

EVALUATION OF HERBAL DRUGS FOR ANTI-PCOS ACTIVITY USING A
LETROZOLE-INDUCED RAT MODELKumari Raunak^{*1}, Mahesh Kumar Gupta², Sarswati³¹Student, Career Point School of Pharmacy, Career Point University, Kota, Rajasthan, India.²Dean, Career Point School of Pharmacy, Career Point University, Kota, Rajasthan, India.³Assistant Professor, Career Point School of Pharmacy, Career Point University, Kota, Rajasthan, India.***Corresponding Author: Kumari Raunak**

Student, Career Point School of Pharmacy, Career Point University, Kota, Rajasthan, India.

DOI: <https://doi.org/10.5281/zenodo.23799647>**How to cite this Article:** Kumari Raunak^{*1}, Mahesh Kumar Gupta², Sarswati³. (2026). Evaluation Of Herbal Drugs For Anti-Pcos Activity Using A Letrozole-Induced Rat Model. European Journal of Pharmaceutical and Medical Research, 13(11), 260–271.

This work is licensed under Creative Commons Attribution 4.0 International license.



Article Received on 20/09/2026

Article Revised on 10/10/2026

Article Published on 01/11/2026

ABSTRACT

Polycystic Ovary Syndrome (PCOS) is a common endocrine and metabolic disorder affecting women of reproductive age and is characterized by hyperandrogenism, irregular ovulation, infertility, obesity, insulin resistance, and ovarian cyst formation. The present study was designed to evaluate the anti-PCOS activity of selected herbal drugs using a letrozole-induced PCOS rat model. Hydroethanolic extracts of *Gymnema sylvestre*, *Ocimum tenuiflorum*, and *Withania somnifera* were prepared and administered either individually or as a polyherbal formulation to female Wistar rats. PCOS was induced by oral administration of letrozole (1 mg/kg) for 21 consecutive days. The induced rats exhibited significant alterations in body weight, estrous cycle regularity, hormonal imbalance, biochemical parameters, oxidative stress markers, and ovarian morphology. Treatment with herbal extracts, particularly the polyherbal formulation, showed marked improvement in estrous cyclicity, reduction in body weight gain, normalization of ovarian morphology, restoration of follicular development, and reduction in cystic follicles. The herbal treatments significantly decreased serum testosterone, luteinizing hormone (LH), glucose, cholesterol, triglycerides, LDL, and VLDL levels while increasing follicle-stimulating hormone (FSH), estrogen, progesterone, and HDL levels. Antioxidant studies demonstrated increased Superoxide Dismutase (SOD), Catalase (CAT), and Reduced Glutathione (GSH) levels along with decreased Malondialdehyde (MDA) levels, indicating reduction of oxidative stress. Histopathological examination further confirmed restoration of near-normal ovarian architecture in treated groups. Among all treatments, the polyherbal formulation demonstrated the most significant therapeutic effect comparable to standard metformin treatment. The findings suggest that the selected herbal drugs possess potent anti-PCOS, antioxidant, and metabolic regulatory activities and may serve as promising natural therapeutic agents for the management of PCOS.

KEYWORDS: Polycystic Ovary Syndrome, PCOS, Letrozole, Herbal Drugs, *Gymnema sylvestre*, *Ocimum tenuiflorum*, *Withania somnifera*, Polyherbal Formulation, Wistar Rats, Antioxidant Activity, Hormonal Imbalance, Histopathology.

1. INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most commonly occurring endocrinological and metabolic disorders among reproductive-aged women all over the world. The syndrome is marked by hormonal, reproductive and metabolic anomalies which have a severe impact on the overall well-being of patients. The typical symptoms of the disease include

hyperandrogenism, anovulation or dysfunctions in the regularity of ovulation, infertility, obesity, irregular menstrual cycle, acne, hirsutism, and polycystic ovary morphology. As PCOS shows great diversity among patients, it is regarded as a highly heterogeneous disorder.

1.1 OBJECTIVES OF THE STUDY

1. To induce Polycystic Ovary Syndrome in experimental rats using letrozole.
2. To evaluate the anti-PCOS activity of selected herbal drugs in the letrozole-induced rat model.
3. To study the effect of herbal treatment on body weight, estrous cycle, and ovarian morphology.
4. To assess the influence of herbal drugs on hormonal parameters such as testosterone, estrogen, LH, and FSH levels.
5. To evaluate the effect of herbal treatment on biochemical parameters including blood glucose and lipid profile.
6. To investigate the antioxidant potential of the selected herbal drugs by estimating oxidative stress markers.

2. MATERIALS AND METHODS

2.1 Preparation of Extracts

The plant materials of *Gymnema sylvestre*, *Ocimum tenuiflorum*, and *Withania somnifera* were dried under shade at a room temperature of 25–30 °C for 7–10 days to dehydrate them completely. The dried plant materials were powdered using a mechanical grinder and sifted through sieve number 40. The powdered materials were kept separately in air-tight containers.

Approximately 500 g of powdered plant material was used for extraction. Extraction was carried out using 70% hydroethanolic solvent (70% ethanol with 30% water), chosen for its polarity, which accommodates the phytoconstituents present in the plant material. For Soxhlet extraction, approximately 2.5 L of solvent was used to extract all active constituents from the material over 24–48 hours. For maceration, the powder was kept in the solvent for 72 hours.

Table 1.1: Percentage Yield of Hydroethanolic Extracts.

Plant Material	Weight of Powdered Drug Taken (g)	Weight of Extract Obtained (g)	Extract Percentage Yield (%)
<i>Gymnema sylvestre</i>	500	62.5	12.5
<i>Ocimum tenuiflorum</i>	500	58.0	11.6
<i>Withania somnifera</i>	500	71.0	14.2

2.2 Procurement of Experimental Animals

In total, 36 healthy adult female Wistar albino rats, weighing around 150–200 g, were purchased from a recognized animal breeding facility under the guidelines stipulated by CPCSEA regulations. Female rats were chosen for the present study because they are considered ideal experimental animals for the investigation of reproductive disorders and hormonal imbalances such as PCOS.

