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EFFECT OF METFORMIN AND *PERGULARIA DAEMIA* EXTRACT ON HORMONAL CHANGES OF PCOS INDUCED WISTAR RATS

R. Poornima, Horne Iona Averal*

P.G and Research Department of Zoology, Holy Cross College (Autonomous), Trichy, India.

ABSTRACT: This work focussed about the combined effect of methanolic extracts of *Pergularia daemia* with Metformin drug in the recovery of hormone levels in letrozole induced PCOS in female albino Wistar rats. Testosterone hormone level increased in PCOS induced group (Group II). In Metformin (Group III) and plant extract treated groups (Group V) the levels were also high whereas in Metformin+Plant extract treated group (Group IV) the testosterone level was very close to the control group. Group II, III and IV showed reduced FSH hormone level than Group I whereas Group IV showed significant ($P<0.01$) results to the control group. LH hormone levels were increased in Group II and twice levels in Group III and also found increased in Group V than compared to the control group. Estradiol and Progesterone levels revealed Group II, III and V showed significantly increased levels of estradiol than Group I whereas Metformin+plant extract (Group IV) showed similar results to control group. Group II showed three different patterns (Metestrous, Diestrous and Proestrous) due to their PCOS condition lack of estrous stage. Group III showed all the four patterns due to their treatment with Metformin, similarly in Group V also showed all the four patterns due to their plant extract treatment. Group IV showed intermediate results as all the four patterns of cycle. These results concluded that Metformin+Plant extract group results showed significant results with the control. Metformin+Plant extract treated recovers the hormone levels and estrous cycle patterns in female albino Wistar rats.

KEYWORDS: PCOS, *Pergularia daemia,* Metformin, Letrozole, Hormones.

Corresponding Author: Dr. Horne Iona Averal*

Dean of Science and Associate Professor, P.G and Research Department of Zoology,

Holy Cross College (Autonomous), Trichy, India.

Email Address:profionahorne@gmail.com

1.INTRODUCTION

PCOS women have abnormalities in the androgen, estrogen metabolism and uncontrolled androgen production. They also showed high serum concentrations of androgenic hormones like testosterone, androstenedione and Dehydroepiandrosterone sulfate. Excess of adrenal androgen production during stress or adolescence and enzyme defect in congenital adrenal hyperplasia initiate the cycle of abnormal LH/ FSH stimulation which lead to the clinical condition of PCOS [1].Hormonal changes related with PCOS are due to increased level of testosterone, androgen, luteinizing hormone (LH), Follicle stimulating hormone (FSH) and insulin. PCOS is arising due to the excessive release of ovarian androgen and in most patients the adrenal glands also contribute to the hyperandrogenemia. The adrenal cortex produces dehydroepiandrosterone sulfate (DHEAS), a metabolite of the adrenal androgen (AA) dehydroepiandrosterone (DHEA), as a marker of AA production. Studies showed that 25% of PCOS women had supranormal DHEAS levels and it resulted in upward raise in most patients with this disorder [2]. The use of metformin for PCOS was first reported in a study conducted at the University of the Andes, Venezuela in 1994[3]. Metformin is thought to have primary effects on increasing peripheral glucose uptake in response to insulin, perhaps at the post receptor level, with some reduction in basal hepatic glucose production[4, 5].Metformin used for the treatment of PCOS had improved the insulin metabolism and reduced the androgen concentration in the serum which was increased due to PCOS and normalization of menstrual cycle. Metformin can also be used as the first line of treatment for ovulation induction and decreases the infertility rate. Both Metformin alone or in combination with clomiphene citrate had been used in the treatment of PCOS and induction of ovulation thereby increases the pregnancy in women with PCOS [6]. It improves fertilization and pregnancy rates in PCOS women those who undergoing *in vitro* fertilization [7].Pushpa and Kalavathy[8] evaluated the effect of Mehani (50 mg/100gm b.wt.) in Wistar rats induced with PCOS and treated with Testosterone+ Metformin and Testosterone+ Mehani. The hormone levels such as LH, FSH, insulin, testosterone, estradiol and progesterone analyzed on treated groups revealed normalization of all the hormones to that of control which indicated Mehani along with Metformin had a curative effect on PCOS treatment. This study focussed to analyze the hormonal changes using the estimation of Lutenizing hormone (LH), Follicle stimulating hormone (FSH), Testosterone, Estradiol, Progesterone levels in the treatment groups and also to determine the stage of estrous cycle by microscopic analysis.Infertility is the failure to pregnancy after 12 months. Female infertility is due to ovulatory problems with absence of menstrual periods [9]. The most common causes of infertility include PCOS, blockage of fallopian tube, tuberculosis, maternal age related problems, problems of uterus and immune fertility [10]. Stein and Leventhal [11] described the association between PCOS and infertility which is caused by anovulation in women with PCOS from 35% to 94% [12]. After infertility treatment women with PCOS have children as normal healthy women [13]. Certain studies on PCOS explained

