



Prospective use of *Tephrosia Purpurea* in Remedial Treatment of PCOS: Study in Wistar Rat

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Abstract

This study evaluates the potential of an herbal plant *Tephrosia purpurea*, in treatment of polycystic ovary syndrome (PCOS) using a rat model. The study used female Albino Wistar rats, which were divided into four groups, each consisting of 5 female rats. A control group, daily received 1% CMC (carboxymethylcellulose), while the other three groups for 28 days were once, daily administered, letrozole at concentration of 1mg/kg body weight, dissolved in 1% CMC 2ml/kg. Vaginal smears were collected daily from all the groups and studied for the estrous cycle. During 28 days of administration of letrozole, changes in estrous cycle were observed. The study indicated that PCOS was induced. After letrozole treatment, five animals of group 2 were treated orally with plant drug *Tephrosia purpurea* at a concentration of 200mg/kg body weight in milk as a carrier vehicle, 3ml/kg weight for 3 consecutive estrous cycles, vaginal smear were examined, it showed normalcy in estrous cycle sequence. Further, to check ovulation and fertility, female rats were mated and they showed pregnancy. Thus the potential of *Tephrosia purpurea*, in treatment of polycystic ovary syndrome (PCOS) using an animal model is confirmed.

Keywords: *Tephrosia purpurea*, polycystic ovary syndrome, carboxymethylcellulose, letrozole, estrous cycle.

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder characterized by anovulation, menstrual disorder, amenorrhea, hirsutism and infertility. It is also known as stein-levithel syndrome¹⁻³. Women with PCOS have impaired metabolism of androgen, estrogen and also in the control of androgen production. PCOS is the most common disorder in women of reproductive age group as well as premenopausal women^{4,5}. PCOS treatment is directed to the ovary for normalizing its functions. Medications are used to regulate the menstrual cycles and to stimulate ovulation. As different drugs used in treatment of PCOS cater to different symptoms, effective treatment to manage PCOS is a challenge. For the time immemorial several medicinal plants and their constituents have been used to prevent multistage carcinogenesis. To a large world population, medicinal plants are the only source to prevent and treat various diseases. Herbal product such as *Ashwagandha*, *Mimosa*, *Aloe vera* etc. has been used by some researchers for PCOS⁶. In this study we intend to study the effect of herbal plant extract of *Tephrosia purpurea* on the regression of PCOS and thus regularize the reproductive cycle of the female rats. *Tephrosia purpurea* is a wild plant known as Sarapunkha in Sanskrit, Purple Tephrosia or wild indigo in English and Aurvi or Kolinji in Tamil. *Tephrosia purpurea* (Linn) Pers. has been used for centuries in Indian traditional medicine for the treatment of various inflammatory disorders. It is beneficial for liver spleen and kidney disorder. Also it has the property to address recovery from different types of wound^{7,8}. Experimental studies suggest that *Tephrosia purpurea* (Linn) Pers. extracts have antiulcer and antitumor promoting effect^{9,10}.

To our best knowledge there is no scientific report on effect of seed powder of *Tephrosia purpurea* (Linn) Pers. on chemically induced PCOS in Albino Wistar rats. Present study is designed to evaluate the effect of seed powder of *Tephrosia purpurea* (Linn) Pers. along with milk in modifying chemically induced PCOS in rat. Milk is used as a vehicle for plant based drug because, according to the ayurveda any medicine given with milk nullifies the effect, increases the potential and the medicine works wonders in the given disease¹¹.

Objective: Medicinal plants form main source of health care due to better acceptability and fewer side effects. Herbal plants have been used since centuries to correct disorders caused by the hormonal imbalance related to female reproductive system¹¹⁻¹³. Current research work is to investigate the effect of seed powder of *Tephrosia purpurea* (Linn) Pers. in treatment of letrozole induced PCOS. The current research work focuses on normalization of estrous cycle in PCOS after treatment with *Tephrosia purpurea* (Linn) Pers.

