International Journal of Pharmacy and Pharmaceutical Science

ISSN Print: 2664-7222
ISSN Online: 2664-7230
IJPPS 2025; 7(2): 677-680
www.pharmacyjournal.org
Received: 10-09-2025
Accepted: 15-10-2025

Richa Agarwal
Associate Professor, Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan, India

Rajesh Asija
Principal, Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan, India

Amrita Kanwar
Research Scholar, Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan, India

Kashish Tiwari
Assistant Professor, Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan, India

Corresponding Author:
Richa Agarwal
Associate Professor, Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan, India

Pharmacological effect of alfalfa seeds on letrozole induced polycystic ovary syndrome in female rat model

Richa Agarwal, Rajesh Asija, Amrita Kanwar and Kashish Tiwari

DOI: https://www.doi.org/10.33545/26647222.2025.v7.i2h.263

Abstract
Polycystic ovary syndrome (PCOS) is an endocrine disorder that disrupts the menstrual cycle and causes infertility. Considering the increasing use of medicinal plants, the present study aimed to evaluate the effects of *Medicago sativa* on letrozole-induced PCOS in female rats. Five groups of rats were evaluated. The control group received 0.5% carboxy methyl cellulose as vehicle, while the Four other groups received letrozole 1 mg/kg orally for 21 days. After PCOS induction, the rats were orally administered *Medicago sativa* extract100, and 200 mg/kg) or metformin (70 mg/kg) for 21 days. Subsequently, body and ovarian weights and serum levels of follicle stimulating hormone, luteinizing hormone (LH), estradiol, progesterone, and testosterone were measured. Finally, the ovarian tissues were isolated for histological examination. There were no significant changes in weekly body weight in any group. After 21 days of letrozole administration, PCOS induction was confirmed by estrous cycle irregularities and increased LH and testosterone levels. After treatment with the hydroalcoholic extract of *Medicago sativa*, testosterone and LH levels were significantly reduced in all groups.

Keywords: Letrozole induced PCOS, alfalfa seeds, polycystic ovary syndrome, metformin

Introduction
Polycystic Ovary Syndrome (PCOS) is a complex endocrine disorder affecting a significant proportion of women of reproductive age worldwide. Although several allopathic treatments such as hormonal contraceptives, insulin sensitizers (e.g., metformin), and ovulation-inducing agents (e.g., clomiphene citrate) are commonly prescribed to manage PCOS, they often provide only symptomatic relief and are associated with various side effects, including weight gain, gastrointestinal disturbances, mood changes, and long-term metabolic complications. Moreover, these treatments are not curative and require prolonged use, which may lead to poor patient compliance.
In contrast, herbal medicines offer a promising alternative due to their natural origin, lower risk of adverse effects, and potential to provide holistic and long-term benefits. The increasing interest in phytotherapy stems from its ability to modulate hormonal balance, improve metabolic function, and reduce oxidative stress-key factors in PCOS pathophysiology. Therefore, there is a growing need to scientifically evaluate and validate the efficacy of traditional herbs like *Medicago sativa* (alfalfa), which may offer a safer, more sustainable, and patient-friendly approach to the management of PCOS.

Materials and Methods
The seeds of the plant collected from the local vendor shop and authenticated by a verified botanist. To prepare extract, 100gm of powder is weighed and packed a powder in a thimble and extraction process was done using Soxhlet apparatus. 70% ethanol was used as a solvent for extraction. After 6-8 hrs of extraction process the obtained extract is evaporated on water bath and the yield was calculated.

Experimental method
The disease in the animal will be induced by letrozole at a dose of 1mg/kg body weight with dissolve in 0.5% Carboxymethylcellulose (CMC) for 21 days. Metformin will be used as a standard drug at a dose of 70mg/kg body weight.

Table 1: Experimental Design and Treatment Protocol for Animal Groups (36 Days)

Sr. No.	Animal group	Treatment duration (36 days)
1.	Normal control	Saline p.o.
2.	Disease control	Letrozole 1mg/kg b.w. dissolved in 0.5% CMC for 21 days.
3.	Standard Control	Metformin 70mg/kg b.w. for 15 days after the 21 days of letrozole treatment.
4.	Treatment - 1	Alfalfa 100mg/kg B.w. for 15 days after the 21 days of letrozole treatment.
5.	Treatment - 2	Alfalfa 200mg/kg B.w. for 15 days after the 21 days of letrozole treatment.

