

An Observational Study of Estrous Cycle Modulation by Paathadikwath in Wistar Albino Rats with Letrozole-Induced PCOS

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a widespread medical condition that affects multiple aspects of women's overall health, with long term consequences. PCOS is characterized by chronic anovulation, polycystic ovaries, hyperandrogenism leading to symptoms like irregular menstrual cycles, infertility, acne and hirsutism. Menstrual irregularities are due to any causes and among them one is disturbance in reproductive hormones like LH, FSH and GnRh, any irregularities of these hormones in longer time will further manifest in development of PCOS. Irregularities of these hormones also make changes per vagina too. Interpretation of vaginal cytological changes which are influenced by alteration of these hormones modifying estrous cycle which is observed for evaluation of test drug PaathadiKwath in wistar albino rats with letrozole-induced PCOS in this study.

Objectives: To assess the efficacy of PaathadiKwath to regulate estrous cycle in letrozole induced PCOS in wistar albino rats.

Methods: Twenty-four healthy female Wistar Albino rats divided into four groups-control, disease control, standard drug and test drug groups consisting of 6 rats in each group. PCOS is induced in all groups with letrozole through oral gavage for 21 days except in the control group. After inducing PCOS, control and disease control groups are treated with normal saline, while standard drug and test drug groups are treated with clomiphene citrate and PaathadiKwath respectively for next twenty-one days. Vaginal smears of all the groups were taken every day and screened for changes in vaginal cytology to screen regularization of estrous cycle changes.

Results: Results of the study shows the changes of estrous cycle which begins to revert after 1-week of administration of Test drug and standard drug groups, compared to control and disease control groups. Findings of the study concludes that Test drug administered in letrozole induced PCOS, shows evidence of regulation of estrous cycle seen through vaginal smear findings.

Keywords: PaathadiKwath, Letrozole, Estrous cycle, Vaginal Smear, PCOS.

1. INTRODUCTION

Polycystic ovary syndrome is the most common endocrinopathy in women^[1]. In women of all ages, it leads to a variety of issues. It is known to harm women's metabolism, reproductive system, and mental health^[2]. It is characterised by excessive androgen production by the ovaries mainly and lower sex hormone binding globulin levels and this altered hormonal environment. Follicle stimulating hormone and luteinizing hormone (LH), which are controlled by the gonadotropin release hormone, are two important hormones that govern the menstrual cycle. The high oestrogen production is largely due to conversion of androgen to oestrogen in the ovary and peripherally. This causes an increase in LH and decrease in FSH, the LH level being 3 to 4 times the FSH level on day 2 of the cycle. Increase in LH induces thecal hyperplasia and increase in androgen synthesis leading to increased peripheral production of oestrogen and reduction in the sex hormone-binding globulin. Gradually it results in PCOS. Approximately 85–90% of women who

experience oligomenorrhea also have PCOS, which is often classified as a cycle length longer than 35 days^[3].

The symptoms and indicators to identify PCOS by Rotterdam criteria are Oligoovulation; hyperandrogenism and/or hyperandrogenemia; and polycystic ovaries on ultrasound.^[4] Overproduction of the LH hormone in PCOS causes a significant impact on cycle regulation, which is inhibited by the change in LH. These hormonal changes also impact on the vaginal cytology. Vaginal cytological variation is seen throughout the reproductive cycle. Oestrogen increases their vascularity and stimulates epithelial activity. So evaluation of sequential vaginal cytology is helpful for assessing the vaginal epithelium in response to hormones in different stages of cycles and diseases.

Many studies have been performed on PCOS using animal models because of its similarity in humans. These are used to study aetiology, clinical features, diagnosis, various treatment modalities, and insulin resistance aspects. In this study interpretation of vaginal cytological changes which are influenced by alteration in hormones modifying estrous cycle which is taken for evaluation of test drug PaathadiKwath. PCOS was induced with letrozole orally for twenty-one days in winter albino rats. PCOS is verified by vaginal cytology in wistar albino rats daily. The estrous cycle changes were observed, along with variations in the ovulation phases as indicated by the quantity of cornified cells and leukocytes in the morphology of the vaginal smear. The diestrus phase, which is primarily composed of leukocyte cells, is where the animals stay motionless in PCOS during the estrouscycle^[5,6].

