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Pharmacological Effects Of Artemisia Roxburghiana Leaves On Letrozole Induced PCOS In Female Wistar Rats

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Abstract
Polycystic ovarian affliction (PCOS), also dubbed as PCOD, is a shape where a woman's hormones are not balanced. It might diminish the chances of conception and result in issues with their periods. If left untreated, it can eventually result in major health issues like diabetes and heart disease. This study evaluates pharmacological effects of Artemisia roxburghiana leaves on letrozole induced PCOS in female Wistar rats. The experimental technique involves generating of letrozole induced PCOS followed by therapy with ethanolic extract of Artemisia roxburghiana. The research focused on analysing the physical parameters, LH, FSH and testosterone level in blood and histology of ovaries. Results demonstrated that ethanolic extract of Artemisia roxburghiana dramatically improved body weight, improved LH FSH level, regulated testosterone level disturbed by letrozole induced PCOS. The findings showed the potential effect of ethanolic extract of Artemisia roxburghiana in the treatment of PCOS, giving a possible alternative to current pharmaceutical treatments.

Keywords: *Polycystic ovarian Syndrome (PCOS), Oestrogen, Artemisia roxburghiana, Wistar rats, testosterone level, behavioural parameters.*

INTRODUCTION

Polycystic ovarian affliction (PCOS), also dubbed as PCOD, is a shape where a woman's hormones are not balanced. It might diminish the chances of conception and result in issues with their periods. **[1] [2] [3]** If left untreated, it can eventually result in major health issues like diabetes and heart disease. A lot of women who have been spotted with PCOS have several little ovarian cysts. **[4] [5]** It is labelled as polycystic ovary syndrome for this reason. Although they cause hormone abnormalities, the cysts are not dangerous. Long-term issues can be avoided and symptoms can be controlled with early diagnosis and treatment. **[6] [7]**

It is marked by a disturbance of reproductive hormones that bring about metabolic irregularities, ovarian cysts, and irregular menstrual periods. The ovaries are mostly impacted by polycystic ovarian disease (PCOD), which disrupts their regular function. **[8] [9]** This disease has a significant effect on a woman's whole health, including her physical, mental, and cognitive well-being, and goes beyond reproductive health. **[10] [11] [12]** The increasing prevalence of PCOD emphasises the necessity of increased awareness and a thorough plan to manage its effects. **[13] [14]** various studies have shown that the effect of natural compounds and medicinal plants effective in the treatment of polycystic ovaries. **[15] [16]**

From time immemorial, several medicinal plants and their constituents have been used to prevent and treat various diseases. Medicinal plants such as Ashwagandha, Mimosa, Aloe vera etc., have been employed in the treatment of PCOS by some researchers earlier. **[17] [18] [19] [20]**

The study aims to demonstrate the efficacy of the ethanolic extract of *Artemisia roxburghiana* as a possible therapeutic agent in the prevention and management of PCOS. **[21] [22]** The study involves the exploration of laboratory breed female Wistar PCOS Rat models possessing ovarian and/or metabolic abnormalities to evaluate the effect of *Artemisia roxburghiana* as a treatment drug and also to identify the characteristics of serum sex steroids. Hence, the female rats were continuously exposed to the letrozole to induce a hyperandrogenic state. **[23]**

MATERIALS AND METHODS

Leaves of *Artemisia roxburghiana* were shade dried and coarsely powdered in a mixer. Extraction was prepared by Soxhlet procedure. Ethanol was used as a solvent in the Soxhlet assembly to extract the *Artemisia roxburghiana* plant. A Soxhlet apparatus containing 50 grammes of powdered leaves and 100 millilitres of ethanol was run for twenty-four hours under constant observation. A rotary evaporator was used to evaporate the solvent, which was then sealed in an airtight bottle and kept at 40 degrees Celsius. Use Whatman Number 1 filter paper to filter the extract. **[24] [25]**

Dose 200mg/kg and 400mg/kg of *Artemisia roxburghiana* was taken according to previous study

Induction of PCOS: Letrozole was used as a disease-inducing agent at dose 1 mg/kg. The stock solution was made by dissolving one 2.5 mg letrozole tablet in ordinary saline and storing it in the refrigerator. **[26]**

Standard drug: Clomiphene citrate is a typical drug used to treat PCOS. The stock solution of clomiphene citrate, which was prepared by dissolving one 50 mg tablet in 500 millilitres of ordinary saline solution, was given to animal (1 mg/kg). **[26]**

The SGRR Medical and Health Sciences in Dehradun, Uttarakhand, is the source of the animals. All rats are housed in five propylene cages with six rats each, at room temperature (25°C), with a day and dark cycle. Water is provided for the animals to drink and a nutritious pellet meal. After completing Form B, the Siddhartha Institute of Pharmacy's institutional animal ethics committee accepted the proposal (Proposal No. SIP/IAEC/PCOL/03/2025) to undertake the animal experimental investigations.

