



RESEARCH ARTICLE

Regularization of Estrous Cycle Using *Pergularia Daemia* and Metformin in the PCOS Induced Rats

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ABSTRACT

Polycystic ovary syndrome (PCOS) is most common heterogeneous, endocrinological and metabolic disorder affecting, reproductive aged women. Nearly 14.6% - 22.8% of PCOS women have the prevalence of menstrual dysfunction and irregularities ranging from amenorrhea to menorrhagia with classic peripubertal onset. In this study, the herbal plant *Pergularia daemia* is used treat PCOS by using female albino wistar rat as a model. The experimental animals were divided into four groups each group consisting of five rats. 1% of CMC (carboxy methyl cellulose) was received by control group (Group I) for 21 days. The remaining three groups were administrated with letrozole (1mg/kg dissolved in 2.0 ml/kg) orally for 21 days. The first letrozole treated group is considered to be Group II, which serves as a PCOS model. The second letrozole treated group is considered as Group III where (2mg/100g) of metformin is treated for 15 days and the final group (Group IV) was treated with methanolic leaf extract of *Pergularia daemia* (0.5 ml) for 7 days. The vaginal smear was collected every day to find changes in estrus cycle. The control group showed no changes in the estrous cycle whereas the PCOS induced group had an altered estrous cycle. The metformin and *Pergularia daemia* treated groups showed improvement but when compared to metformin, the *Pergularia daemia* treated group showed more regularity in the cycle. Thus the methanolic extract of *Pergularia daemia* has the potentiality to regain the estrous cycle in the PCOS induced rats.

KEYWORDS

Pergularia Daemia, Albino Wistar Rat, Letrozole, Estrous Cycle, Metformin

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex disorder characterized by elevated androgen levels, menstrual irregularities, and/or small cysts one or both ovaries¹ and other symptoms are abdominal obesity, acanthosis nigricans and hyper androgenism (acne or seborrhea and insulin resistance)²⁻⁶. Long-term consequences lead to endometrial cancer, type-2 diabetes mellitus, dyslipidemia, hypertension, and cardiovascular disorder⁷⁻⁹.

PCOS women have inappropriate gonadotropin secretion. Comparison between the follicular phase of normal menstrual cycle and PCOS women showing elevated LH secretion with comparatively constant low FSH secretion^{10,11,12}. Metformin has been used clinically a potential drug for PCOS but it has been known to have side effects. Traditional medicine is mostly used in many developing countries. *Pergularia daemia* is commonly known as “Veliparuthi” in Tamil Nadu. It is a perennial herb that grows along road side of India. The whole plant is used to treat various ailments like anthelmintic, antipyretic, laxative and expectorant, diarrhea

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and also treat infantile and malarial fever⁽³⁾. In this study *P. daemia* is used to treat PCOS condition in rats.

MATERIAL AND METHODS

Experimental Animals

Albino wistar strain rats (*Rattus norvegicus*) were used as experimental animals for the present study. The body weights of the animal ranging from 120-180g were used. The animals were kept in a sterile and aerated area. They were fed with pellets which have been purchased from Sai Durga Enterprises, Chennai. Institutional Animal Ethical committee approved the animal house of Holy Cross College, Trichy.

Experimental Design

The study was conducted on 20 female albino wistar rats, divided into four groups. The control group (Group I) that received only 1% aqueous solution of Carboxy Methyl Cellulose (CMC) (Sigma- Aldrich, USA) once daily p.o. The three treatment group rats were administered with Letrozole (Sigma- Aldrich, USA) at a concentration of 1.0 mg/Kg p.o dissolved in 1% of CMC (2.0 ml/Kg) once daily for the induction of polycystic ovaries. The treatment period was 21 days. The first PCOS induced group was left for normal recovery and serves as a PCOS model (Group II). The second PCOS induced group (Group III) was treated with metformin (Sigma-Aldrich, USA) for 15 days. The last PCOS induced group (Group IV) was administered with 0.5 ml of methanolic leaf extract of *Pergularia daemia* for 7 days. The vaginal smear was taken daily from all the experimental groups to study the changes in the estrous cycle.

Stock Preparation

1mg/ kg Letrozole dissolved in 2.0ml/kg of 1% CMC (Carboxy Methyl Cellulose).

