulinemia contribute to the metabolic anomalies linked to the syndrome that promote androgen production.[9]

Oral contraceptives, insulin sensitizers like metformin, ovulation-inducing medications like clomiphene citrate, and modifying their lifestyle are the current alternatives for managing PCOS.[10], [11] Although these therapies provide symptomatic relief, they are often associated with adverse effects and fail to address the multifactorial nature of the disorder. Consequently, there is growing interest in identifying safer and more effective therapeutic alternatives, particularly those derived from medicinal plants.

Animal models have been essential for investigating the pathogenesis of PCOS and assessing new therapeutic approaches. Among these, the letrozole-induced PCOS model is widely accepted because it closely resembles the endocrine and ovarian changes seen in human PCOS. Letrozole, a non-steroidal aromatase inhibitor,

decreases Estrogen production and induces androgen buildup, leading to hyperandrogenism, interrupted Oestrous cyclicity, and the creation of ovarian cysts.[12], [13]
Therefore, this model is extensively used for preclinical evaluation of anti-PCOS agents.
Medicinal plants are widely used in traditional healthcare systems because of their broad pharmacological characteristics and very low risk profiles. Bauhinia Variegata Linn. (Fabaceae), also known as Kachnar, is a well-known medicinal plant found throughout India. Different parts of the plant are traditionally been used to treat metabolic problems, malignancies, inflammatory illnesses, and gastrointestinal ailments.[14], [15]
Flavonoids, tannins, terpenoids, glycosides, and phenolic compounds with antioxidant, anti-inflammatory, antidiabetic, hypolipidemic, and immunomodulatory properties have been identified by phytochemical analyses.[16], [17] These pharmacological properties suggest that Bauhinia variegata may help alleviate hormonal imbalance, oxidative stress, and metabolic disturbances associated with PCOS.
This study was undertaken to evaluate the therapeutic potential of Bauhinia variegata stem extract in a letrozole-induced PCOS rat model. The study aims to investigate its effects on hormonal parameters, ovarian morphology, Oestrous cyclicity, and histopathological alterations, thereby exploring its potential as a natural therapeutic option for the management of Polycystic Ovary Syndrome.

2. Material and Method

2.1 Animals and IAEC Approval

Healthy Adult Female Sprague-Dawley rats (150-250g) were obtained from the Mahaveera Enterprises, Shrinivas Colony, Bagh Amberpet, Hyderabad, Telangana-500013 (Reg. no1656/PO/Bt/S/12/CCSEA). Each animal was kept in a solid-bottom polypropylene cage with a temperature maintained at (24±1) °C, a relative humidity of 30–70%, and a dark/light cycle of 12 hours each. The animals were kept in pathogen-free environments and acclimated for two weeks. The animals received unrestricted water and a standard pellet diet. Every experiment was conducted strictly in accordance with the Institutional Animals Ethical Committee's guidelines (IAEC) (Reg. no 648/PO/Re/S/02/CCSEA) (Protocol no. PJLCP/2025-26/IAEC/03). Constituted as per the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CCSEA), Ministry of Environment and Forests; Government of India; New Delhi. In every experimental design, every attempt was taken to minimize animal suffering.

2.2 Drugs and Chemicals Procurement

Bauhinia variegata stem was collected from TCS, MIHAN, Nagpur, Maharashtra. Letrozole (Letzotem 2.5 mg) was procured from Medplus Health Services Limited, Clomiphene citrate (Clofert 25) was procured from MANEESH PHARMACEUTICALS LTD. Saline solution (0.9% w/v) was procured from Kamla Amrut Pharmaceuticals Ltd., Carboxymethyl cellulose was procured from SAMAR CHEMICALS and Diethyl ether (98%) was procured from LOBA CHEMIE Pvt. Ltd.

2.3 Instruments and Methods

A Digital Microscope (model number: LEICA DM2000 LED) was utilized, along with Leica image capture software for image analysis. Weighing balance was used to measure the animal's body weight and ovary weight, a centrifuge was used to separate the serum from blood for hormone level estimation.