The animals were taken into 5 groups (6 animal in each group), one normal control and three groups of letrozole induced PCOS. For a time period of 21 days animals received treatment mentioned in table no. 1.

Table no. 1 Treatment protocol **[27]**

Group name	Treatment	Dose	Route
Normal saline	Drinking water	-	Oral
Diseased	Letrozole	1mg/kg	Oral
Standard	Letrozole+ Clomiphene citrate	1mg/kg+1mg/kg	Oral
Treatment 1	Letrozole + AR 1	1mg/kg+200mg/kg	Oral
Treatment 2	Letrozole + AR 2	1mg/kg+400mg/kg	Oral

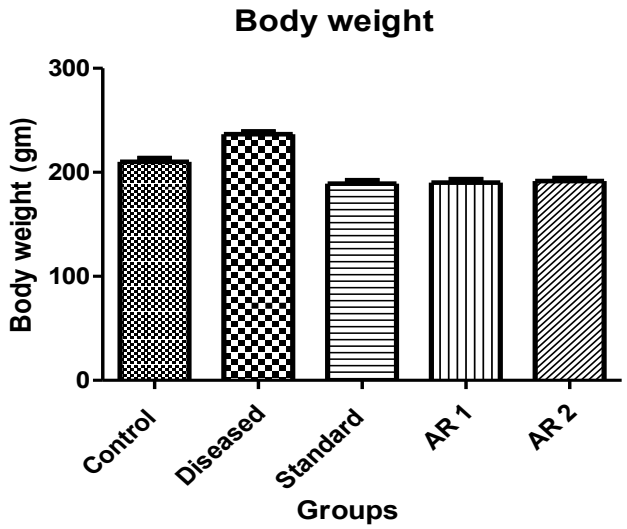
At the end of the treatment biochemical parameters were performed to check LH level FSH Level fasting blood sugar, testosterone level. Physical parameters were also evaluated and animals were sacrificed for histological studies to observe the recovery from PCOS after the treatment.**[28] [29]**

Histopathology: Sections of 5 µm thickness were obtained and stained with haematoxyline eosin as per standard procedure after the ovaries were dehydrated by placing them in 30%, 50%, 70%, 90%, and 100% alcohol gradations, fixed in an aqueous Bouin's fluid for 24 hours, and then randomly selected 10 good sections from each ovary in each group were examined under a microscope.

RESULTS

Body weight

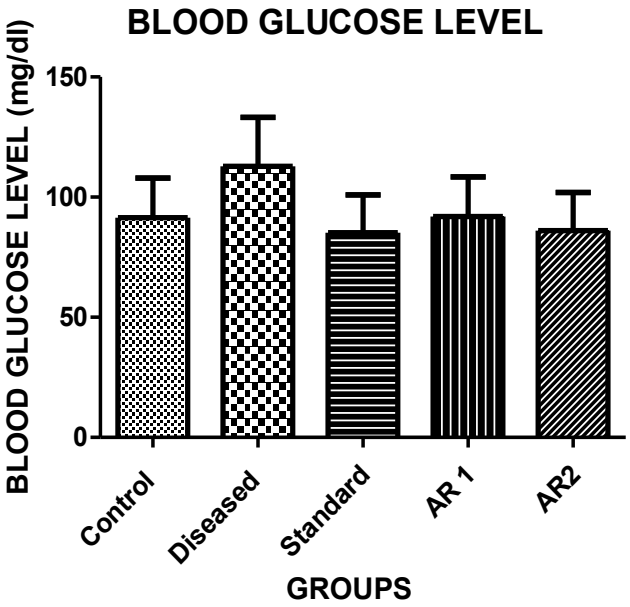
The administration of levozole resulted in a considerable increase in the animals' body weight across all groups. The standard group received the conventional medication, clomiphene citrate, which results in a decrease in body weight. Following therapy, body weight also dropped with both low and high doses of *Artemisia roxburghiana.*