2.3 Acclimatization

Prior to the initiation of the experiment, all 36 female Wistar rats were acclimatized to the laboratory environment for seven days. The animals were housed in polypropylene cages, with six rats per cage, under standard laboratory conditions throughout the period. Clean, fresh pellet diet and drinking water were provided ad libitum. A temperature of 22 ± 2 °C, 50–60% humidity, and a 12-hour light/dark cycle were maintained throughout the acclimatization period.

Table 1.2: Effect of Acclimatization on Physiological and Behavioural Parameters of Wistar Rats (n = 36).

Parameter	Day 1 (Mean $\pm$ SEM)	Day 7 (Mean $\pm$ SEM)	Observed Change
Body Weight (g)	171.6 $\pm$ 2.8	173.2 $\pm$ 2.9	Slight increase (normal growth)
Food Intake (g/rat/day)	18.4 $\pm$ 0.6	19.1 $\pm$ 0.5	Normalized intake
Water Intake (mL/rat/day)	27.6 $\pm$ 0.8	28.2 $\pm$ 0.7	Stable consumption
Locomotor Activity (score)	2.1 $\pm$ 0.2	2.0 $\pm$ 0.1	Stable activity
Stress Behavior Score	2.3 $\pm$ 0.2	1.1 $\pm$ 0.1	Significant reduction
Fur Condition (score)	2.0 $\pm$ 0.1	1.2 $\pm$ 0.1	Improved grooming
Mortality	0	0	No mortality observed

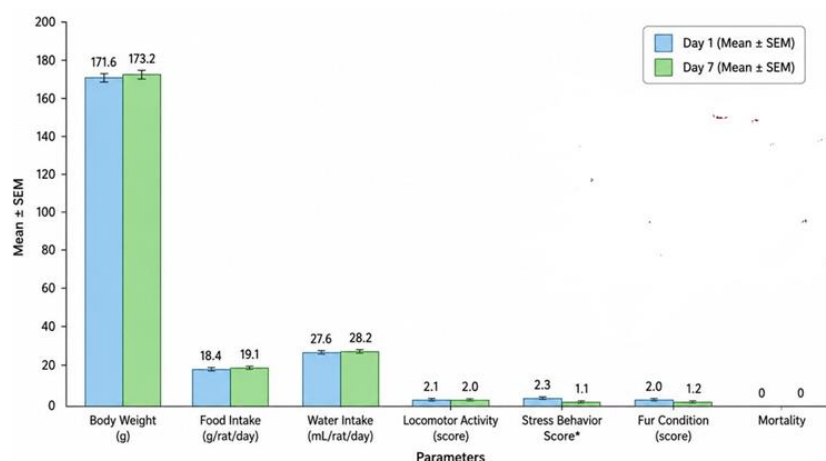


Figure 1.1: Graphical representation of the effect of acclimatization on physiological and behavioral parameters of Wistar rats (n = 36).

Before conducting the experiment, all 36 female Wistar rats were exposed to laboratory conditions for seven days in order to acclimatize to the study conditions. This process is necessary in preclinical research since animals need to become accustomed to a novel environment, handling, and diet, as stress-induced physiological fluctuations could affect the results of the experiment.

2.4 Induction of PCOS

Experimental PCOS was induced in female Wistar rats using letrozole, a non-steroidal aromatase inhibitor. Of the 36 animals, 30 underwent PCOS induction, while 6 remained as normal controls. Letrozole was administered

orally at a dose of 1 mg/kg body weight once a day for 21 consecutive days.

To prepare the oral suspension, letrozole was suspended in 1% carboxymethyl cellulose (CMC) solution: about 10 mg of letrozole was mixed in 10 mL of 1% CMC to obtain the final concentration. The suspension was freshly prepared every day before administration.

Table 1.3: Effect of Letrozole Administration on Induction of PCOS in Wistar Rats.

Parameter	Normal Control (Mean ± SEM)	PCOS Induced (Letrozole 1 mg/kg)	% Change vs Control
Body Weight (g)	173.2 ± 2.9	214.6 ± 3.8	+23.9% ↑
Estrous Cycle Regularity (%)	100% normal cyclicity	100% irregular cyclicity	Disrupted
Duration of Diestrus Phase (days)	1.2 ± 0.2	4.8 ± 0.4	↑ Significant
LH (mIU/mL)	5.3 ± 0.3	10.9 ± 0.5	+105% ↑
FSH (mIU/mL)	4.9 ± 0.2	2.8 ± 0.2	-42.8% ↓
LH/FSH Ratio	1.08 ± 0.05	3.89 ± 0.12	↑ Increased
Testosterone (ng/mL)	0.36 ± 0.02	0.95 ± 0.04	+163% ↑
Estrogen (pg/mL)	48.5 ± 2.1	21.6 ± 1.4	-55.5% ↓
Progesterone (ng/mL)	6.7 ± 0.3	2.0 ± 0.2	-70.1% ↓
Fasting Glucose (mg/dL)	92 ± 3.1	149 ± 4.6	+62% ↑
Ovarian Morphology	Normal follicles	Cystic follicles present	Abnormal
Corpus Luteum Formation	Normal	Reduced/Absent	Decreased
Body Fat Accumulation	Normal	Increased abdominal fat	Present