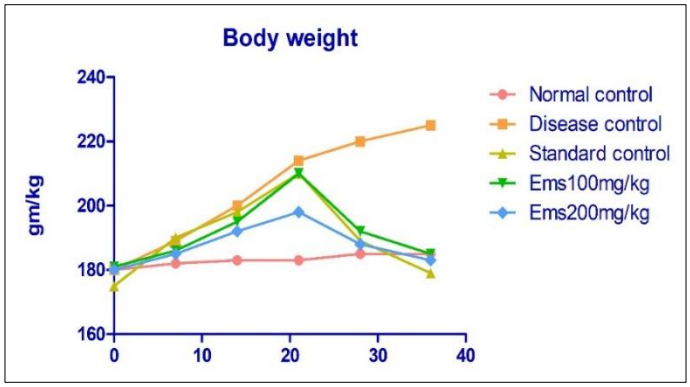
A comprehensive set of evaluation parameters were selected based on morphological, biochemical, hormonal, and histopathological aspects. These parameters help in confirming the successful induction of PCOS and evaluating the efficacy of the test treatment.

Table 2: Evaluation Parameters for Assessing PCOS Induction and Treatment Efficacy

Sr. No.	Evaluation Parameters
1.	Body weight
2.	Biochemical parameters - serum hormone level of FSH, LH, estradiol, testosterone, progesterone
3.	Intraperitoneal glucose tolerance test
4.	Histopathological analysis of ovary - H & E staining