Paathadikwath^[7] is mentioned in sushrutasamhita in vatakaphajartavadusti. Paathadikwath contains Patha(Cissampelospareira Linn.), Shunthi(Zingibarofficinalis Roxb.), Maricha (Pipernigrum Linn.), Pippali (Piperlongum Linn.), and Kutaja (Holarrhenaantidysenterica Roxb.ex. Fleming) in equal proportion. It has been reported, drugs of PaathadiKwath possess a wide variety of biological effects like anti-inflammatory activity, analgesics, anti-oxidant activity, hepatoprotective activity, anti-hyperglycemic activity^[7,8] anti-hyperlipidemic activity, anti-tumor activity^[9,10]

2. OBJECTIVE

To assess the efficacy of PaathadiKwath to regulate estrous cycle by vaginal smear with letrozole induced PCOS in wistar albino rats – An experimental model.

3. MATERIAL AND METHODS

The study was planned in Yashwant Ayurvedic College for Post Graduate Training & Research Centre, Kodoli, Maharashtra and carried out at Animal Experimental Laboratory CRF, KLEU's Shri B.M.Kankanwadi Ayurveda Mahavidyalaya, Shahapur, Belagavi, Karnataka after obtaining institutional and animal ethical clearances.

3.1 Experimental Source:

Female Wister Albino Rats procured from licensed breeders through Animal Experimental Laboratory CRF, KLEU's Shri B.M.Kankanwadi Ayurveda Mahavidyalaya, Shahapur, Belagavi, Karnataka.

3.2 Experimental study:

Twenty-four healthy, ten weeks old young female Wistar Albino rats weighing 150-200gms and showing a regular estrous cycle of 4-5days are randomly selected and divided into four groups consisting of 6 rats each, in each group.

3.3 Criteria's of study:

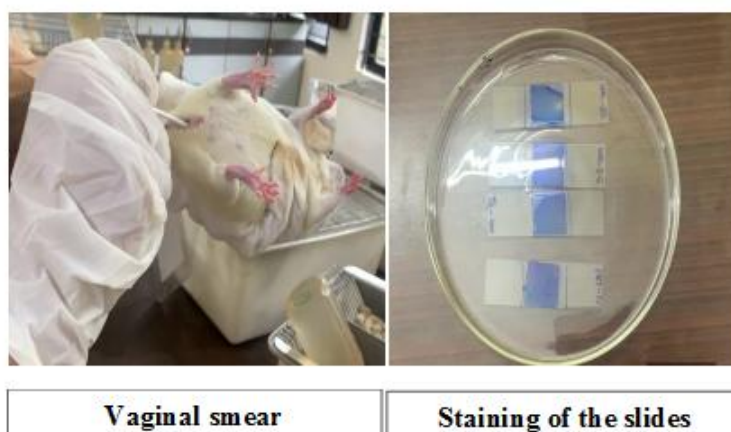
Table No: 1

Inclusion Criteria	Exclusion Criteria
Animals weighing between 150gms-200gms will be selected	Weight range below 150gms and above 200gms.
Active and healthy rats.	Rats which were used for other studies previously.
	Pregnant and disease rats

3.4 Plant material:

Paathadikwath raw ingredients were collected from GMP certified pharmacy of Shri B.M.K Ayurveda Mahavidhyala, Belagavi, Karnataka. Drug confirmation and authentication done by an expert.

Fig. no: 1 Vaginal smear procedure and slide preparation:



3.5 Vaginal smear:

Each animal was taken off the cage, a wet cotton swab was inserted into the vagina of the animal while carefully holding the animal in one hand. The wet cotton swab was gently rotated and removed out of the animal. Using the wet cotton swab, a smear was created on a clean grease-free microscope slide. The slides were air-dried and stained with methylene blue and observed under a binocular microscope at magnification of 40X to identify different stages of estrous cycle in smear.