increased rate of miscarriage due to high of concentration of LH in high follicular phase inducing harmful effects on conception and miscarriage [14, 15]. The works of Bhattacharya *et al*. [16] showed a positive correlation between prevalence of PCOS with high levels of testosterone and LH: FSH ratio.

2. MATERIALS AND METHODS

2.1. Experimental animal and Treatment

Female Albino Wistar Rats are procured from King's Institute of Preventive Medicine and Research, Guindy, Chennai (India) acclimatized in Holy Cross College animal house in polypropylene cages (8 × 12× 8 inch) with a steel grid on the top for fifteen days prior to the experiment with water and feed at *ad libitum.* Metformin and Letrozole were purchased from the Sigma Aldrich Company. The methanolic leaf extracts of the *Pergularia daemia,* used for this study.

2.2. Hormonal studies

Five groups (n=6) such as Control group (Group I), PCOS induced group (Group II), Metformin treated group (Group III), Metformin +Plant leaf extract treated group (Group IV) and Plant leaf extract treated group (Group V) were maintained. After 15 days, the Wistar rats blood collected by intravenous method used for hormonal analysis**.**The hormonal estimation were done by kit method and their procedures for Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Testosterone, Estradiol (E2) and Progesterone (P) by Uotila*et al.*, [17], Marshall [18], Tietz [19], Ratcliffe*et al.*, [20] and Csapo*et al.*, [21] respectively.

2.3. Estrous Cycle

Rats vaginal secretion collected every morning (8-9 am for 36 days) using cotton tipped swabs softened with a drop of saline. The swabs gently rotated and withdrawn and the vaginal smears prepared immediately after withdrawal on a glass slide. Air dried slides added with 3-4 drop of methanol (10 minutes) and then stained by methylene blue (3 drops) for 15 minutes. Rinsed in tap water and examined under microscope. The stage of cyclicity was determined by microscopic analysis of the predominant cell type in vaginal smears obtained daily in the experiment [22].

2.4. Statistical Analysis

Data were represented in Mean±Standard Deviation (SD). One way Analysis of Variance (ANOVA) were used to test the significance between the control and the treated groups by SPSS (17.0 version) tool.