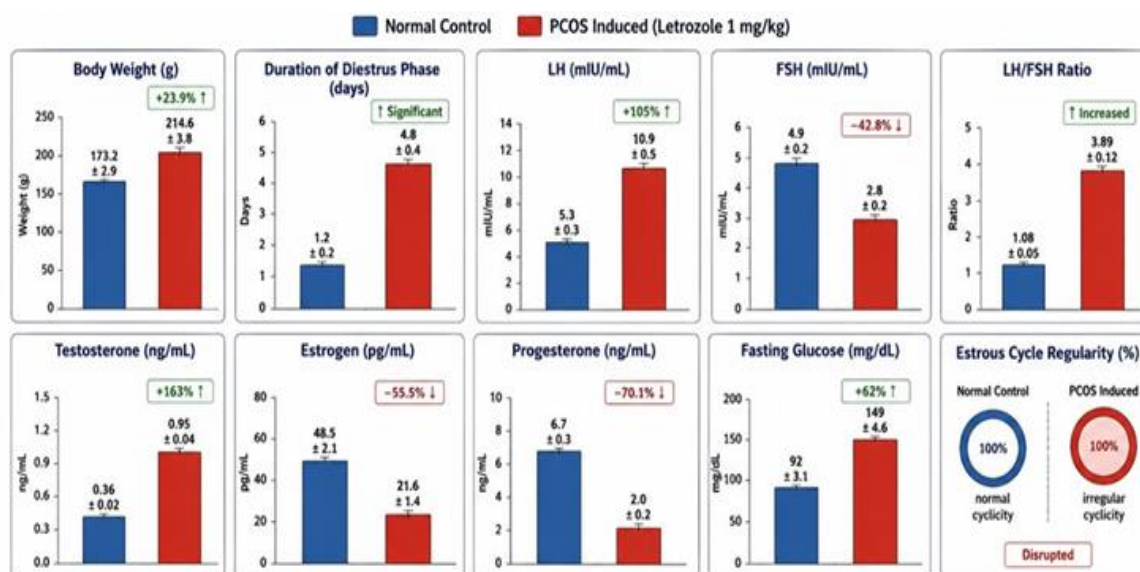


Figure 1.2: Comparative biochemical and hormonal alterations in normal and letrozole-induced PCOS rats.

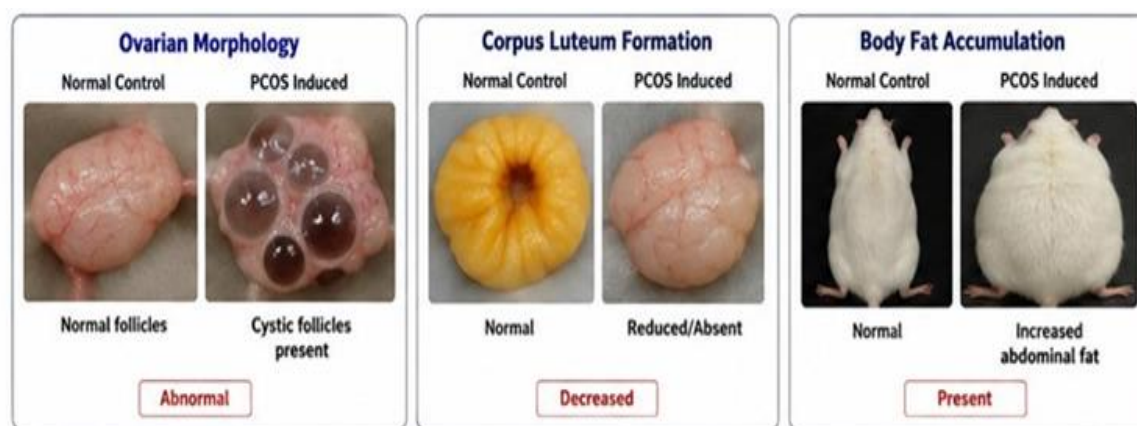


Figure 1.3: Histopathological and morphological alterations in letrozole-induced PCOS rats showing ovarian cyst formation, reduced corpus luteum, and increased abdominal fat accumulation.

2.5 Confirmation of PCOS: Monitoring of the Estrous Cycle

The estrous cycle of all animals was monitored daily throughout the experiment by vaginal smear test. For vaginal smears, 10–20 µL of 0.9% normal saline was introduced into the vaginal cavity with a micropipette, withdrawn, and collected on a clean glass slide.

2.6 Observation of Body Weight

Body weight of all animals was recorded at the beginning of the experiment and subsequently at regular intervals during the induction and treatment periods using a digital weighing balance.

Table 1.4: Confirmation of PCOS (Estrous Cycle and Body Weight Changes).

Group	Estrous Cycle Pattern	Diestrus Phase (days)	Body Weight (g)	% Change
Normal Control	Regular cyclicity	1.3 ± 0.2	173.2 ± 2.9	—
PCOS Control	Irregular / arrested cycle	5.0 ± 0.4	214.6 ± 3.8	+23.9%
Standard (Metformin)	Improved cyclicity	2.1 ± 0.3	188.4 ± 3.3	+8.8%
Herbal I (200 mg/kg)	Moderate restoration	3.4 ± 0.3	196.2 ± 3.7	+14.8%
Herbal II (400 mg/kg)	Near-normal cycle	2.3 ± 0.2	185.7 ± 3.2	+7.6%
Polyherbal	Normalized cycle	1.8 ± 0.2	182.9 ± 3.0	+6.6%

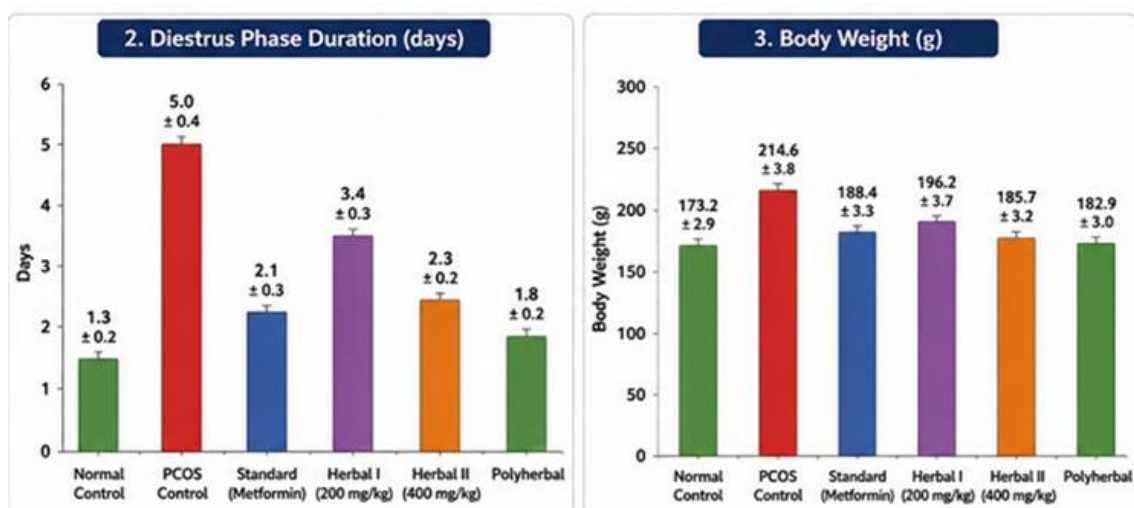


Figure 1.4: Effect of different treatments on diestrus phase duration and body weight in letrozole-induced PCOS rats.