3.6 Stages of estrous cycle:

The estrous cycle is defined as the reproductive cycle in rats and it is similar to the human menstrual cycle which involves ovarian cycle and uterine cycle. In rats it lasts for 4-5 days and has four stages namely proestrous, estrus, metestrus and diestrus. In humans, there are three phases of the menstrual cycle; menstruation, proliferative (Follicular) and secretory (Luteal). Proestrous corresponds to the follicular phase of the human menstrual cycle. estrus to ovulation phase of human menstrual cycle. metastus, diestrus stage correlates with early and late stages of the human secretory phase of the menstrual cycle^[11]. Three types of cells are found in vaginal secretions of wister albino rats, namely leucocytes, cornified epithelial cells and nucleated epithelial cells. Diagnosis of phases^[12] are based on the proportion of the cell in the vaginal secretion.

a) Proestrous Stage:

The presence of tiny, round, nucleated epithelial cells that are similar in shape amongst each other defines the proestrous. And there are also numerous in them. They are mostly nucleated epithelial cells for 8 to 12 hours and some are cornified epithelial cells.

b) Estrous Stage:

Extensive cornified epithelial cells without nuclei and some well-developed nucleated epithelial cells.

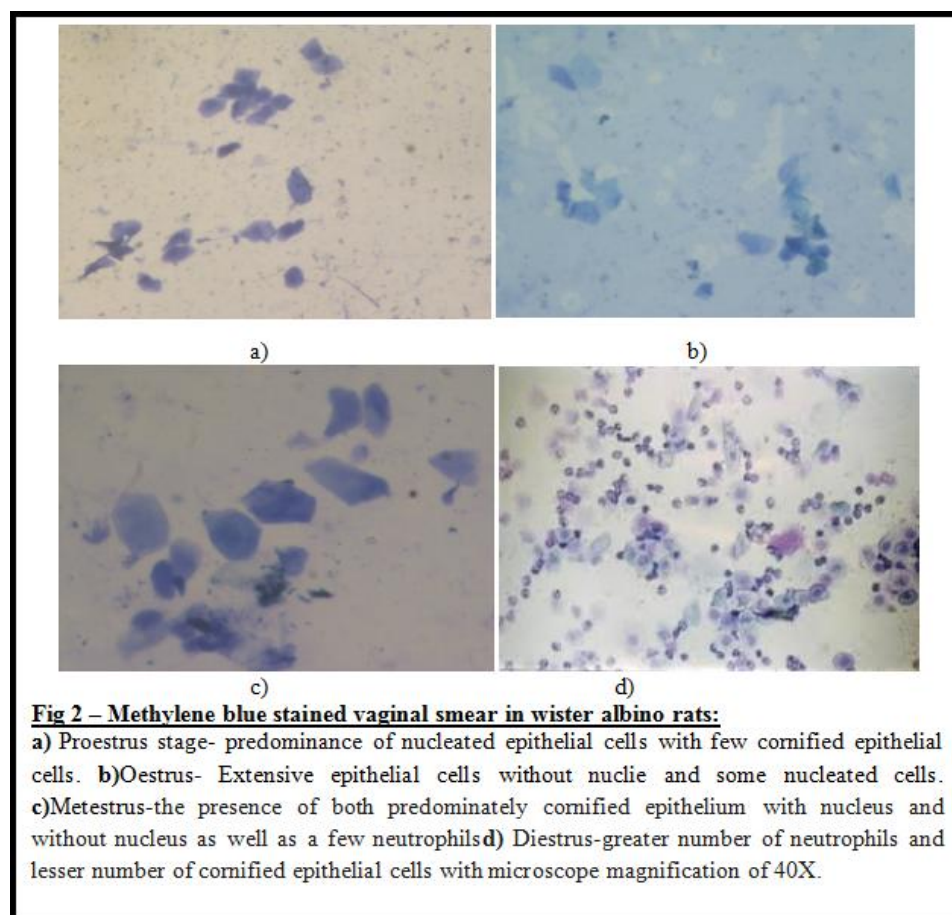
c) Metestrus Stage:

The metestrous is defined by the presence of both predominately cornified epithelium with nucleus and without nucleus as well as a few neutrophils.

d) Diestrus Stage:

Diestrous is featured by having a greater number of neutrophils and lesser number of cornified epithelial cells.