3. RESULTS AND DISCUSSION

The FSH hormone levels in Group II, III and IV showed significant reduction as 9.26±0.41, 10.36±0.61 & 11.13±0.66mIU/ml respectively, than Group I (13.21±0.56mIU/ml). Group IV has 12.1±0.65mIU/ml levels of FSH which is significant (F= 40.379; P<0.01) to the control values (Figure 1, Table 1). LH hormone levels were increased as three times in Group II (39.06±4.97mIU/ml) and twice levels in Group III (27.54±1.75mIU/ml) and also found increased in Group V (19.2±2.53mIU/ml) than compared to the control group (12.88±0.76mIU/ml). But in Group IV (13.86±1.00mIU/ml) showed very close results (F=100.029, P<0.01) to the control group (Figure 2).During the menstrual cycle the sharp increase in serum levels of estradiol in the proliferative phase, which occurs due to the recruitment of ovarian follicles, stimulates the release of luteinizing hormone (LH) from the pituitary gland, which culminates with ovulation. However, if serum levels remain constant, LH is not released [23]. The *Cimicifugaracemosa*extract on PCOS rats produced significantly reduced LH level and also LH/FSH ratio is brought down, which in turn increases the circulating FSH level and decreases the androgen level elevated during PCOS condition, hence improved the follicular growth, thickening of endometrium, ovulation and increased the pregnancy and implantation rate [24].Similarly in Estradiol analysis, Group II (62.92±2.13pg/ml), Group III (57.05±2.02pg/ml) and Group V (51.43±0.75pg/ml) showed significantly (F=98.016) increased levels of estradiol that Group I (47.41±1.60pg/ml) whereas Metformin+plant leaf extract (49.08±0.77pg/ml) showed nearby results to control group (Figure 3). Progesterone levels found decreased in PCOS induced group (3.40±0.53ng/ml) than control group (7.98±0.36ng/ml). The levels were retrieved in the other three treated groups (Group III, IV and V) as 7.11±0.21, 8.06±0.28 and 8.00±0.14ng/ml (Figure 4). Soumya*et al.* [25] reported the effect of flower extract of *Cocusnucifera*in reducing Letrozole-induced PCOD condition in female rats. The C. *nucifera*flower extract treated groups showed normalized estrous cyclicity and increased blood glucose level, normalized lipid profile, balanced antioxidant status and they also brought down the LH and FSH levels which lead to recovery of ovaries of polycystic condition. Sushma Reddy *et al.*[26] demonstrated the beneficial effect of curcumin extracted from *Curcuma longa* in Letrozole induced polycystic ovary syndrome in female Wistar rats. The ovary of PCOS rats revealed multiple subcapsular cysts, absence of granulose layer and corpus luteum with the indication of anovulation. In control group (Group I), the testosterone hormone levels (Figure 5) found as 93.04±2.82ng/ml and it was nearly 150% increased in PCOS induced group (Group II) as 257.03±10.65ng/ml. In Metformin (Group III) and plant leaf extract treated groups (Group V) the levels were 152.26±6.29 and 116.78±3.12ng/ml respectively whereas in Metformin+Plant leaf extract treated group (Group IV) the testosterone level found as 103.05±5.47ng/ml which was very close to the control group. This result evidenced that Metfromin+Plant leaf extract group showed significant results (F=666.773; P<0.01) to the control group.

Table 1: Hormonal studies in control, PCOS induced and *Pergularia daemia* extract treated female albino Wistar rats

Experimental groups	FSH (mIU/ml)	LH (mIU/ml)	Estradiol (pg/ml)	Progesterone (ng/ml)	Testosterone(ng/ml)
Group I-Control	13.21±0.56	12.88±0.76	47.41±1.60	7.98±0.36	93.04±2.82
Group II-PCOS induced	9.26±0.41	39.06±4.97	62.92±2.13	3.40±0.53	257.03±10.65
Group III-Metformin treated	10.36±0.61	27.54±1.75	57.05±2.02	7.11±0.21	152.26±6.29
Group IV-Metformin + Plant leaf extract treated	12.1±0.65	13.86±1.00	49.08±0.77	8.06±0.28	103.05±5.47
Group V-Plant leaf extract treated	11.13±0.66	19.2±2.53	51.43±0.75	8.00±0.14	116.78±3.12