Preparation of Plant Extract

The leaves of *Pergularia daemia* were air-dried at room temperature (37°C) for 2 weeks, after which they were ground to a uniform powder of 40 mesh size. 100 g of air dried powder was extracted with methanol (40-60° C) in a Soxhlet extractor for 18-20 hours and solution was

evaporated to dryness under reduced pressure and controlled temperature by using rotary evaporator. The extract was stored in a refrigerator at 4 °C in air-tight bottle until further use.

Vaginal Smear

During the experimental period, every morning between 8.00–9.00am, vaginal smear was performed for the determination of estrous cycle. Vaginal secretions were collected using cotton-tipped swabs softened with a drop of saline. After 1-2 inches of the swab had been inserted, the end had been rotated through 2-3 revolutions, which will allow the cotton tip to pick an adequate load of cells. The swab was then gently withdrawn. The smears were prepared immediately after withdrawal of the swab by rolling the cotton tip along the length of a glass slide. Generally two tracks were rolled on two slides. As soon as the smears were prepared and dried out, were dipped 3-5 times in a container of 70% alcohol in order to fix expeditiously. Methylene blue stain (MERCK, Mumbai) was used in the current study. It consists of 0.5% aqueous methylene blue solution. After dipping in the methylene blue stain, the slides were rinsed in tap water and examined. Using this technique the characterization of each phase of the estrous cycle was done based on the proportion among three types of cells observed in the vaginal smear; epithelial cells, cornified cells, leukocytes and keratinocytes. The cells are seen under the light microscope.

RESULTS

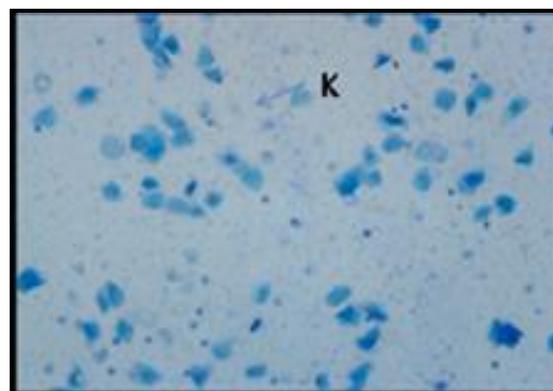


Figure 1a: Estrous stage

Table 1: Comparison of Estrous cycle

Days	Group I (Control)	Group II (PCOS Induced by letrozole)	Group III (Metformin)	Group IV (<i>P. daemia</i>)
1	Estrous	Metestrous	Metestrous	Proestrous
2	Estrous	Metestrous	Metestrous	Proestrous
3	Estrous	Diestrous	Diestrous	Estrous
4	Metestrous	Diestrous	Diestrous	Estrous
5	Metestrous	Diestrous	Diestrous	Estrous
6	Diestrous	Diestrous	Proestrous	Metestrous
7	Diestrous	Diestrous	Proestrous	Metestrous
8	Proestrous	Proestrous	Proestrous	Metestrous
9	Proestrous	Proestrous	Metestrous	Diestrous
10	Estrous	Metestrous	Metestrous	Diestrous
11	Estrous	Metestrous	Metestrous	Proestrous
12	Estrous	Diestrous	Diestrous	Estrous
13	Metestrous	Diestrous	Diestrous	Estrous
14	Metestrous	Diestrous	Proestrous	Estrous
15	Diestrous	Proestrous	Proestrous	Metestrous
16	Diestrous	Proestrous	Estrous	Metestrous
17	Proestrous	Metestrous	Metestrous	Metestrous
18	Proestrous	Metestrous	Diestrous	Diestrous
19	Estrous	Diestrous	Diestrous	Diestrous
20	Estrous	Diestrous	Proestrous	Proestrous
21	Estrous	Diestrous	Proestrous	Estrous
22	-	-	Estrous	Estrous
23	-	-	Metestrous	Estrous
24	-	-	Metestrous	Metestrous
25	-	-	Proestrous	Metestrous
26	-	-	Proestrous	Metestrous
27	-	-	Estrous	Proestrous
28	-	-	Metestrous	Proestrous
29	-	-	Metestrous	-
30	-	-	Diestrous	-
31	-	-	Diestrous	-
32	-	-	Proestrous	-
33	-	-	Proestrous	-
34	-	-	Estrous	-
35	-	-	Metestrous	-
36	-	-	Metestrous	-