3.7 Experimental design:



4. RESULTS

Table no: II

Group 1	Control Group n=6	No Induction	Normal Saline
Group 2	Disease control Group n=6	Letrozole ^[13] in the dose of 1mg/kg/day will be dissolved in 1% CMC (carboxymethylcellulose- 2ml/kg/day) given orally for 21 days	Normal Saline
Group 3	Standard Drug Group n=6	Letrozole in the dose of 1mg/kg/day will be dissolved in 1% CMC given Orally for 21 Days	Clomiphene citrate ^[14] 1mg/kg/day for 21 days
Group 4	Test Drug Group n=6	Letrozole in the dose of 1mg/kg/day will be dissolved in 1% CMC given orally for 21 days	PaathadiKwath drug 1.8ml/200gms/divided dose (Twice/day) for 21 days

4.1 Control Group

Throughout the experiment, the cyclic changes were regular in the control group indicating proestrus, estrus, metaestrus, and diestrus phases at regular time periods.

4.2 Disease Control Group

Except for the first two cycles, the wistar albino rats stayed in the diestrus phase. However, from the 9th day onwards, the estrus cycle was delayed, and the animals remained in diestrus stage. Moreover, on the 31st and 32nd day the animals progressed to proestrus stage and on the 33rd day it changed to diestrus stage.

4.3 Test Drug Group

The estrous cycle was regular in the initial stage, and on the 10th day the animals were in the diestrus phase of the cycle. Till the 21st day the animals were induced to develop PCOS. On 26th day, the cycle changed to proestrus and remained so for 2 days, changed to diestrus and continued for two days and then the estrous cycle was regular from 30 to 42nd day.

4.4 Standard Drug Group

The estrous cycle was normal till the 12th day and continued to be stable in the diestrus phase till 22nd day. On the 28th day, proestrus phase was observed and continued for 3 days and transformed to estrus for 1 day and thereafter continued with diestrus. The estrous cycle stayed regular from 36th day to 42nd day.

5. DISCUSSION

5.1 Discussion on estrous cycle stages, letrozole and clomiphene citrate:

Proestrous, estrous, metaestrous, and diestrous are the four phases that typically make up the estrous cycle, which lasts four to five days. These stages are characterized by specific morphological changes in the vaginal epithelium, which can be accessed via vaginal cytology to identify the phase of oestrus cycle accurately.^[15,16] estrus cycle is regular reproductive cycle in mature wister albino rats. It is under control of H-P-O axis through gonadotropins. Female cyclicity is observed through vaginal changes which in turn confirms the normal functioning of the reproductive system. Any disturbance in oestrus cycle reflects changes in vagina and it indicates the disturbance in oestrogen and progesterone^[17]. Letrozole is an aromatase inhibitor that prevents testosterone converting into estradiol, thereby increasing blood and ovarian androgen levels. It induces changes similar to PCOS like anovulation thickening of theca cells in animal. The majority of research uses letrozole to cause PCOS in rat models and alter vaginal smear cytological characteristics, which show that the estrous cycle is stopped in the diestrous phase^[18]. Clomiphene citrate is selective oestrogen receptor modulator, it binds of oestrogen receptor throughout the cycle. Depletion of oestrogen receptor at level of hypothalamus and results in reduction in circulatory oestrogen. Which in turn enhances release of compensatory pulsatile gonadotrophic releasing hormone secretion and increase ovarian follicular activity.^[19]

5.2 DISCUSSION ON PAATHADIKWATH:

Paathadikwath has paatha is used to treat various ailments related to menstrual irregularities, dysmenorrhea, uterine bleeding. It acts as anti-inflammatory, anti-oxidant^[20], anti-tumor activity, antispasmodic activity, antifungal activity. Pippali has piperine, it is bio enhancer, act as anti-inflammatory, hypoglycemic, hypolipidemic properties. Maricha has 5 alpha reductase inhibitors that act as an anti-androgenic effect. Shunti acts as an anti-inflammatory, and antioxidant property. The antioxidant property of paathadikwath helps in proper maintenance of oestrogen production and progesterone regulating whole H-P-O axis. Anti-oxidant properties helps for regulating hypothalamo pituitary ovarian axis^[21], ovarian activity and helped in modulation of estrous cycle in wistar albino rats.

6. CONCLUSION

PCOS is the fastest growing health hazard amongst women of reproductive age. In many letrozole induced PCOS studies vaginal smear has shown estrous cycle arrested in diestrus phase. In present study, vaginal smear shown the regulation in estrous cycles occurred 2 days earlier in Letrozole induced PCOS in wistar albino rats treated with Paathadikwath compared to Standard drug group.

Conflicts of Interests:

There is no conflict of interests that could influence the author's interpretation of the data or influence the acceptance of the manuscript.

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