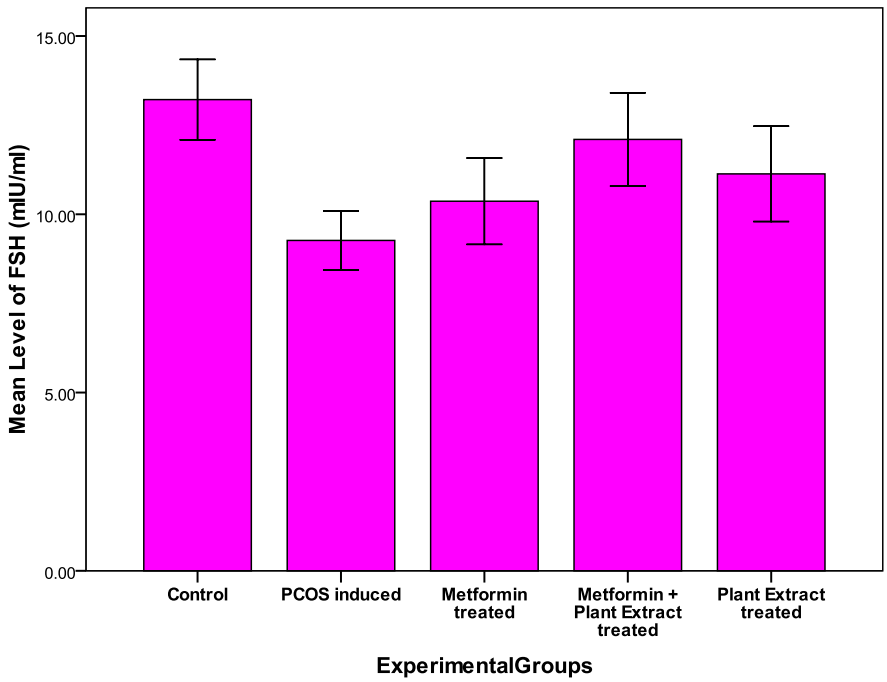


Figure 1: FSH levels in control and various groups of albino Wistar rats

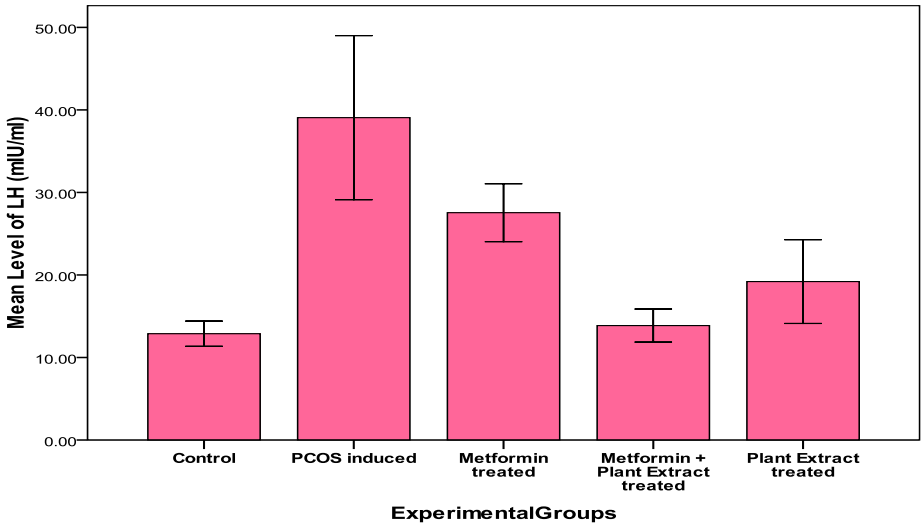


Figure 2: LH levels in control and various groups of albino Wistar rats

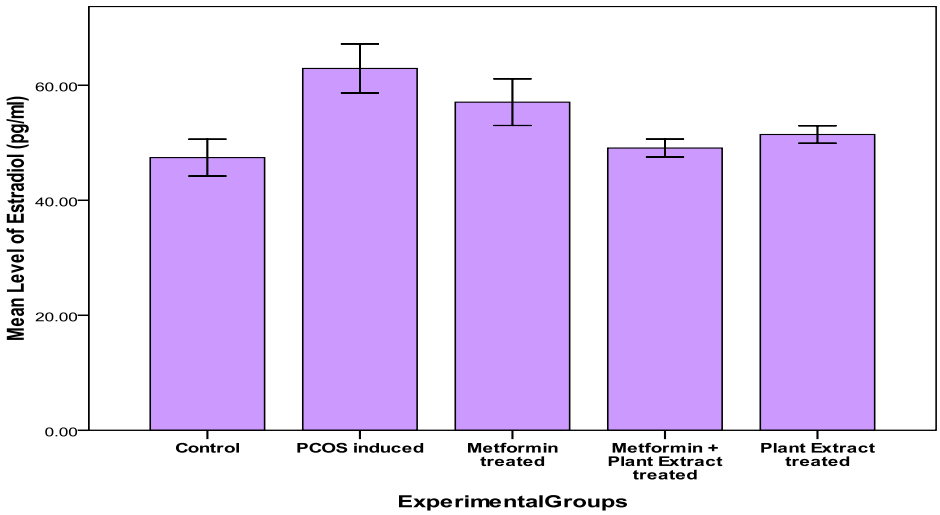


