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Normalizing of estrous cycle in polycystic ovary syndrome (PCOS) induced rats with *Tephrosia purpurea* (Linn.) Pers.

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Abstract: This study evaluates the potentiality of an herbal plant *Tephrosia purpurea* (Linn.) Pers. in the treatment of Letrozole induced polycystic ovary syndrome (PCOS) using female *Albino wistar* rats. Seed powder of *T. purpurea* at concentration of 200 mg/kg body weight with milk as a carrier vehicle 3ml/kg body weight, given for at least 3 consecutive estrous cycles. Vaginal smear, FSH, LH, Testosterone, and Estrogen were analyzed to determine the fluctuations in sex steroid level in PCOS induced rats. The plasma testosterone and estrogen level were found to be significantly increased in rats with PCOS whereas Follicle stimulating hormone and Leutinizing hormone did not show any changes. When compared with control the PCOS induced rats showed characteristic ovary with high incidence of ovarian cyst. All the parameters assessed were significantly improved after the treatment with *T. purpurea* and achieved level close to 80% normalcy. Effect of *T. purpurea* significantly reduced histopathological changes in ovary and endocrinological and biochemical changes induced by hyperandrogenism. Further to check ovulation and fertility female rats were mated and pregnancy was confirmed. Thus potential of *T. purpurea* in the treatment of PCOS using an animal model suggested being a good alternative therapy in the treatment of PCOS.

Keywords: Carboxymethylecellulose, Estrogen, Estrous cycle, Letrozole, Polycystic ovary syndrome, Testosterone, *Tephrosia purpurea*

INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine disorder characterized by anovulation, amenorrhea, hirsutism and infertility. It is also known as stein Levithel syndrome (Shearman, 1985; Speroff *et al.*, 1999; Fallon, 2006). PCOS is the most common disorder in women of reproductive age group as well as premenopausal women (Janssen *et al.*, 2004; Zarger *et al.*, 2005). PCOS treatment is directed to the ovary for normalizing its functions. Medications are used to regulate the menstrual cycles to stimulate ovulation and normalize hormonal level. As different drugs used in treatment of PCOS cater to different symptoms, effective treatment to manage PCOS is a challenge. In spite of tremendous progress in the development of modern medicine, plants continue to be an important source of drug for the treatment of several diseases and hence demands for plant drug have increased (Pandey *et al.*, 2003). To a large worldly 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 (Craig, 1999).

Herbal plant drug form main source of health care due to more effectiveness, lower cost and well tolerated by the patient having fewer unintended consequences and fewer side effects than traditional medicine and may be safer to use. Herbal plants have been used since centuries to correct disorders caused by the hormonal imbalance related to female reproductive system (Nadkarni, 1982**;** Shearman, 1985; Khare, 2004). *Tephrosia purpurea* (Linn) Pers. is a wild plant known as Sarapunkha in Sanskrit, Purple Tephrosia or wild indigo in English and Aurvi or Kolinji in Tamil (Nadkarni, 1982). *T. purpurea* has been used for centuries in Indian traditional medicine. This plant found in Bordi, Aswali region and it is used by the tribal community for treating female reproductive disorder, dog bite, as a fodder of cattle and various inflammatory disorders. It is beneficial for liver spleen and kidney disorder (Deshpande *et al.*, 1968; Saleem *et al.*, 1999). Also it has the property to address recovery from different wounds. (Hans *et al.*, 2000; Joshi and Oleacea, 2000) and there is no scientific report on effect of seed powder of *T. purpurea* on chemically induced (Letrozole) PCOS in Albino Wistar rats. Keeping this in view, the present study was designed to evaluate the effect of seed powder of *T. purpurea* along with milk in modifying, letrozole induced PCOS in rat.

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Table 1. Comparison of estrous cycle of different treatment groups of albino rat.

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

MATERIALS AND METHODS

Collection of plant material: *Tephrosia purpurea* (Linn.) Pers. was collected from Barta mountain of Aswali region, Bordi, Maharashtra, India and was authenticated by blatter Herbarium, St Xavier's College, Mumbai, India. After collection the seeds were dried under room temperature 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 using force feeding needle.