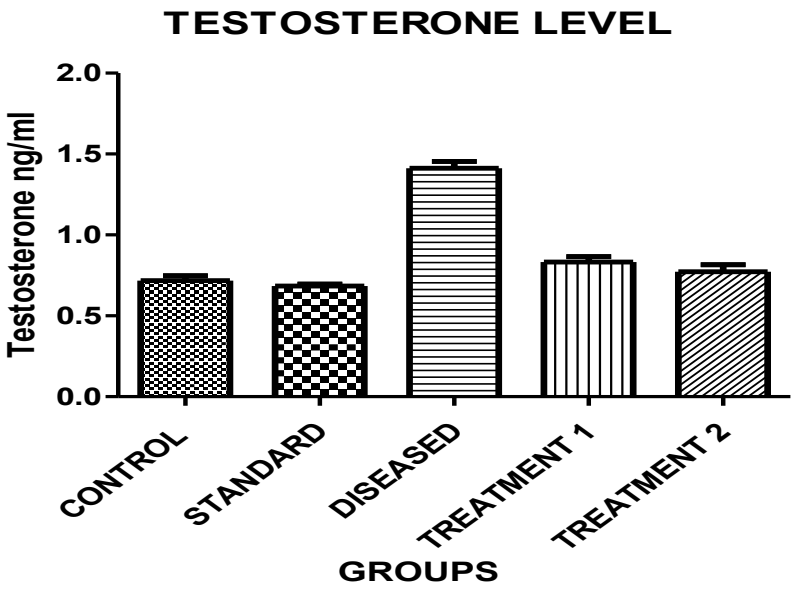
Graph no.1: depicting the difference in body weight of all group

Blood glucose level

PCOS-positive rats showed an increase in their cholesterol and glucose levels, while PCOS - positive mice given clomiphene citrate and different doses of *Artemisia roxburghiana* showed a decrease in their glucose levels.