Figure 3: Estradiol levels in control and various groups of albino Wistar rats

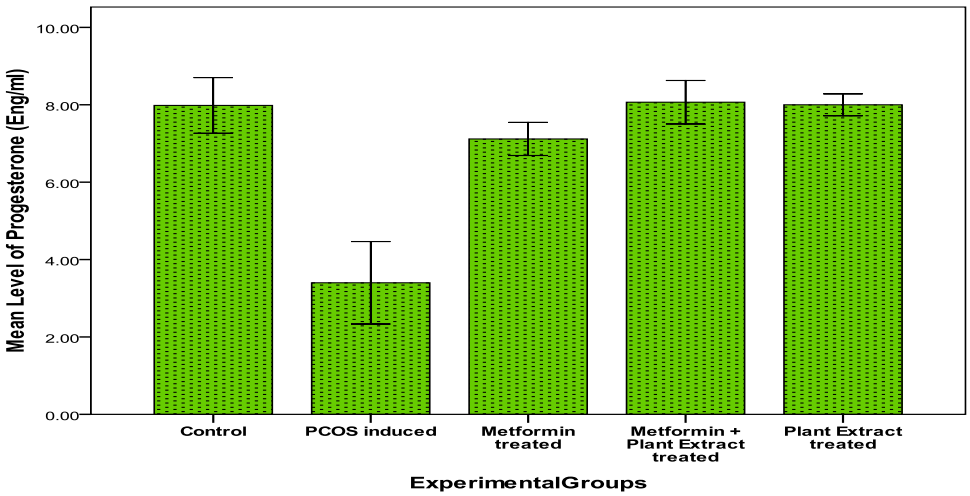


Figure 4: Progesterone levels of control and various groups of albino Wistar rats

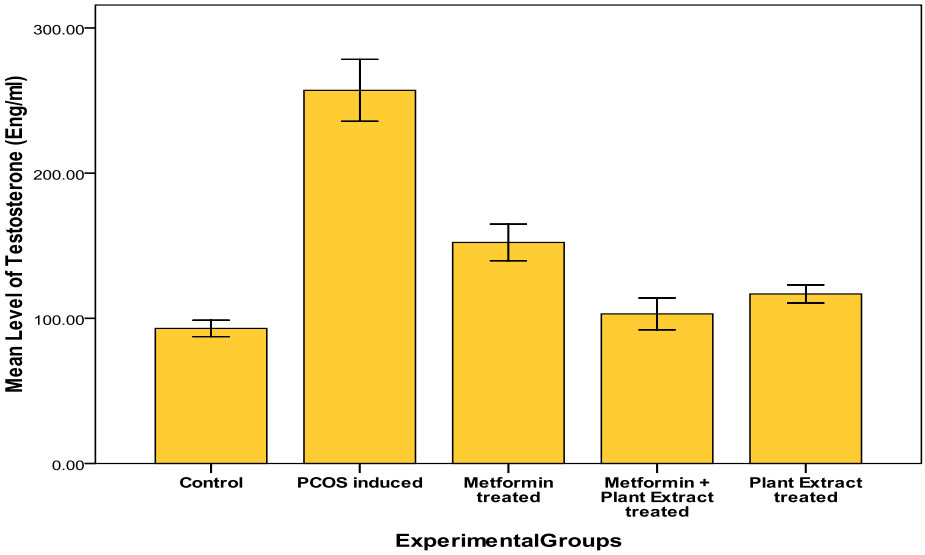
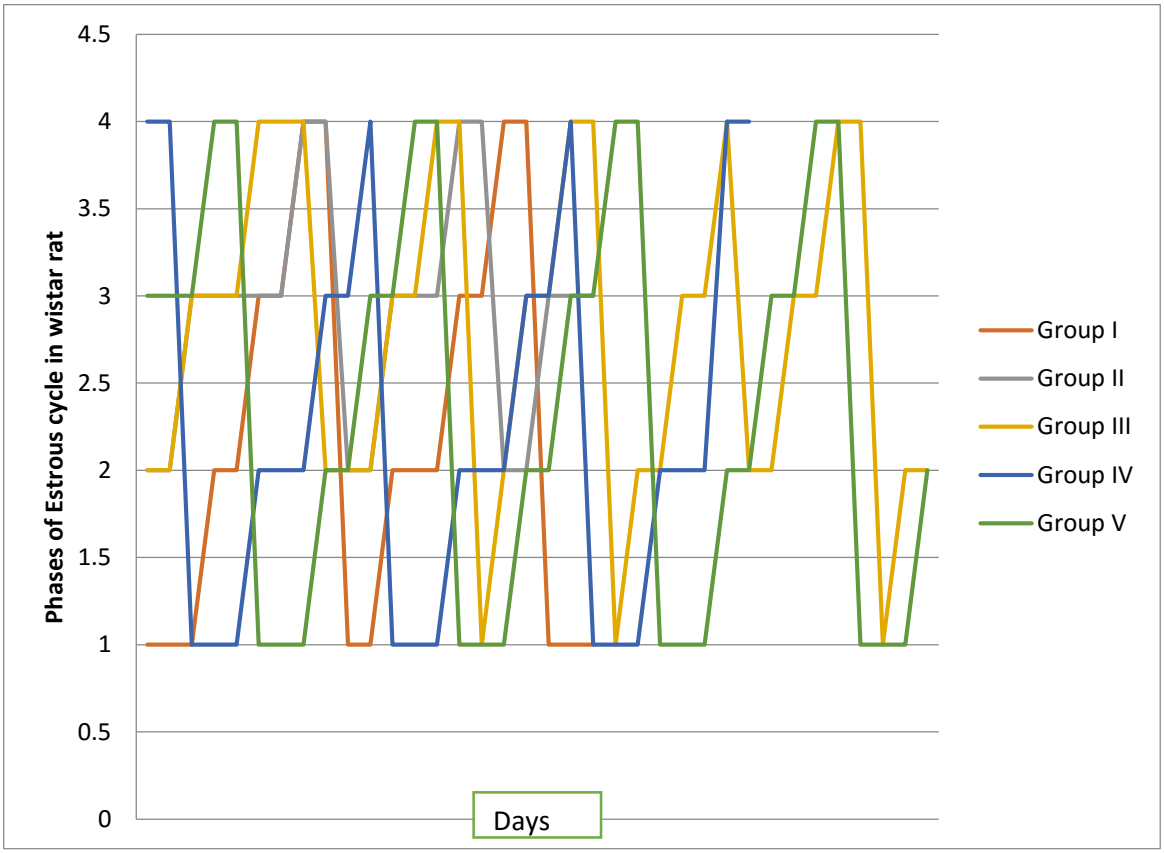