2.4 Collection and Authentication of Bauhinia variegata

Stems of Bauhinia variegata were procured from TCS, MIHAN, Nagpur, Maharashtra. Authentication of Bauhinia variegata stem was done at Botany department at Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur. Authentication no. 383/2025.

a) Extraction of Bauhinia variegata stem powder

The Bauhinia variegata stem powder was extracted with 95% ethanol using Soxhlet extraction process for 1 week. Filtered extract was evaporated at room temperature, while maintaining the safe conditions.

b) Extraction yield

$$\%\ \textbf{Practical yield} = \frac{\textbf{Practical yield}}{\textbf{Theoretical yield}} \times \textbf{100}$$

Table 1: Practical yield of Bauhinia variegata stem

Extract	Wt. of dried powder taken (gm)	Wt. of extract obtained (gm)	% Practical yield
Bauhinia variegata	93	12	12.9%

2.5 Phytochemical Screening

Bauhinia variegata stem extract was subjected to phytochemical evaluation tests.

2.5.1 Test for alkaloid

a) Mayer's test – Add 2 ml of test sample into 2 ml of Mayer's reagent. Cream colour precipitate developed.

b) Dragendorff's test – Mix 2 ml of test sample with 2 ml Dragendorff's reagent to get reddish-brown precipitate.

2.5.2 Test for flavonoids

a) Shinoda test – Two ml of the sample solution will turn pink, crimson, or purple when magnesium powder and a few drops of strong HCl or H2SO4 are added.

2.5.3 Test for phenolic compounds and tannins

a) Ferric chloride test – Mix 2 ml of test solution with 5% of ferric chloride solution gives blue-black or blue-green colour reaction.

b) Lead acetate test – 1 ml of test solution was treated with a 10% w/v solution of basic lead acetate in distilled water. A white precipitate developed.

2.5.4 Test for saponins

a) Foam test – The sample was vigorously shaken with water. A foam that resembles a honeycomb may be a sign that saponins are present.

2.5.5 Test for carbohydrates

a) Molish test: Thoroughly mix two drops of alcoholic α-naphthol solution in 2 ml of test solution, now add 1 ml of concentrated H_2SO_4 gradually to the test tube. The appearance of a reddened coloration indicates the presence of carbohydrates.

2.5.6 Test for terpenoids

a) Salkowski test – Mix test sample solution with 2 ml of CHCl3. 3 ml of conc. Sulfuric acid was added. After a few minutes, a reddish-brown layer formed. The two layers formed indicated the presence of terpenoids.

2.6 Hormonal Estimation

After the period of 21 days post induction of letrozole (1mg/kg), Blood from the induction group was collected and after 28 days post induction + treatment; their blood was collected for hormone level estimation.

2.7 Oestrous Cyclicity

In rats, Oestrous cycle is the key aspect to check whether the female rat has reached puberty and could conceive or not. To check this cycle, vaginal smear was collected using a thin cotton swab which was dipped in 0.9% saline, and was observed under light microscope with 10x magnification value. There are 4 phases in Oestrous cycle; Proestrus, Estrus, Metestrus, Diestrus.

Table 2: Various phases of Oestrous cycle

Phase	Time duration	Interpretation
Proestrus	12 hrs	Nucleated Epithelial cells with a few leucocytes
Estrus	9-15 hrs	Large number of Cornified Squamous epithelial cells
Metestrus	10-14 hrs	Leucocytes with a few Cornified nucleated cells
Diestrus	60-70 hrs	Leucocytes with some epithelial cells

3. Results

3.1 Phytochemical Screening

Table 3: Phytochemical results of Bauhinia variegata Stem extract (+ indicates present; - indicates absent)

Sr. no.	Phytochemical Test	Inference
1.	Alkaloids Test	+
2.	Flavonoid Test	+
3.	Saponin Test	-
4.	Molisch Test	+
5.	Tannins Test	+
6.	Terpenoids Test (Lupeol)	+