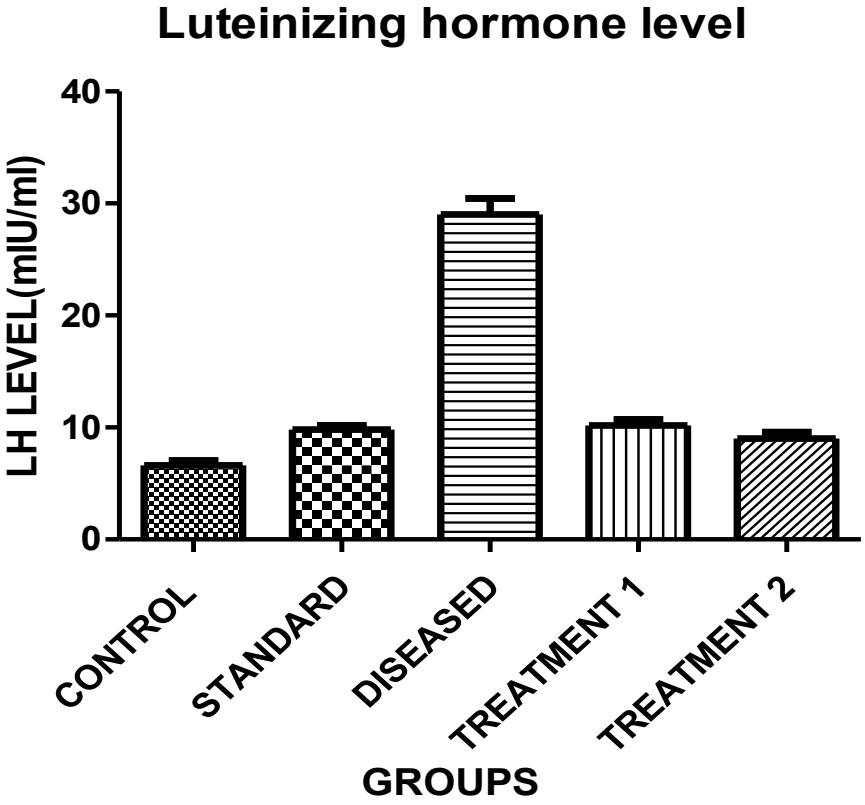


Graph no.2: depicting the difference in blood glucose level of all groups



Graph no. 3: depicting the blood testosterone level among various group

LUTEINIZING HORMONE LEVEL



Graph no. 4: depicting the blood luteinizing hormone level among various group

Ovarian Histopathological Findings

Normal group

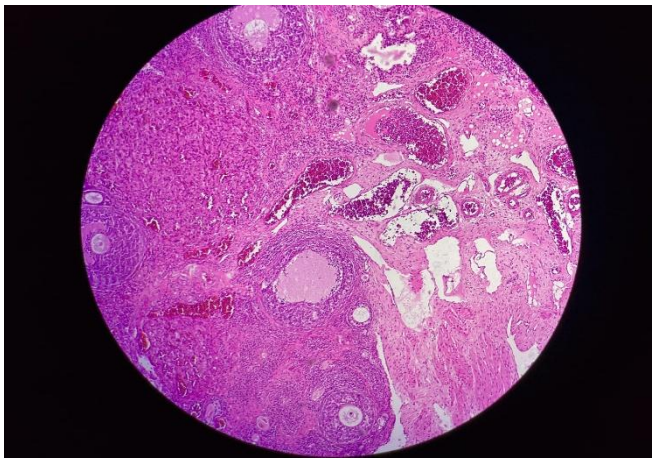


Fig no.4 Histopathology of Standard ovary

The ovarian histology exhibits a strong and well-coordinated pattern of follicular development and regression, and it is completely within normal bounds. A physiologically active ovarian cycle is represented in the reality of luteal structures, such as corpus luteum and regressing corpora albicantia, normal atretic follicles, and preantral and antral follicles growing in unison. An ovary that is generally normal with no pathologic abnormalities or cystic change is further confirmed by the intact oocyte, well-preserved tunica albuginea, and lack of any follicular cystic expansion.

Standard group

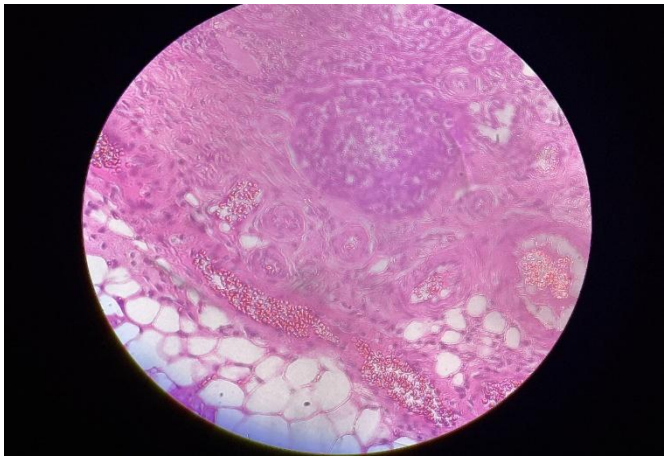


Fig no.5 Histopathology of Standard ovary

Significant structural and functional realignment is highlighted by the ovarian sections' strong indications of an early reversal of polycystic ovarian pathology: Significant decrease in cystic follicles elevated corpora lutea count, The tunica albuginea has somewhat thickened, which is consistent with mild Vascular congestion

Diseased group

The following characteristics of advanced polycystic ovarian morphology are revealed by the histological evaluation:

Absence of corpora lutea, which confirms chronic anovulation and prolonged cycle disruption.

In line with Stein-Leventhal pathology, there are several subcapsular cystic follicles that exhibit chronic follicular arrest and cystic dilatation.

The classic PCOS "polycystic" appearance on gross and microscopic examination is caused by stromal hyperplasia, which is characterised by expanded and fibrotic ovarian stroma. The process is non-destructive, as There isn't any proof of fibrosis, inflammation, haemorrhage, or neoplastic changes.

These results present bilateral polycystic ovarian syndrome, which is characterised by many cystic follicles, stromal proliferation, and total lack of luteal structures. This supports a chronic anovulation condition without any inflammatory or malignant after effects.

TREATMENT 1

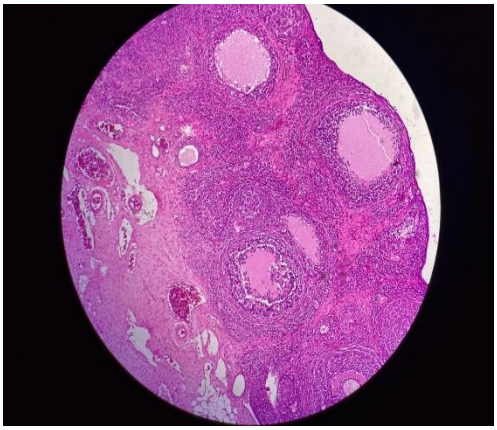


Fig no.8 Histopathology of treatment 1 ovary

Morphology: Cystic follicles, which are primarily seen in subcapsular region, are noticeably many in the ovarian sections.