Material and Methods

Materials required for experimental purpose are chemicals like letrozole, 1% CMC (Carboxymethylcellulose) and giemsa stain. Model animal were female Albino Wistar rat 150-200gm weight were purchased from Haffkins institute, Mumbai, India and maintained at N. B. Mehta Science College Bordi. The animals were divided into 4 groups, each group of 5 animals in polypropylene cage with rice husk bedding and provided standard pellet diet and water *ad libitum* and maintained under normal condition. Plant material *Tephrosia purpurea* (Linn)

Pers. was collected from Aswali region, Bordi, Maharashtra, India. Authenticated by Department of Botany, St Xavier's college Mumbai, India. After collection of the seeds they were dried under normal condition to maintain its active principle and secondary metabolites present in it. Seeds were ground using mixer grinder these powdered seeds were stored in air tight container. Dried seed powder of plant was used for experimental purpose. And was given with milk as a vehicle using force feeding needle.

Experimental protocol: Albino wistar female rats of 150-200gm were divided into 4 groups. Group I- A and B, group II - A and B, group III and IV, five rats per group. Group I- letrozole + natural recovery, group II- letrozole + plant drug, group III- letrozole control and group IV-normal control table-1. All rats were acclimatized for 14 days to laboratory conditions before starting with experiment. Group I, II and III were administrated 1mg/kg body weight letrozole with 1 % CMC 2mg/kg body weight and group IV animal were given 1% CMC (Carboxy methyl cellulose) for 28 days¹⁴. During these periods vaginal smears were collected daily for estrous cycle determination table-3 and figure-2, 3. On the day subsequent to last dose of administration, five rats each of group III and IV were sacrificed for evaluation of reproductive system status. Group I there were ten animals all were kept for natural recovery for at least 15 days, examining their estrous cycle whether it has return to normalcy. Out of ten rats, five rats were sacrificed for further evaluating whether PCOS reverts naturally? The remaining 5 were kept for mating with male rat to check its fertility. In group II, there were ten animals, after induction of PCOS by letrozole rats were treated with plant drug i.e. seed powder of *Tephrosia purpurea* (Linn) Pers. 0.2gm/kg body weight with milk 3ml/kg body weight for at least 3 consecutive estrous cycles i.e. 15 days. During this period vaginal smear were collected daily for estrous cycle

determination, table-4 and figure-4. On 15th day after plant drug treatment five rats were sacrificed for further evaluation and other five rats were kept with male for mating.

Examination of estrous cycle: A cotton bud dipped in normal saline was inserted gently in the vaginal opening of the female rats and a swab was obtained. The cotton bud was rolled on a clean grease free slide to make a smear and allowed to air dry. Few drops of methanol were added to fix the cells in the smear. The slide was air dried. Giemsa stain was added to the slide to cover the smear. The slide was kept covered in petridish for 5 minutes. After 5 minutes distilled water was added to the giemsa and gently rocked. A green scum appeared on top of the slide. The slide was stained for 10 minutes in dilute giemsa. The stained slide was drained and then washed in gentle stream of tap water. The washed slide was air dried and observed under the microscope in 40x objective.

Results and Discussion

Tephrosia purpurea (Linn) Pers. showed potent effect on rats estrous cycle by normalizing it, after being induced with PCOS, which was proven after comparing estrous cycle of different groups rats table-3 and figure-3. Normal estrous cycle of rat lasts for 4-5 days showing four phases in sequential order like estrous, metaestrous, diestrous and proestrous figure 2-a,b,c and d respectively. Letrozole treated rats showed irregularity in its phases, estrous cycle determination, which changed to normal sequence of estrous cycle after treating rat with plant drug *Tephrosia purpurea* (Linn) Pers table-4 and figure-4. Reproductive system and ovaries weight of group I, II, III and IV were compared table-2. Comparison of estrous cycle of all groups was done table-3 and figure- 3. After completing treatment, female rats were kept with male rats for mating and pregnancy was confirmed figure -1.



Figure-1

Photo showing delivery after inducing PCOS with Letrozole treatment and recovery with plant drug *Tephrosia purpurea*(Linn)pers