3.2 Hormones Level Estimation

Table 4: Hormone level estimation results

Groups	NC	DC	Standard	BVSE (200mg/kg)	BVSE (400mg/kg)
LH	0.19500 ± 0.0022361	0.30333 ± 0.0042164$^{\delta\delta\delta}$	0.21500 ± 0.0099163***	0.26667 ± 0.010541*	0.22667 ± 0.0095452***
FSH	0.37833 ± 0.0047726	0.26167 ± 0.0079232$^{\delta\delta\delta}$	0.36667 ± 0.015202***	0.30167 ± 0.00307320	0.31500 ± 0.013102**
Progesterone	14.595 ± 0.17925	23.033 ± 0.32318$^{\delta\delta\delta}$	17.627 ± 0.20089***	21.663 ± 0.60336*	19.867 ± 0.34319***
Testosterone	29.150 ± 0.70178	48.433 ± 0.81432$^{\delta\delta\delta}$	30.583 ± 0.26760***	38.233 ± 0.94434***	33.033 ± 0.89393***
Estrogen	28.205 ± 0.55791	2.1100 ± 0.038816$^{\delta\delta\delta}$	17.917 ± 0.22794***	15.073 ± 0.15097***	16.240 ± 0.37601***

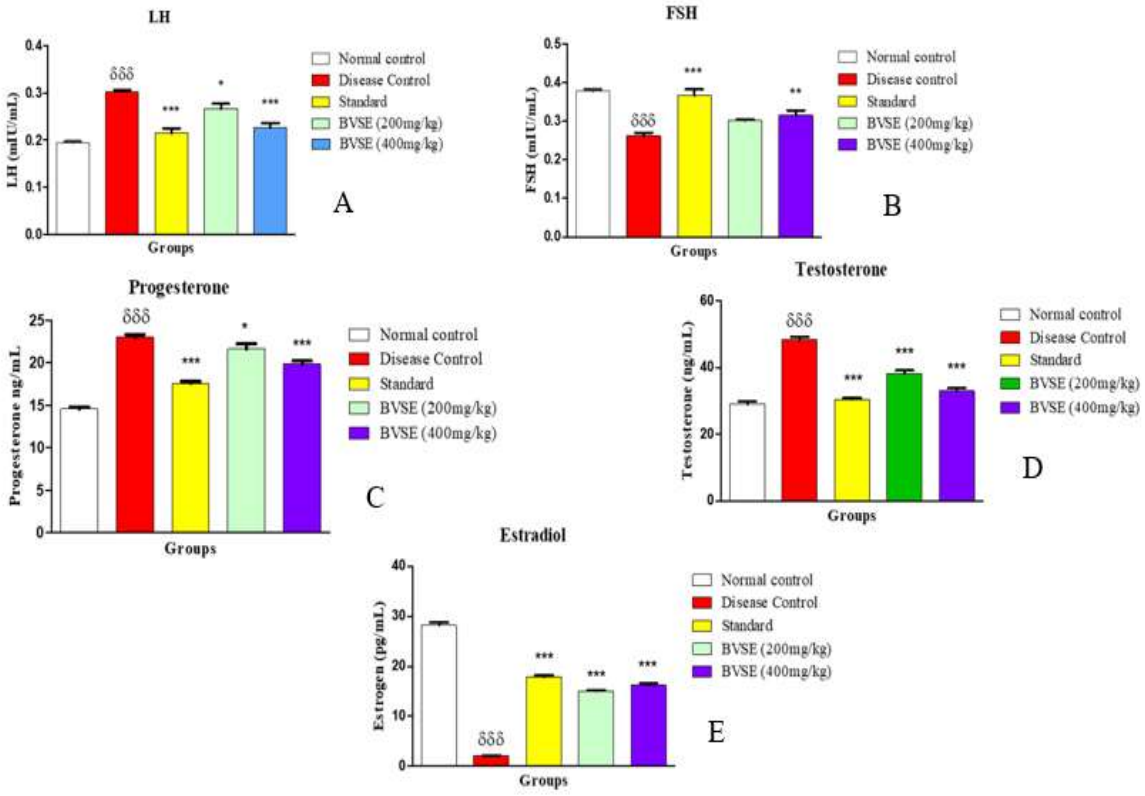


Figure 1: A) LH B) FSH C) Progesterone D) Testosterone E) Estrogen levels: All data are expressed as Mean ± SEM (n=6 in each group) and analysed by one-way ANOVA followed by Tukey's multiple comparison test. $^{\delta\delta\delta}$P<0.001 DC vs NC; p values express as *P<0.05, **P<0.01, ***P<0.001 when compared DC vs STD and treatment group of BVSE(200mg/kg) and BVSE(400mg/kg).