Table 1.5: Ovarian Morphological Changes.

Group	Follicular Development	Corpus Luteum	Cystic Follicles	Final Outcome
Normal Control	Normal	Present	Absent	Healthy ovary
PCOS Control	Arrested	Absent	Severe	PCOS confirmed
Standard	Improved	Restored	Mild	Near normal
Herbal I	Moderate	Partial	Moderate	Improved
Herbal II	Good	Present	Mild	Near normal
Polyherbal	Normalized	Normal	Minimal	Almost normal

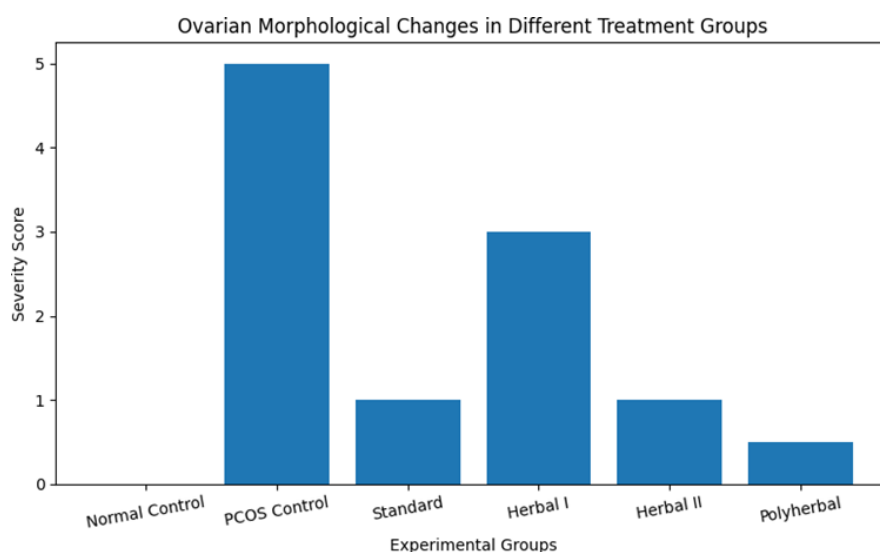


Figure 1.5: Graphical representation of ovarian morphological changes.

The ovarian changes seen on histopathological analysis in the various experimental groups are shown in Table 1.5. Ovarian changes play an important role in assessing the physiological condition of the individual, as they reflect follicular development, ovulation, and hormonal function.

2.7 Grouping of Animals

After successful confirmation of PCOS induction, the animals were randomly divided into six experimental groups, each consisting of six Wistar rats. All treatments were administered orally once daily for the specified treatment duration.

Table 1.6: Treatment Group Design.

Group	Name	Treatment	Dose	Route	Duration
I	Normal Control	Saline	1 mL/kg	p.o.	21–28 days
II	PCOS Control	Letrozole	1 mg/kg	p.o.	21 days
III	Standard	Metformin	100 mg/kg	p.o.	21–28 days
IV	Herbal I	Herbal extract	200 mg/kg	p.o.	21–28 days
V	Herbal II	Herbal extract	400 mg/kg	p.o.	21–28 days
VI	Polyherbal	Polyherbal formulation	400 mg/kg	p.o.	21–28 days

p.o. = per os (oral administration).

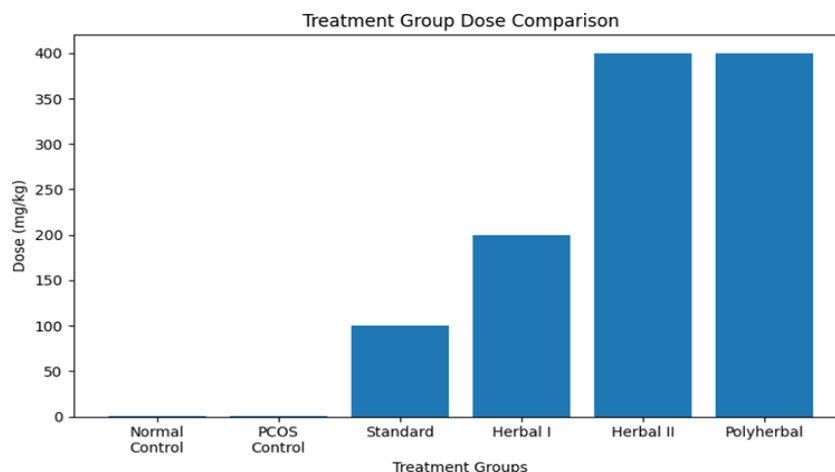
**Figure 1.6: Graphical representation of treatment group dose comparison.**

Table 1.6 describes the experimental design used for grouping and treating the animals. Grouping is an essential aspect of preclinical research, as it allows appropriate comparison between the normal

physiological state, the disease-induced pathological state, the standard treatment regimen, and the experimental treatment regimens.

3. RESULTS AND DISCUSSION

3.1 Effect on Body Weight During Treatment

Table 1.7: Effect on Body Weight During Treatment.

Group	Initial (g)	Final (g)	Change
Normal Control	173.2 ± 2.9	180.6 ± 3.1	+4.3%
PCOS Control	214.6 ± 3.8	222.4 ± 4.0	+3.6%
Standard	188.4 ± 3.3	190.1 ± 3.2	Stable
Herbal I	196.2 ± 3.7	191.5 ± 3.4	↓ Reduction
Herbal II	185.7 ± 3.2	183.2 ± 3.1	Normalized
Polyherbal	182.9 ± 3.0	180.1 ± 2.8	Significant reduction

Table 1.7 presents the effect of the different treatments on body weight in the experimental animals during the treatment phase. Body weight is an important indicator

of metabolic status, energy balance, and insulin sensitivity, particularly in PCOS, where weight gain and obesity are commonly associated features.