Results and Discussion

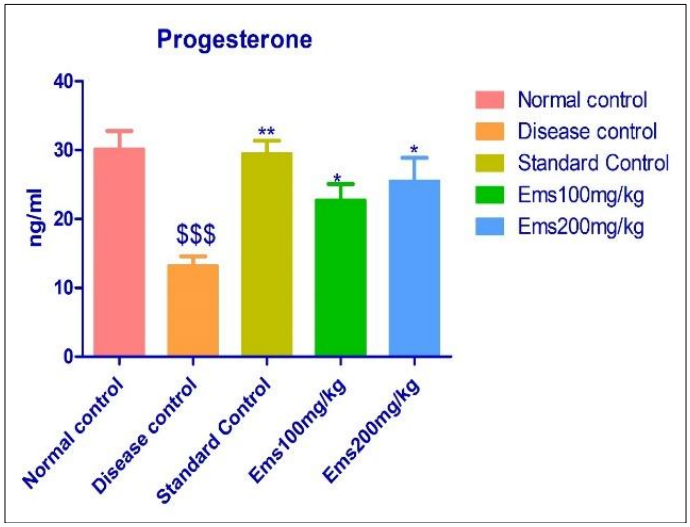
Effect of EMS on body weight of animals

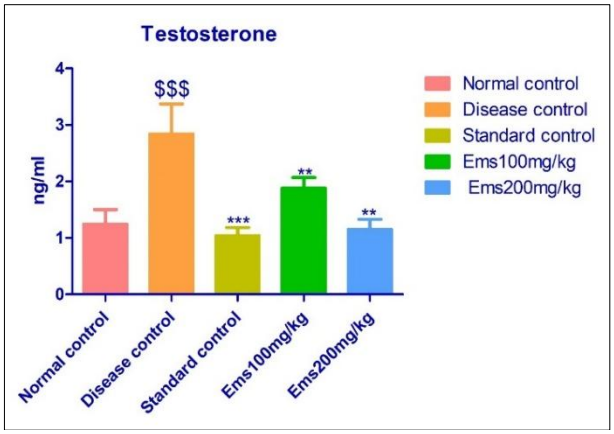


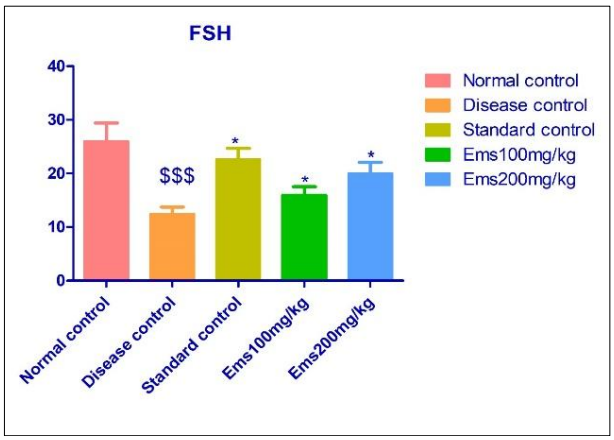
Among them, the higher dose (200 mg/kg) produced a more pronounced effect, nearly comparable to the standard treatment, while the lower dose (100 mg/kg) exhibited moderate improvement. These findings suggest that *Medicago sativa* extract exerts a dose-dependent effect in controlling PCOS-induced body weight gain, likely due to its phytoconstituents that modulate oxidative stress, insulin resistance, and hormonal imbalance.

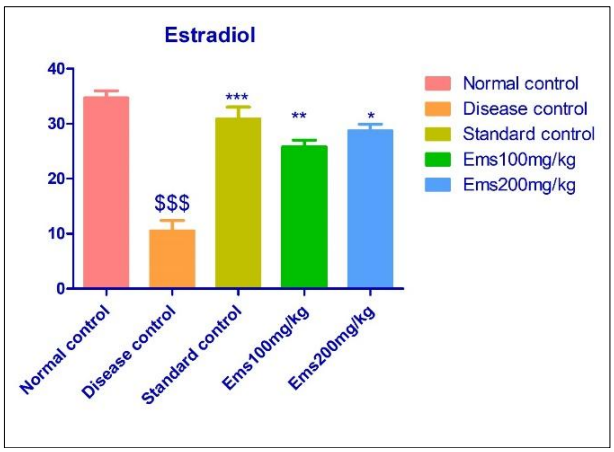
Table 3: Effect of EMS on reproductive organ weight of animals

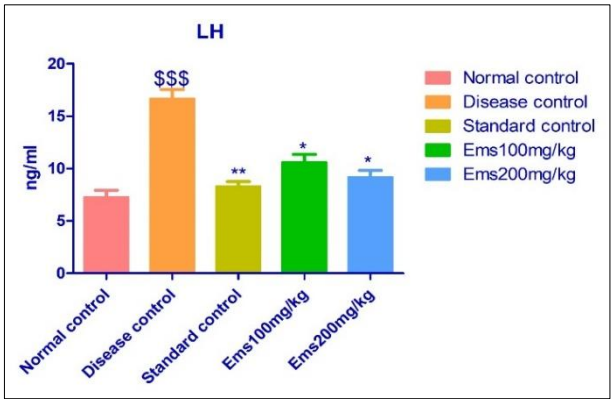
Animal Group	Ovary weight	Uterus weight
Normal control	94.97±8.91	147.54±9.00
Disease control	157.59±6.35	125.34±7.39
Standard Control	127.25±4.32	144.76±10.18
Ems100mg/kg	134.44±3.67	140.24±8.73
Ems200mg/kg	126.32±5.64	141.35±5.39







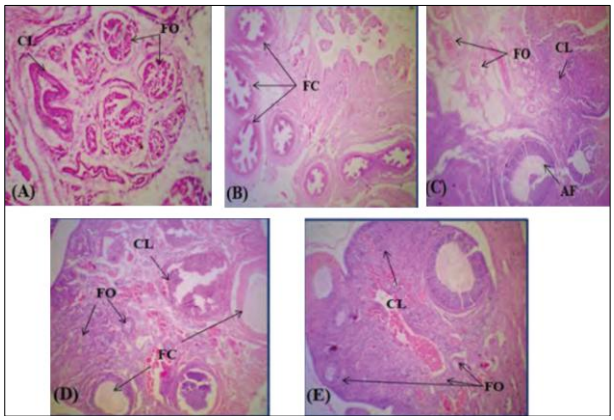




Effect of hormonal level of EMS

Each value represents the mean ± SEM of six observations per group. Statistical analysis was performed using One way ANOVA followed by the Bonferroni post hoc test. The level of Estradiol, FSH and Progesterone was significantly decreased in the disease group and Increased level was observed in other groups after treatment. While the level of testosterone and LH was significantly increased in the disease group and decreased level was observed in other groups after treatment. Statistical significance denoted as (*-*p< 0.05, **-p<0.01 and ***-p<0.001* compared to the disease control group.