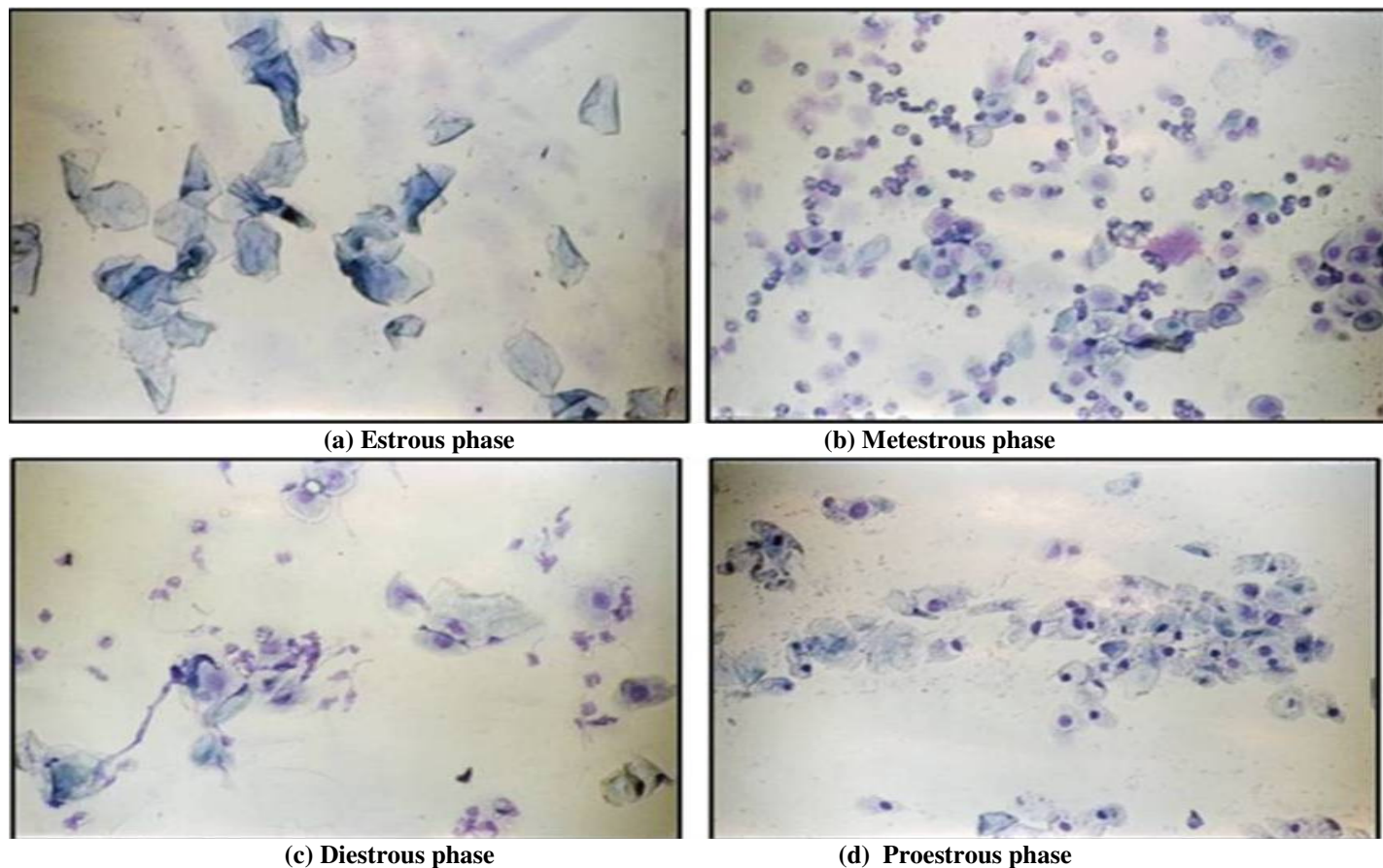


Figure-2
 Estrous cycle of rat

Table-1
 Representing protocol

Treatment	GroupIA (5rats) Letrozole +Natural recovery	GroupIB (5rats) Letrozole + Natural recovery	GroupIIA (5rats) Letrozole+ Plant drug	GroupIIB (5rats) Letrozole+ Plant drug	Group III (5rats) Letrozole Control	GroupIV (5 rats) Normal Control
Letrozole	1-28 days	1-28 days	1-28 days	1-28 days	1-28 days	-
Plantdrug (<i>Tephrosia Purpurea</i>)	-	-	29-45days	29-45days	-	-
1%CMC, Carboxy Methyl cellulose	-	-	-	-	-	1-28 days
Natural recovery	29-45days	29-45days	-	-	-	-
Sacrifice	5 rats after 45 days	-	5 rats after 45 days	-	After 28 days	After 28 days
Mating	-	5 rats	-	5 rats	-	-