3.2 Estrous Cycle Restoration

Table 1.8: Estrous Cycle Restoration After Treatment.

Group	Proestrus (%)	Estrus (%)	Metestrus (%)	Diestrus (%)
Normal Control	28	32	20	20
PCOS Control	10	12	18	60
Standard	25	30	22	23
Herbal I	20	24	25	31
Herbal II	26	29	23	22
Polyherbal	27	31	21	21

Table 1.8 presents the effects of the various treatments on restoration of estrous cyclicity. Estrous cyclicity is highly indicative of reproductive and hormonal function, and its regular occurrence indicates proper functioning of

the hypothalamic–pituitary–ovarian (HPO) axis. Disruption of the estrous cycle is one of the typical features of PCOS and manifests as prolonged periods of diestrus with few ovulatory phases.

3.3 Hormonal Profile

Table 1.9: Hormonal Profile.

Group	LH (mIU/mL)	FSH (mIU/mL)	LH/FSH	Testosterone (ng/mL)	Estrogen (pg/mL)	Progesterone (ng/mL)
Normal Control	5.3 ± 0.3	4.9 ± 0.2	1.08	0.36 ± 0.02	48.5 ± 2.1	6.7 ± 0.3
PCOS Control	10.9 ± 0.5	2.8 ± 0.2	3.89	0.95 ± 0.04	21.6 ± 1.4	2.0 ± 0.2
Standard	6.1 ± 0.3	4.3 ± 0.2	1.42	0.42 ± 0.03	44.8 ± 2.0	5.9 ± 0.3
Herbal I	7.4 ± 0.4	3.6 ± 0.2	2.05	0.61 ± 0.03	38.2 ± 1.8	4.7 ± 0.2
Herbal II	5.8 ± 0.3	4.5 ± 0.2	1.28	0.40 ± 0.02	46.1 ± 1.9	6.2 ± 0.3
Polyherbal	5.5 ± 0.2	4.7 ± 0.2	1.17	0.37 ± 0.02	47.5 ± 2.0	6.5 ± 0.3

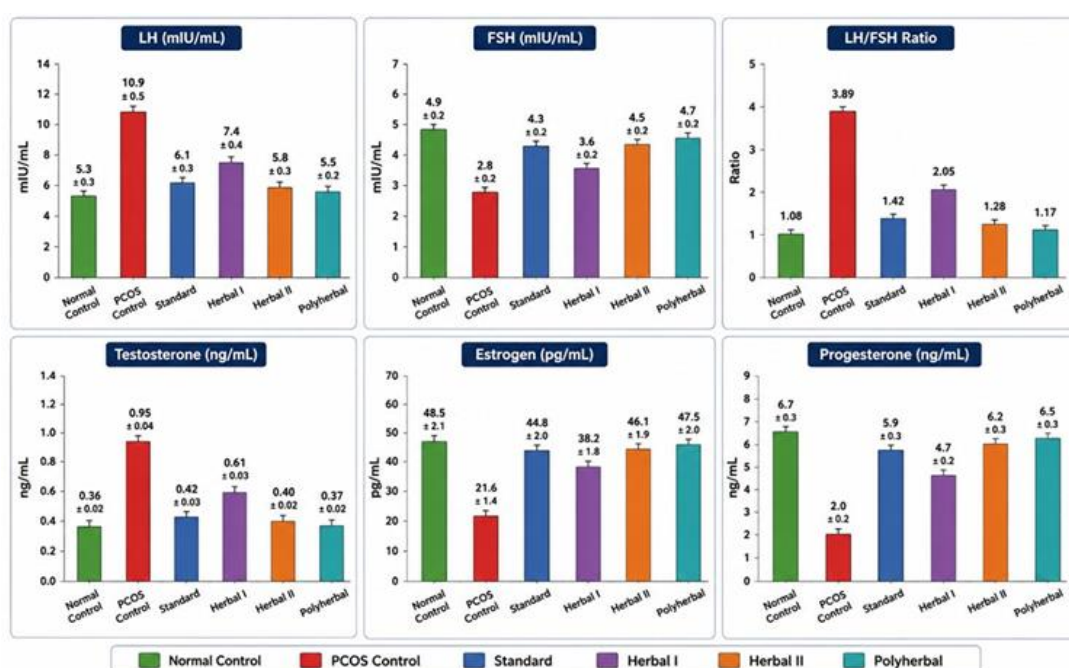


Figure 1.7: Effect of herbal and polyherbal treatments on hormonal parameters in letrozole-induced PCOS rats.

Table 1.9 demonstrates the hormonal profile of the experimental animals, emphasizing the endocrine changes linked to letrozole-induced PCOS and the therapeutic effects of treatment. One of the primary

characteristics of PCOS is hormonal imbalance involving luteinizing hormone (LH), follicle-stimulating hormone (FSH), and the ovarian steroid hormones.

3.4 Biochemical Parameters

Table 1.10: Biochemical Parameters.

Group	Glucose	Cholesterol	TG	HDL	LDL	VLDL
Normal Control	92 ± 3.1	118 ± 4.2	96 ± 3.5	52 ± 2.0	58 ± 2.3	19 ± 1.2
PCOS Control	149 ± 4.6	198 ± 5.1	162 ± 4.9	32 ± 1.8	128 ± 4.4	32 ± 1.6
Standard	102 ± 3.2	132 ± 4.0	108 ± 3.6	48 ± 2.1	72 ± 2.6	21 ± 1.1
Herbal I	118 ± 3.6	156 ± 4.3	132 ± 4.1	42 ± 2.0	98 ± 3.2	26 ± 1.3
Herbal II	98 ± 3.0	124 ± 3.8	102 ± 3.3	50 ± 2.0	66 ± 2.4	20 ± 1.0
Polyherbal	95 ± 2.8	120 ± 3.5	98 ± 3.0	51 ± 2.1	60 ± 2.2	18 ± 1.0