Experimental design: Animal model, female *Albino wistar* rats weighing 150-200gm body weight were

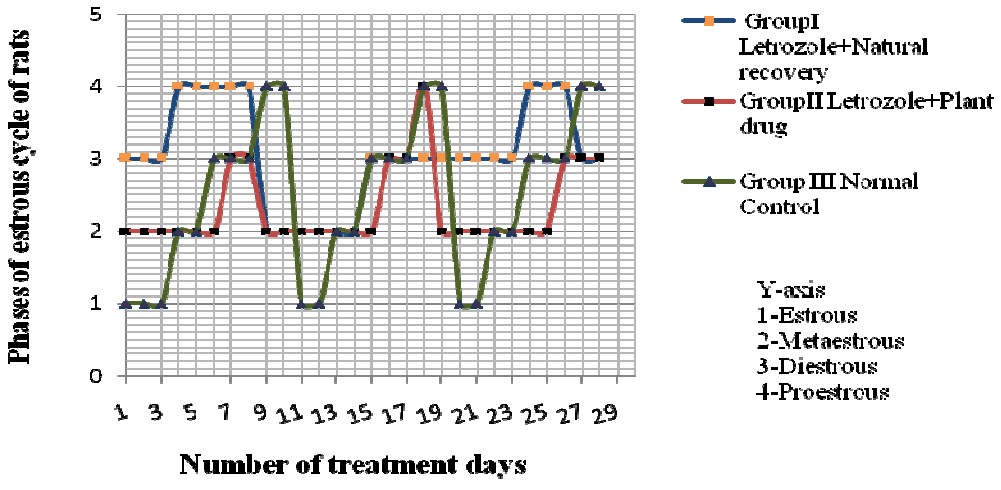

Fig.1. *Phases of estrous cycle in female albino rats at different days of treatment.*

Table 2. 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
46	Diestrous	Estrous
47	Proestrous	Metaestrous
48	Proestrous	Metaestrous
49	Estrous	Diestrous
50	Metaestrous	Diestrous
51	Metaestrous	Diestrous
52	Metaestrous	Proestrous
53	Diestrous	Estrous
54	Diestrous	Metaestrous
55	Diestrous	Metaestrous
56	Proestrous	Diestrous
57	Proestrous	Diestrous
58	Estrous	Diestrous
59	Metaestrous	Proestrous
60	Metaestrous	Estrous
61	Diestrous	Metaestrous
62	Diestrous	Metaestrous
63	Proestrous	Diestrous
64	Estrous	Diestrous
65	Metaestrous	Diestrous
67	Metaestrous	Proestrous
68	Diestrous	Estrous
69	Diestrous	Metaestrous
70	Diestrous	Metaestrous
71	Proestrous	Diestrous

purchased from Haffkins Institute, Mumbai, India. Maintained at N.B. Mehta Science College, Bordi. The animals were kept in polypropylene cage with rice husk bedding and provided standard pellet diet and water *ad libitum* and maintained under normal condition. The rats were divided into 3 groups. Group I and II contained 15 rats per group and group III contained 4 rats and named as group I- letrozole followed by natural recovery, group II- letrozole +Plant drug and group III was considered as control. All rats were acclimatized for 14 days to laboratory conditions before commencement of experiment. Group I and II rats were administrated 1mg/kg body weight Letrozole with 1 % CMC 2mg/kg body weight and group III rats were given 1% CMC (Carboxymethyl cellulose) for 28 days (Kafali *et al.,* 2004). During this period vaginal smears were collected daily for estrous cycle determination. On the day subsequent to last dose of letrozole administration, 3 rats of group I and 4 rats of group III were sacrificed for evaluation of reproductive system weight and hormonal level. Group I there were 15 animals, 12 were kept for natural recovery, and examining their estrous cycle. Out of 12, 3 rats were sacrificed at interval of 15 days for further evaluating whether PCOS reverts naturally up to 71days. The remaining 3 were kept for mating to check its fertility. In group II, there were 15 animals, after induction of PCOS by letrozole rats were treated with plant drug i.e. seed powder of *T. purpurea* 0.2gm/kg body weight with milk 3ml/kg body weight from 29th day onwards for at least 3 consecutive estrous cycles (i.e. up to 43rdday). During this period vaginal smear were collected daily for estrous cycle determination. On the day subsequent to last dose of plant drug *T. purpurea* 3 rats were sacrificed for further evaluation and out of 12, 3 rats were sacrificed at interval of 15 days for evaluating their percentage of normalcy in hormonal level up to 71 days. Remaining 6 rats were kept for mating.

For biochemical analysis food was discontinued 12-15 hours prior to sacrifice for each group,but water was provided ad libitum Animal were sacrificed as per the experimental design, with overdose of ether. The blood sample was collected by puncture, in preheparinised eppendrof tubes using preheparinised syringes. The eppendrof were centrifuged at 4000 rpm and plasma was separated. This plasma was used for estimation of hormonal essay.

Plasma Testosterone, LH and FSH were estimated by competitive chemiluminescent immunoassay using automated instrument ADIVA Centaur, Bayer Diagnostic Europe Limited for TSTO, LH and FSH kit respectively (Yilmaz *et al.,* 2001). Plasma Estrogen was estimated by chemiluminescent micro particle immunoassay using E2-kit (Taieb *et al*., 2007).