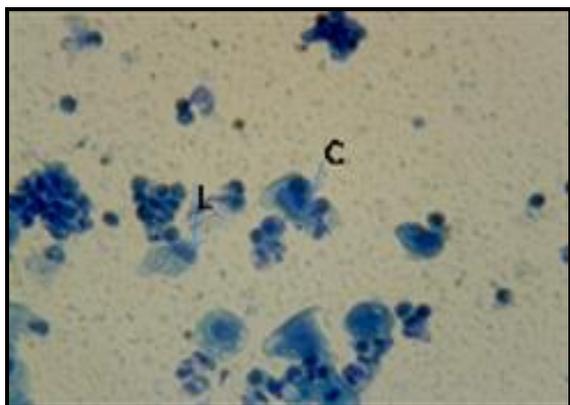


Figure 1b: Metestrous stage

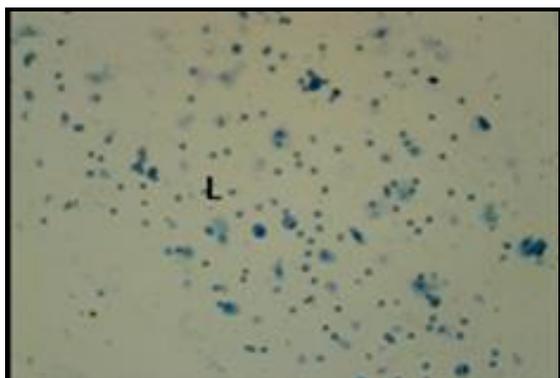


Figure 1c: Diestrous stage

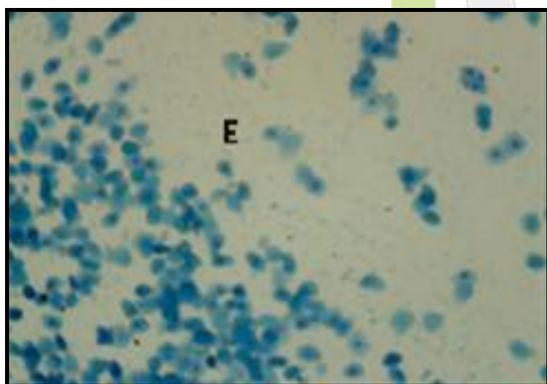


Figure 1d: Proestrous stage

Figure 1 (a,b,c,d): vaginal smear observed under the light microscope showing Keratinocytes (K), Leukocytes (L), Cornified cells (C), Epithelial cells (E)

DISCUSSION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting 5- 10% of women in their reproductive age. PCOS is frequently associated with anovulatory infertility^{3,9}. Due to anovulation, 75% of women are affected by infertility. In 1995, Balen *et al.*,¹⁴ explained that

among PCOS women, 47% are affected by oligomenorrhea. In PCOS women, clinical signs of Obesity and enlarged ovaries with small multiple cysts leads to anovulation and pregnancy complication^{12,13,15}. In the present study, the estrous cycle of the PCOS induced rat was observed. The control group has a normal estrous cycle whereas the PCOS induced rats showed changes in the estrous cycle. After the administration of the metformin, there was a positive change in the estrous cycle but when compared to the plant treatment, the metformin was not that much effective. The methanolic extract of *P. daemia* treated group had a good recovery of the estrous cycle. Thus by comparing the two kinds of treatments, the methanolic extract of *P. daemia* is better than metformin in regaining the estrous cycle of PCOS induced rats by letrozole (Tab 1).

Estrous cycle phases are determined based on the proportion of four different cell types (Fig 1) such as epithelial, cornified leukocytes, Keratinocytes, The cells with nucleus is known as epithelial cell, without nucleus are cornified and small round ones are leukocytes. Proestrous consists predominance of nucleated epithelial cells; an estrous stage primarily consists of anucleated cornified cells. The diestrous stage consists of predominant leukocytes. There is frequent occurrence of diestrous stage in the letrozole induced PCOS rat, indicating the abnormality in the estrous cycle. The metformin and the *P. daemia* treated animals regained their estrous cycle where the occurrence of estrous was seen more in the *P. daemia* treated group. This shows that the methanolic extract of the *P. daemia* plant has the efficacy to re-establish the estrous cycle of PCOS induced rats.

CONCLUSION

The methanolic leaf extract of *P. daemia* has potential effect on normalizing menstrual irregularities in letrozole induced PCOS albino wistar female rats. It was concluded that the estrous cycle was disturbed in the polycystic ovary condition. After the administration of *P.daemia* plant, there was restoration of the estrous cycle.

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