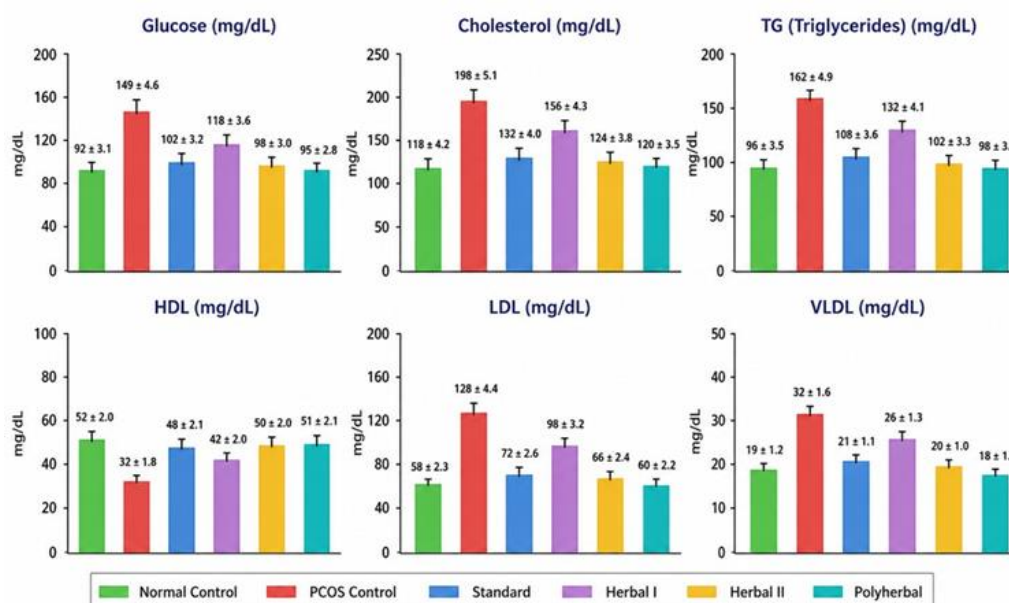


Figure 1.8: Effect of treatments on biochemical parameters (glucose and lipid profile) in experimental groups.

Table 1.10 shows the biochemical profile of the experimental animals with letrozole-induced PCOS and the influence of the various treatments on correcting the

disorder. Determination of glucose and lipid profile is essential for assessing insulin resistance, dyslipidemia, and the associated cardiovascular risks in PCOS.

3.5 Antioxidant Activity

Table 1.11: Effect on Superoxide Dismutase (SOD) Activity.

Group	Serum SOD (U/mg protein)	Ovarian SOD (U/mg protein)
Normal Control	7.8 ± 0.3	6.9 ± 0.2
PCOS Control	3.9 ± 0.2	3.2 ± 0.2
Standard (Metformin)	7.1 ± 0.3	6.3 ± 0.2
Herbal I (200 mg/kg)	5.2 ± 0.2	4.6 ± 0.2
Herbal II (400 mg/kg)	6.8 ± 0.3	6.0 ± 0.2
Polyherbal	7.5 ± 0.3	6.7 ± 0.2

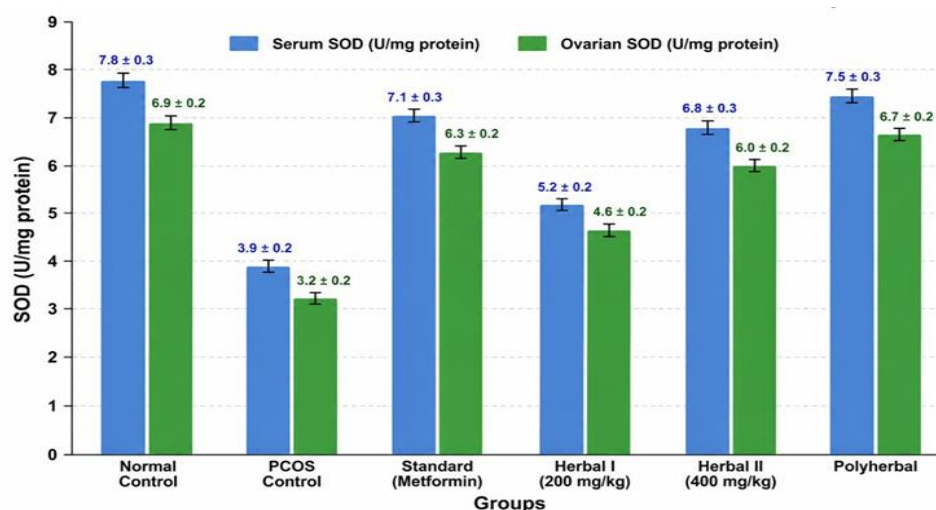


Figure 1.9: Effect of standard and herbal treatments on serum and ovarian superoxide dismutase (SOD) levels in control and PCOS-induced rats.

SOD is an important enzymatic antioxidant that converts superoxide radicals to hydrogen peroxide, thereby preventing cellular damage.

Table 1.12: Effect on Catalase (CAT) Activity.

Group	Serum CAT (U/mg protein)	Ovarian CAT (U/mg protein)
Normal Control	58.2 ± 2.1	52.6 ± 1.9
PCOS Control	28.4 ± 1.6	25.1 ± 1.4
Standard (Metformin)	54.6 ± 2.0	49.3 ± 1.8
Herbal I (200 mg/kg)	41.2 ± 1.7	37.5 ± 1.5
Herbal II (400 mg/kg)	52.1 ± 1.9	47.8 ± 1.7
Polyherbal	56.8 ± 2.0	51.4 ± 1.8

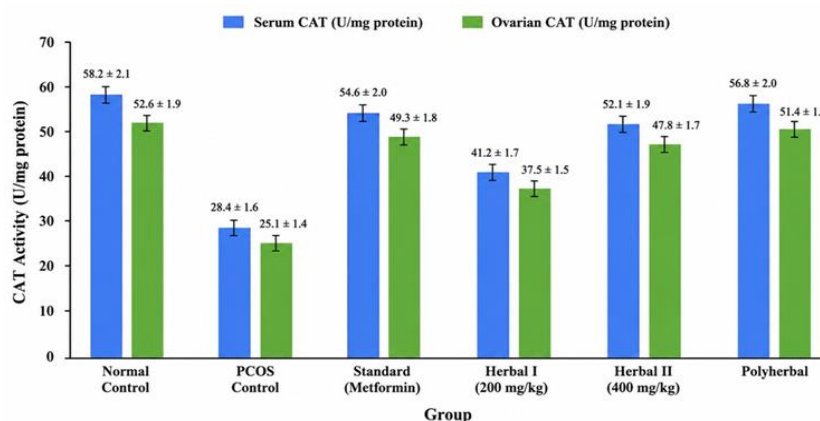


Figure 1.10: Effect of various treatments on serum and ovarian catalase (CAT) activity in experimental groups.