RESULTS AND DISCUSSION

After induction of PCOS with letrozole (Group I and II), rats showed irregularity in their estrous cycle, compared to normal rats (Group III control) when studied up to 29th day (Table 1, Fig. I). Letrozole treated PCOS induced rats (Group I) showed irregularity in its estrous cycle compared to (group II), when allowed for natural recovery (Table 2, Fig. 2) *T. purpurea* treated PCOS induced rats (Group II) showed reduced ovary and reproductive system weight (Table 3) and decrease level of hormone testosterone and estrogen (Table 4), when compared with control (Group III) and naturally recovered rats (Group I).Similar findings were obtained for testosterone and ovary weight using *Mimosa pudica* (Jadhav *et al.,*

Table 3. Ovary and reproductive system weight of female albino rat. (Average of three replicates).

Parameters	Group I	Group II	Group III
Ovary weight/body (gm)	0.49gm	0.38gm	0.45gm
Weight of female reproductive system/body (gm)	2.233gm	1.631gm	1.807gm
Total no of matured follicles	1	1	5

Table 4. Hormonal level of different treatment groups of female albino rat.

Day	parameters	Group I (15rats) Letrozole + Natural Recovery	Group II(15rats) Letrozole + Plant drug treatment	Group III(4rats) Normal Control
29th	Testosterone(ng/dl)	253.55 ±4.04	-	38.07±1.16
	Estrogen(pg/ml)	1297.88±1.16	-	110.21±1.01
	FSH(mlU/ml)	>0.3	>0.3	>0.3
	LH(mlU/ml)	>0.07	>0.07	>0.07
43rd	Testosterone(ng/dl)	180.03±1.57	140.05±0.55	-
	Estrogen(pg/ml)	627.57 ±4.91	325.07±5.33	-
57th	Testosterone(ng/dl)	160.45±5.77	129.08±0.95	-
	Estrogen(pg/ml)	431.65±6.79	297.21±1.16	-
71st	Testosterone(ng/dl)	147.33±2.07	97.07±0.92	-
	Estrogen(pg/ml)	398.07±1.01	255.77±0.70	-

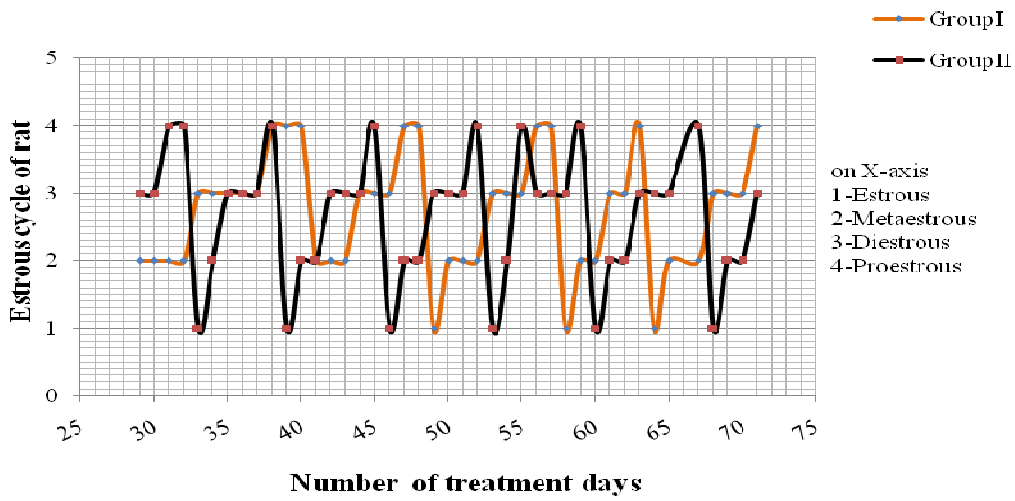

Fig.2. *Estrous cycle in female albino rat at different days of treatment.*

2013). Hormone LH and FSH did not show any drastic changes after plant drug treatment as compared to letrozole treated rats (Table 4) probably because these hormones are secreted from pituitary gland and plant which was used targeted on ovarian hormones. After completing treatment female rats kept with male rats for mating and pregnancy was confirmed.

Conclusion

The seeds of *T. purpurea* had potential effect on PCOS bringing the reproductive cycle of the rats to normalcy. It was concluded that the estrous cycle of the rats was disturbed when they were in polycystic ovary condition. The irregular cycles were preceded by persistent vaginal cornification (PVC). The estrous cycle restored to 80% normalcy in the animals treated with the seed powder of *T. purpurea*. Letrozole also led to increase production of testosterone which improved after the plant drug treatment.

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