3.3 Morphology

It was seen that the Disease Control group ovaries showed bulging out of cyst which was formed over the cells. This indicated successful induction inside the ovaries. Treatment group showed a smaller number of cysts as compared to the induction group.

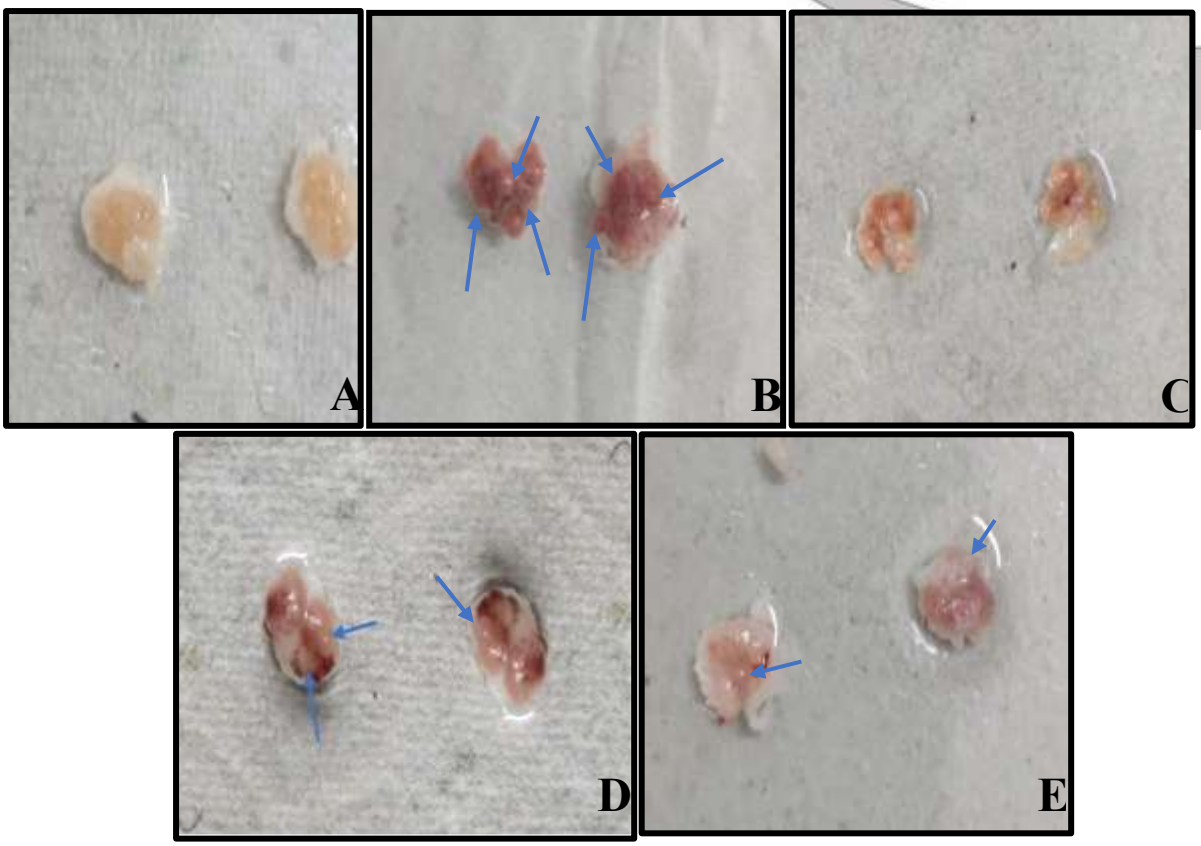


Figure 2: Morphology of Ovaries

A: Ovaries of a normal female rat; B: Ovaries of Disease control rat which was letrozole induced; C: Ovaries of Standard treatment group i.e., Clomiphene citrate; D: Ovaries of Low dose treatment group of BVSE; E: Ovaries of High dose treatment group of BVSE.

↑ Indicates Cyst.

3.4 Histopathology

Histopathological studies showed that the disease control group. Infiltration of the fluid inside the follicles has been reduced in the higher dose of BVSE as compared to the low dose. Developing follicles were seen.