Histopathological analysis of ovary: H&E Staining



Histopathological microscopic images of ovary sections of rats (40x)

a) Normal control, (B) Disease control, (C) Standard control, (D) EMS100mg/kg (E) Ems200mg/kg

b) AF- Atretic Follicles; CL- Corpus luteum; FC-Follicular cysts; FO-Follicles

Table 4: Effect of Ems on Blood glucose level of animals

Days	Normal group	Disease group	Standard group	Ems100mg/kg	Ems200mg/kg
0	74.66±3.0	78.00±3.02	73.66±1.25	73.66±1.60	73±3.02
7	77.50±1.20	82±1.20	80.00±2.36	82±1.22	81±1.20
14	78.66±1.38	86±1.3	85±2.24	87±1.54	84±1.38
21	78.00±1.22	92±1.22	90±3.34	91±0.42	90±1.22
28	79.33±1.60	95±1.60	83.0±2.21	88±0.63	86±1.60
36	79.00±2.2	97±2.2	81±3.21	87±0.54	85±2.2

EMS 200 mg/kg produced a more pronounced reduction, showing a dose-dependent effect. The statistical significance observed at different time points further confirms the efficacy of the treatment. Overall, this indicates that PCOS induction elevates blood glucose levels, while treatment with standard drug and Medicago sativa extract effectively reduced these levels, with the higher dose producing better results.

Conclusion

The findings of this study suggest that Medicago sativa leaves extract exhibits potent therapeutic effects in letrozole-induced PCOS in rats. The extract improved both reproductive and metabolic abnormalities associated with PCOS, with the higher dose (200 mg/kg) showing effects comparable to the standard drug metformin. These results support the traditional use of Medicago sativa in reproductive health and highlight its potential as a natural, plant-based intervention for PCOS management.

References

1. Al-Snafi A, Salman H, Khadem, Hussein HA, Alqahtani A, Batiha G, *et al*. A review on *Medicago*

sativa: A potential medicinal plant. International Journal of Biological and Pharmaceutical Sciences Archive. 2021:22-33.
2. Rana MG, Katbamna RV, Padhya AA, Dudhrejiya AD, Jivani NP, Seth NR. *In vitro* antioxidant and free radical scavenging studies of alcoholic extract of *Medicago sativa* L. Rom J Biol Plant Biology. 2010;2:95-99.
3. Al-Snafi A. Encyclopedia of chemical constituents and pharmacological effects of Iraqi medicinal plants. Rigi Publication; 2015.
4. Choi KC, Hwang JM, Bang SJ, Kim BT, Kim JH, Chae M, *et al*. Chloroform extract of alfalfa inhibits lipopolysaccharide-induced inflammation by downregulating ERK/NF signaling and cytokine production. J Med Food. 2013;16(5):410-420.
5. Alanazi A, Kabra A, *et al*. Gastroprotective evaluation of *Medicago sativa* L. on diabetic rats. Journal of Saudi Pharmaceutical. 2023;31(11).
6. Gatouillat G, Magid AA, Bertin E, Okiemy-Akeli MG, Morjani H, Lavaud C, *et al*. Cytotoxicity and apoptosis induced by alfalfa (*Medicago sativa*) leaf extracts in sensitive and multidrug-resistant tumor cells. Nutrition and Cancer. 2014;66(3):483-491.
7. Maharjan R, Nagar PS, Nampoothiri L. Effect of *Aloe barbadensis* Mill. formulation on Letrozole-induced polycystic ovarian syndrome rat model. Journal of Ayurveda and Integrative Medicine. 2010;1(4):273-279.
8. Feyzollahi Z, Mohseni Kouchesfehani H, Jalali H, Eslimi-Esfahani D, Sheikh Hosseini A. Effect of *Vitex agnus-castus* ethanolic extract on hypothalamic KISS-1 gene expression in a rat model of polycystic ovary syndrome. Avicenna Journal of Phytomedicine. 2021;11(3):292-301.