Figure 5: Testosterone levels of control and various groups of albino wistar rats

3.2. Estrous cycle

The various estrous cycle patterns were observed in Control (Group I) and other treated groups in 36 days (Figure 6). Group I showed patterns for 26 days with all the four patterns whereas in Group II also patterns observed for 26 days with three different patterns (Metestrous, Diestrous and Proestrous) due to their PCOS condition lack of estrous stage. Group III showed all the four patterns of estrous cycle upto 36 days due to their treatment with Metformin, similarly in Group V also showed all the four patterns for 36 days due to their plant leaf extract treatment. Group IV showed intermediate results as all the four patterns of cycle for 28 days.The seed powder of *Tephrosiapurpurea*(200 mg/kg b.w.)had potential effect on PCOS treatment in restoring reproductive cycle to normalcy which was disturbed in rats during PCOS condition. The irregular cycles were preceded by persistent vaginal cornification (PVC) [27]. Demirel*et al*. [28] in his experiment analyzed the relative proportion of leukocytes, epithelial and cornified cells to evaluate estrous cycle. The Letrozole induced PCOS rats showed constant estrous stage compared to irregular estrous cycle in control, while *Corylusavellana* seed (Hazelnut) oil treatment in PCOS induced rats produced significant normalization of estrous cycle.Letrozole treated PCOS rats showed reduced proestrous, estrous and metestrous phase with increased diestrous phase indicating irregular estrouscyclicity reflected by the presence of leukocytes in the vaginal smear. While the rats treated with hydroalcoholicextractof *Withaniasomnifera*and*Tribulusterrestris*for 28 days resulted in the normalization of estrous cycle in the animal groups [29].Wang *et al.,*[30] reported that experimental animal rat showed approximately 4–5 days of estrous cycle, including proestrous, estrous, metestrous, and anestrous and the average pregnancy period lasted for 21 days (19-23 days). According to the results of Brawer *et al*. [31] and Sasikala and Shamila[32], PCOS induced rats showed diminished corpora lutea, anovulation and complete absence of estrous cycle.



1=Estrous; 2=Metestrous; 3=Diestrous 4=Proestrous

Figure 6: Various estrous cycle patterns observed in 36 days in control and other treated groups

4. CONCLUSION

This work focussed about the combined effect of methanolic extracts of *Pergularia daemia* with Metformin drug in the recovery of hormone levels in letrozole induced PCOS in female albino Wistar rats. Metformin group and plant extract group showed normalcy of the hormone levels in treated rats whereas Metformin+Plant extract group results showed high significant results with the control. This proved that Metformin+Plant extract treated recovers the hormone levels and estrous cycle patterns in female albino Wistar rats.

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CONFLICT OF INTEREST

No

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