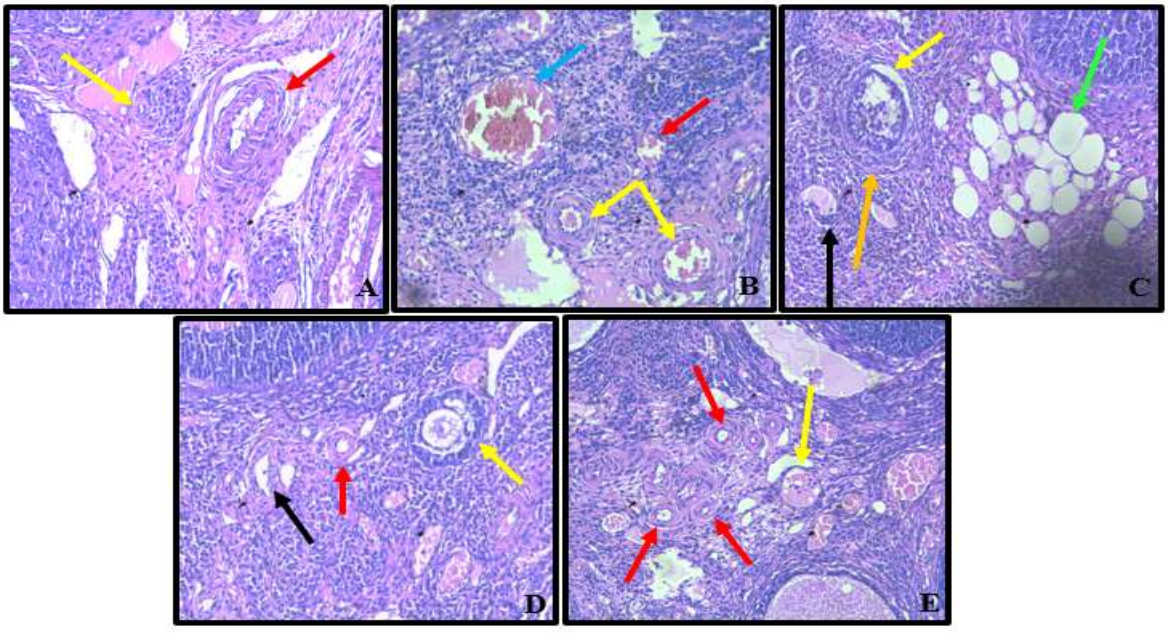


Figure 3: Histopathology of ovaries of all groups (10x)

A: Normal control; B: Disease control; C: Standard; D: BVSE Low dose; E: BVSE high dose. ↑ Primordial follicle; ↑ Primary Follicle; ↑ Secondary follicle; ↑ Tertiary antral follicle; ↑ Theca externa; ↑ Lipid vacuoles

3.5 Vaginal Smear Cytology

The disease control group was observed to spend more time in Diestrus phase as it is also known as cleaning phase, while the other treatment groups showed normal Oestrous cycle. (Table no. 2).

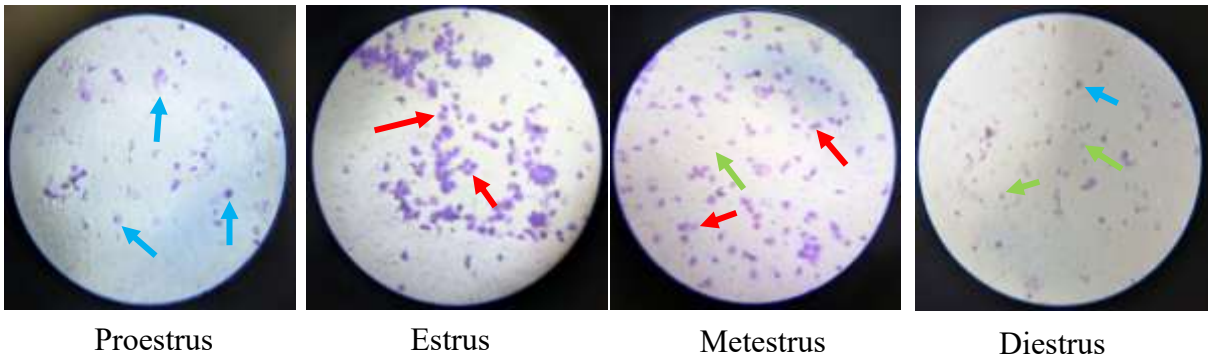


Figure 4: Oestrous cycle (10x)