Follicular Development: The reality of mature oocytes and corpus luteum structures has decreased, showing that ovulatory processes have been disturbed.

Capsular Changes: The ovary's distinctive sclerotic look is a result of the tunica albuginea becoming noticeably thicker.

Vascular Observations: There is evidence of vascular congestion and the production of fibrinous clots, which may specify underlying chronic ischaemic or inflammatory processes.

Impression: The histological characteristics showed structural changes those are consistent with Polycystic Ovarian Disease (PCOD). A moderate response to treatment interventions is also indicated by the changes that have been seen.

TREATMENT 2

Follicle Dynamics: A moderate decline in the quantity of cystic antral follicles indicates that polycystic shape is regressing early.

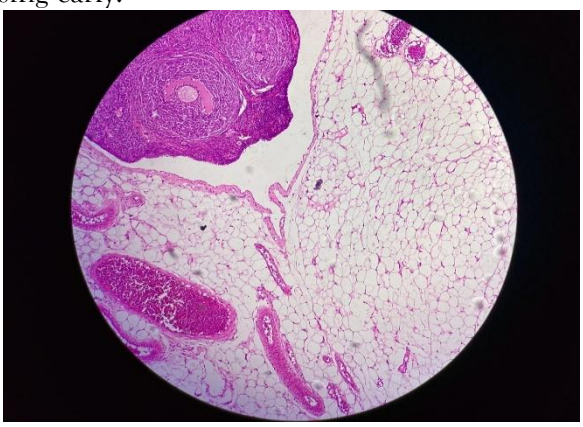


Fig no.9 Histopathology of Treatment 2 ovary

Ovulatory Activity: Reactivated ovulation is demonstrated by a discernible increase in corpus luteum structures and mature, normal-sized oocytes.
Capsular Features: The tunica albuginea continues to thicken slightly.
Haemostatic and Vascular Indicators: persistent arterial blockage accompanied by the development of localised fibrinous clots.
Recovering from Polycystic Ovary Disease
Histological characteristics show a positive change from the traditional PCOS morphology; a decrease in cystic follicles and a rise in ovulatory markers indicate ovarian healing and a response to treatment**.**

DISCUSSION

A global survey found that infertility, irregular menstruation, trouble losing weight, and excessive hair growth were the regular health issues among individuals with polycystic ovarian syndrome. The study examines the pharmacological effects of an ethanolic extract of *Artemisia roxburghiana* on female rats with PCOS produced by letrozole. The study's primary objective is to investigate how oral administration of an ethanolic extract of *Artemisia roxburghiana* affects PCOS.
Artemisia roxburghiana is a potent medicinal herb because of its abundance of bioactive secondary metabolites, which include polyphenols, flavonoids, anthocyanins, tannins, and terpenoids. Long ago, plant was used safely in many cultures to control menstruation, including amenorrhoea, and cure dementia and rheumatism as an anti-inflammatory. In letrozole-induced PCOS mice, body weight, serum glucose, and testosterone levels were carefully monitored at baseline, day 21 (the end of letrozole treatment), and day 49, indicating strong metabolic improvements. These recent experimental investigations further verify its promise in controlling PCOS. At the conclusion of the trial, ovarian histopathology and hormonal tests showed a notable recovery of ovarian architecture and endocrine balance.
Rats given 400 mg/kg of *Artemisia roxburghiana* ethanolic extract demonstrated promise in treating letrozole-induced PCOS, although more thorough investigation is needed to assess its therapeutic potential.
Conclusion: Letrozole was used to develop PCOS in our model over a 21-day induction period, and then two different doses of ethanolic extract of *Artemisia roxburghiana* was used for 28 days of treatment. *Artemisia roxburghiana* has long been used to cure a variety of illnesses.
Interestingly, the extract successfully reversed the morphological abnormalities in ovarian tissue caused by PCOS, normalising ovarian architecture, especially at the higher dose of 400 mg/kg. Blood sugar and body weight also stabilised, and elevated levels of luteinizing hormone and testosterone went back to normal. Additionally, the plant extract helped normalise irregular menstruation, skin infections, hirsutism, vaginal dryness, and hair loss.
The efficacy of the high dose was comparable to that of the conventional reference treatment. Though the outcomes are encouraging, more thorough research is required to completely determine plant extract's safety profile, therapeutic potency, and underlying mechanisms.

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