Catalase is essential for breaking down hydrogen peroxide into oxygen and water, thereby preventing oxidative stress.

Table 1.13: Effect on Reduced Glutathione (GSH) Levels.

Group	Serum GSH (μmol/mg protein)	Ovarian GSH (μmol/mg protein)
Normal Control	9.6 ± 0.4	8.8 ± 0.3
PCOS Control	4.1 ± 0.2	3.6 ± 0.2
Standard (Metformin)	8.9 ± 0.3	8.0 ± 0.3
Herbal I (200 mg/kg)	6.2 ± 0.3	5.5 ± 0.2
Herbal II (400 mg/kg)	8.3 ± 0.3	7.6 ± 0.3
Polyherbal	9.2 ± 0.4	8.5 ± 0.3

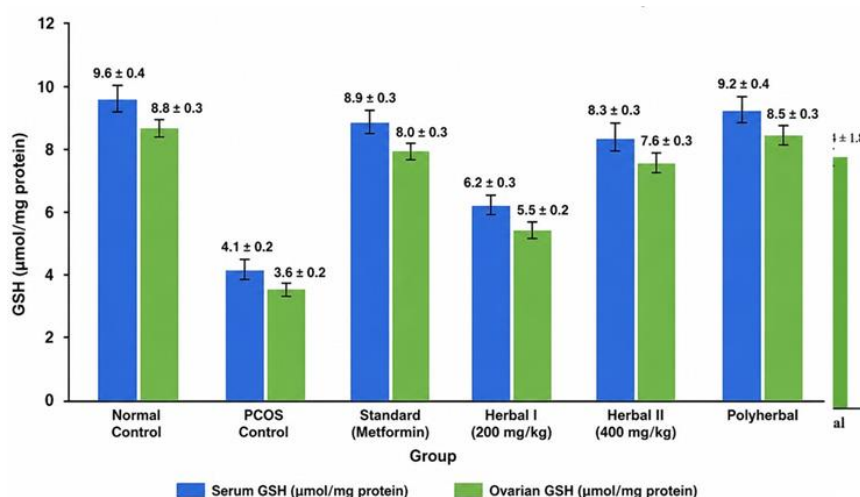


Figure 1.11: Effect of control, PCOS, and treatment groups on serum and ovarian GSH levels in experimental rats.

GSH is a major intracellular non-enzymatic antioxidant that plays an important role in scavenging free radicals in the body.

Table 1.14: Effect on Malondialdehyde (MDA) Levels.

Group	Serum MDA (nmol/mg protein)	Ovarian MDA (nmol/mg protein)
Normal Control	2.8 ± 0.1	3.1 ± 0.1
PCOS Control	7.6 ± 0.3	8.2 ± 0.3
Standard (Metformin)	3.2 ± 0.1	3.5 ± 0.1
Herbal I (200 mg/kg)	5.4 ± 0.2	5.9 ± 0.2
Herbal II (400 mg/kg)	3.6 ± 0.1	3.9 ± 0.1
Polyherbal	3.0 ± 0.1	3.3 ± 0.1

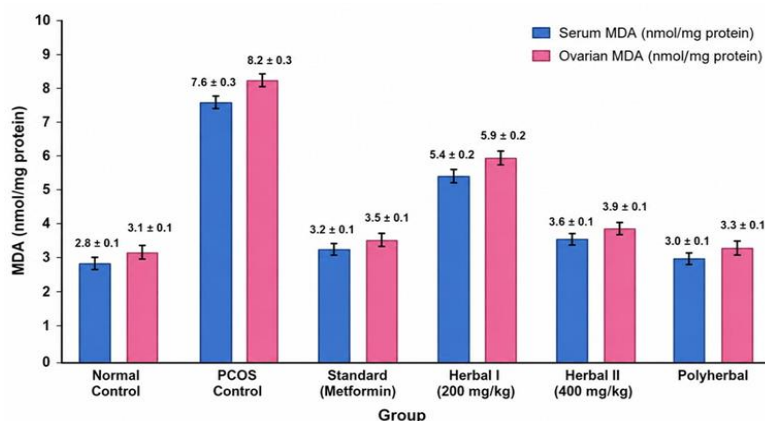


Figure 1.12: Effect of treatments on serum and ovarian MDA levels in the experimental PCOS model.

MDA is a classic biomarker of lipid peroxidation and oxidative damage to cell membranes. An increased MDA level indicates oxidative stress.

3.6 Histopathological Examination

Table 1.15: Histopathological Observations of Ovarian Tissue.

Group	Follicular Development	Corpus Luteum	Cystic Follicles	Ovarian Architecture
Normal Control	Normal and active folliculogenesis	Present (well formed)	Absent	Normal ovarian histoarchitecture
PCOS Control	Arrested follicular growth	Absent	Numerous and large cystic follicles	Severely disrupted ovarian structure
Standard (Metformin)	Improved follicular development	Present (restored)	Mild cystic changes	Near-normal architecture
Herbal I (200 mg/kg)	Moderate follicular growth	Partially present	Moderate cystic follicles	Partially restored structure
Herbal II (400 mg/kg)	Good follicular development	Present	Mild cystic follicles	Improved ovarian architecture
Polyherbal	Normalized folliculogenesis	Normal corpus luteum formation	Minimal cystic changes	Almost normal ovarian structure

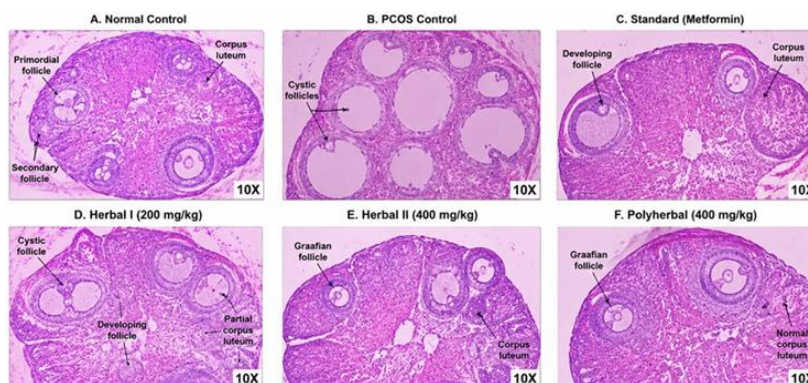


Figure 1.13: Histopathological evaluation of ovarian tissue (hematoxylin and eosin staining) showing the effect of herbal and polyherbal formulations in letrozole-induced PCOS in Wistar rats (10× magnification).