↑ Nucleated epithelial cells; ↑ Cornified squamous epithelial cells; ↑ Leucocytes

4. Discussion

Letrozole administration successfully induced Polycystic Ovary Syndrome (PCOS) in female rats, as it causes significant hormonal imbalances, cystic ovarian shape, disturbed oestrous cyclicity, and histopathological changes. In normal physiology, CYP17A1 (a dual function enzyme responsible for 17-α-hydroxylase and 17,20-lyase activity) plays a vital role in conversion of progesterone into 17-α-hydroxyprogesterone, and then further into an important precursor of androgen synthesis; androstenedione.

Further, androstenedione is converted into testosterone by catalytic activity of 17β-Hydroxysteroid dehydrogenase enzyme. Aromatase (CYP19A1) converts these two into Estrogen and Estradiol. Being that letrozole is an aromatase inhibitor, prevents androgens like testosterone and androstenedione from being converted into Estrogen and Estradiol, which lowers the level of Estrogen in the ovaries. Due to this blockade, testosterone accumulate in the ovarian theca cells, which is a condition known as hyperandrogenism; a symptom of PCOS. This decrease in level of Estrogen initiates Negative feedback to the Hypothalamus and pituitary. In response to this feedback, GnRH pulse frequency increases, therefore, LH secretion increases. LH binding to its receptor causes hyperactivity of CYP17A1 enzyme, which causes stimulation in androgen synthesis.

Meanwhile, treatment with Bauhinia variegata stem extract (BVSE) significantly reduced these pathological changes. BVSE treatment decreased LH, testosterone, and progesterone levels while raising FSH and Estrogen levels. Furthermore, BVSE reduced ovarian cyst formation, increased follicular growth, and restored normal oestrous cyclicity.

Lupeol, a primary triterpenoid constituent of BVSE, has a high affinity for CYP17A1, which is found in ovarian theca cells, as well as the human progesterone receptor. Lupeol acts as a neuromodulatory antagonist by targeting these molecular structures, thereby inhibiting the LH levels which was increased by letrozole.

Lupeol inhibits CYP17A1 and restored systemic homeostasis, which inhibits the conversion of progesterone into androgens, thus steroidogenesis activity reduced, androgen synthesis decreased. Folliculogenesis improved i.e. follicles restored.

Lupeol also shows binding affinity towards 1E3K (Human Progesterone receptor) stimulation of this receptor causes release of progesterone, excessive progesterone causes reduction in the Pulse frequency of GnRH from the anterior pituitary. [18]GnRH low levels cause decreased secretion of LH , decrease in activity of CYP17A1, thus less production of androgens. It stimulates the release of FSH, so Follicular development is promoted.

5. Conclusion

Bauhinia variegata stem extract contains Triterpenoid (Lupeol) active constituent which effectively improved hormonal imbalance, ovarian morphology, and Oestrous cyclicity in letrozole-induced PCOS rats. The high dose (400 mg/kg) showed greater efficacy and produced effects comparable to low dose (200mg/kg). The study indicates that BVSE possesses promising anti-androgenic activity and may serve as a potential natural therapeutic agent.

6. Future Scope

- Evaluation of long-term safety and toxicity.
- Development of standardized herbal formulations.
- Clinical studies to establish efficacy and safety in humans.

Article Type: Laboratory Based Study (Preclinical animal study)

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Arnav Badole declares no conflicts of interest.
Dr. Shweta A. Rathod declares no conflicts of interest.
Sohan Dhone declares no conflicts of interest.

Credit Authorship Contribution Statement:
Arnav Badole: Conceptualization, Investigation, Methodology, Data Curation, Formal Analysis, Writing Original Draft.
Dr. Shweta A. Rathod: Supervision, Validation, Visualization, Project Administration, Writing Review & Editing.
Sohan Dhone: Resources, Data Interpretation, Data Curation, Writing – Review & Editing.

Attestation Statement:
The authors attest that the data presented in this manuscript are original, have not been previously published, and are not under consideration elsewhere. The authors agree to provide the study data to the editors upon reasonable request. All applicable institutional guidelines for animal care and use were followed.

Competing Interests: The authors declare no competing interests.

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