Table-2
 Representing weight of rats reproductive system

Parameters	Group I	Group II	Group III	Group IV
Ovary weight/body(gm)	0.49gm	0.38gm	0.51gm	0.45gm
Weight of Female reproductive system/body (gm)	2.233gm	1.631gm	3.233gm	1.807gm

Note: Average weight of ovary and female reproductive system of 10 rats

Table-3
Comparison of estrous cycle of all groups

Days	Group I Letrozole+Natural recovery	Group II Letrozole+Plant drug	Group III Letrozole Control	Group IV Normal Control
1	Diestrous	Metaestrous	Proestrous	Estrous
2	Diestrous	Metaestrous	Proestrous	Estrous
3	Diestrous-Proestrous	Metaestrous	Proestrous	Estrous
4	Early Proestrous	Metaestrous	Proestrous	Metaestrous
5	Proestrous	Metaestrous	Estrous	Metaestrous
6	Proestrous	Metaestrous	Estrous-Metaestrous	Diestrous
7	Proestrous	Metaestrous –Diestrous	Metaestrous	Diestrous
8	Proestrous	Metaestrous-Diestrous	Metaestrous	Diestrous
9	Metaestrous	Metaestrous	Metaestrous	Proestrous
10	Metaestrous	Metaestrous	Metaestrous -Diestrous	Proestrous
11	Metaestrous -Diestrous	Metaestrous	Metaestrous -Diestrous	Estrous
12	Metaestrous -Diestrous	Metaestrous	Diestrous	Estrous
13	Metaestrous -Diestrous	Metaestrous	Diestrous	Metaestrous
14	Metaestrous	Metaestrous	Diestrous	Metaestrous
15	Diestrous	Metaestrous	Diestrous	Diestrous
16	Diestrous	Diestrous	Diestrous-Proestrous	Diestrous
17	Diestrous	Diestrous	Diestrous -Proestrous	Diestrous
18	Diestrous	Diestrous –Proestrous	Diestrous -Proestrous	Proestrous
19	Diestrous	Metaestrous	Proestrous	Proestrous
20	Diestrous	Metaestrous	Proestrous	Estrous
21	Diestrous-Proestrous	Metaestrous	Proestrous	Estrous
22	Diestrous -Proestrous	Metaestrous	Proestrous	Metaestrous
23	Diestrous -Proestrous	Metaestrous	Metaestrous	Metaestrous
24	Proestrous	Metaestrous	Metaestrous	Diestrous
25	Proestrous	Metaestrous	Metaestrous	Diestrous
26	Proestrous	Metaestrous-Diestrous	Metaestrous	Diestrous
27	Metaestrous	Metaestrous-Diestrous	Metaestrous	Proestrous
28	Metaestrous	Metaestrous-Diestrous	Metaestrous -Diestrous	Proestrous

Table-4
Comparison of estrous cycle of Group I and Group II

Days	Group I	Group II
29	Metaestrous	Diestrous
30	Metaestrous	Diestrous
31	Metaestrous	Proestrous
32	Metaestrous	Proestrous
33	Diestrous	Estrous
34	Diestrous	Metaestrous
35	Diestrous	Diestrous
36	Diestrous	Diestrous
37	Diestrous	Diestrous
38	Proestrous	Proestrous
39	Proestrous	Estrous
40	Proestrous	Metaestrous
41	Metaestrous	Metaestrous
42	Metaestrous	Diestrous
43	Metaestrous	Diestrous
44	Diestrous	Diestrous
45	Diestrous	Proestrous