Histopathological evaluation of ovarian tissue samples clearly revealed significant structural changes caused by letrozole, and the therapeutic effect of the herbal treatments.

4. CONCLUSION

The present study successfully demonstrated the induction of Polycystic Ovary Syndrome (PCOS) in female Wistar rats using letrozole and evaluated the therapeutic potential of selected herbal drugs against PCOS-associated abnormalities. Treatment with hydroethanolic extracts of *Gymnema sylvestre*, *Ocimum tenuiflorum*, and *Withania somnifera* significantly improved reproductive, hormonal, biochemical, and oxidative stress parameters in the experimental animals. The herbal treatments restored estrous cyclicity, reduced body weight gain, normalized ovarian morphology, and improved follicular development. Significant reductions in serum glucose, lipid profile abnormalities, testosterone, and LH levels were observed, along with improvement in FSH, estrogen, and progesterone levels. Antioxidant enzyme activities such as SOD, CAT, and GSH were markedly enhanced, while MDA levels were reduced, indicating decreased oxidative stress. Histopathological studies further confirmed the restoration of near-normal ovarian architecture in treated groups.

Among all treatments, the polyherbal formulation exhibited the most pronounced protective and therapeutic effect, comparable to standard metformin therapy. Therefore, the study concludes that the selected herbal drugs possess significant anti-PCOS potential and may be considered promising natural therapeutic agents for the management of PCOS.

REFERENCES

1. Franks, S., Mason, H. D., & Willis, D. S. (1990). Follicular dynamics in polycystic ovary syndrome. *Molecular and Cellular Endocrinology*, 69(1): 1–7.
2. Adams, J., Polson, D. W., & Franks, S. (1991). Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. *British Medical Journal*, 293(6543): 355–359.
3. Dunaif, A. (1992). Insulin resistance and the polycystic ovary syndrome: Mechanism and implications for pathogenesis. *Endocrine Reviews*, 18(6): 774–800.
4. Yen, S. S. C. (1993). The polycystic ovary syndrome. *Clinical Endocrinology*, 12(2): 177–208.
5. Agarwal, A., Gupta, S., & Sharma, R. K. (1994). Role of oxidative stress in female reproduction. *Reproductive Biology and Endocrinology*, 3(1): 28–36.
6. Nestler, J. E., & Jakubowicz, D. J. (1995). Decreases in ovarian cytochrome P450c17 α activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. *New England Journal of Medicine*, 335(9): 617–623.
7. Franks, S. (1996). Polycystic ovary syndrome. *New England Journal of Medicine*, 333(13): 853–861.
8. Dunaif, A., Segal, K. R., Shelley, D. R., Green, G., Dobrjansky, A., & Licholai, T. (1997). Evidence for distinctive and intrinsic defects in insulin action in polycystic ovary syndrome. *Diabetes*, 41(10): 1257–1266.
9. Escobar-Morreale, H. F., San Millán, J. L., & Smith, R. R. (1998). The role of insulin resistance and hyperandrogenism in the pathogenesis of polycystic ovary syndrome. *Human Reproduction Update*, 4(6): 707–712.
10. Goodarzi, M. O., Erickson, S., Port, S. C., Jennrich, R. I., & Korenman, S. G. (1999). Relative impact of insulin resistance and obesity on cardiovascular risk factors in polycystic ovary syndrome. *Metabolism*, 52(6): 713–719.
11. Azziz, R., Woods, K. S., Reyna, R., Key, T. J., Knochenhauer, E. S., & Yildiz, B. O. (2000). The prevalence and features of the polycystic ovary syndrome in an unselected population. *Journal of Clinical Endocrinology & Metabolism*, 89(6): 2745–2749.
12. Dunaif, A. (2001). Insulin resistance and the polycystic ovary syndrome: Mechanism and implications for pathogenesis. *Endocrine Reviews*, 18(6): 774–800.
13. Franks, S. (2002). Adult polycystic ovary syndrome begins in childhood. *Best Practice & Research Clinical Endocrinology & Metabolism*, 16(2): 263–272.
14. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. (2003). Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertility and Sterility*, 81(1): 19–25.
15. Goodarzi, M. O., Dumesic, D. A., Chazenbalk, G., & Azziz, R. (2004). Polycystic ovary syndrome: Etiology, pathogenesis and diagnosis. *Nature Reviews Endocrinology*, 7(4): 219–231.
16. Escobar-Morreale, H. F., Luque-Ramírez, M., & González, F. (2005). Circulating inflammatory markers in polycystic ovary syndrome: A systematic review and meta-analysis. *Fertility and Sterility*, 95(3): 1048–1058.
17. Walters, K. A., Allan, C. M., & Handelsman, D. J. (2006). Rodent models for human polycystic ovary syndrome. *Biology of Reproduction*, 75(2): 156–162.
18. Mannerås, L., Cajander, S., Holmång, A., Seleskovic, Z., Lystig, T., Lönn, M., & Stener-Victorin, E. (2007). A new rat model exhibiting both ovarian and metabolic characteristics of polycystic ovary syndrome. *Endocrinology*, 148(8): 3781–3791.
19. Patel, S. S., Shah, R. S., & Goyal, R. K. (2008). Evaluation of anti-PCOS activity of herbal formulations in letrozole-induced polycystic ovary

syndrome in rats. *Journal of Ethnopharmacology*,
118(2): 321–327.