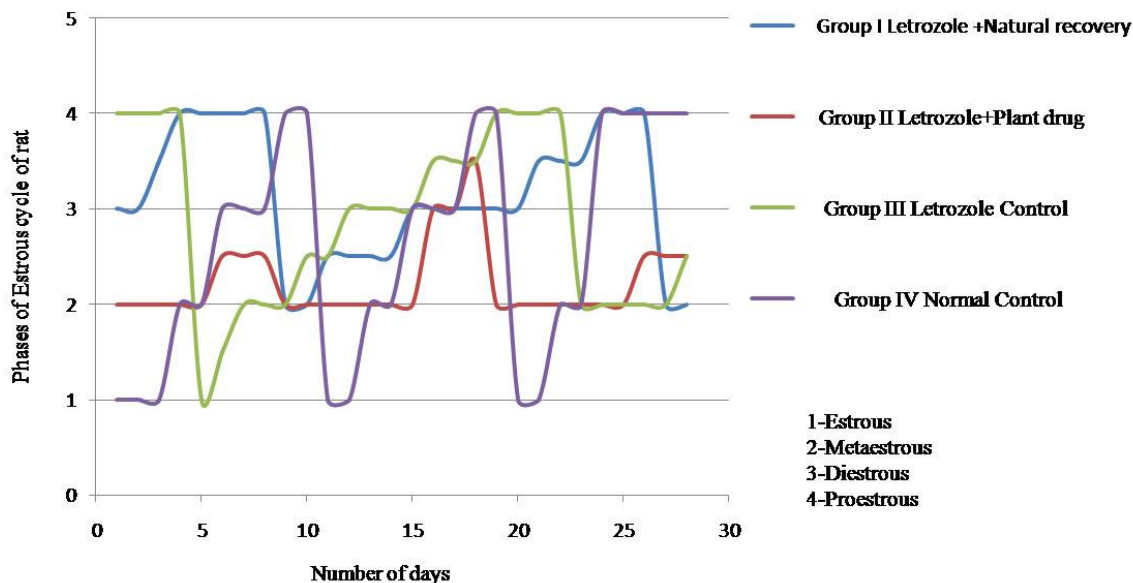


Figure-3
 Representing Phases of estrous cycle of all groups

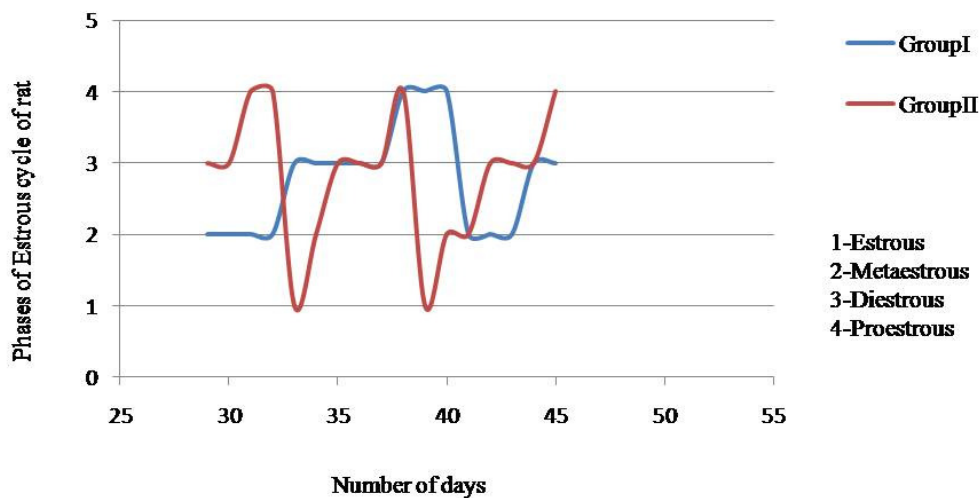


Figure-4
 Representing estrous cycle of group I and group II

Conclusion

Normal rats have an estrous cycle of 4-5 days. First phase is estrous lasts 9-15hrs vaginal smear shows presence of cornified epithelial cells figure- 2a, second phase metaestrous lasts 10-14hrs vascularity of the uterus is lost and vaginal smear shows leucocytes along with cornified cells figure-2b, smear shows the presence of only leucocyte, third phase diestrous lasts 60-70hrs phase figure-2c and fourth phase proestrous last for 12 hrs which is a preparatory stage for the next estrous¹⁵ figure- 2d. Estrous cycle of the rats was disturbed when they were in polycystic ovary condition irregular cycles were preceded by

persistent vaginal cornification (PVC). The estrous cycle was restoring to 80% normalcy in the animals treated with the seed powder of *Tephrosia purpurea* (Linn) Pers. Thus Seeds of *Tephrosia purpurea* (Linn) Pers. has potential effect on PCOS bringing the reproductive cycle of the rats to normalcy. Also the reproductive system and ovary weight of normal rats increased after letrozole treatment which normalizes after plant drug treatment table-2. Herbal remedies are also commonly used for self-medication¹⁶. Further studies of *Tephrosia purpurea* (Linn) Pers. need to be carried out to check other related parameters of PCOS .

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