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Evaluation Of Ovulation Effect of Pippalyadi Yoga in Letrozole-Induced Polycystic Ovarian Syndrome.

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Abstract
Infertility is typically defined as inability or failure to conceive after one year period of continuous exposure to normal unprotected coitus. Mainly the causes in females are anouvulation, blocked fallopian tube, hormonal imbalance. Common in males: low sperm count, abnormal sperm morphology. In this regard we took Pippalyadi yoga is herbal formulation mentioned in "Chakradatta" indicated in Vandhya (disorders of female Infertility). Hence this formulation are expected to contribute positive and better result PCOS. In the present study letrozole induced PCOS method was followed. Female albino rats, letrozole (to induce PCOS) clomiphene citrate (standard drug) Pippalyadi yoga (test drug) were served as materials for the study. After inducing PCOS & after treatment of PCOS condition rats were sacrificed and Hormonal assay, histomorphometric changes were observed and recorded. The test drug Pippalyadi yoga has shown a significant result and similar to that of standard drug Clomiphene citrate result in letrozole-induced PCOS.

Keywords
Letrozole, Clomiphene citrate, Pippalyadi yoga, Infertility, PCOS.

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INTRODUCTION:
Pippalyadi Yoga is one of the Churna Kalpana. Which contains Pippali, Adraka, Krishnamaricha, Nagakeshara. Pippalyadi Yoga is indicated in treatment of Vandhya mentioned in "Chakradatta"[1]
Pippali is guru, sheeta, mitigates pitta, sleshma (Kapha), vrana (Ulcers) and arsha (diseases of blood), kashaya, causes shushka, improves color and purifies vagina (Yoni shodhana).[2] & used for diseases of the respiratory tract, as a sedative (In insomnia and epilepsy), as a cholagogue (in obstruction of bile duct and bladder), as *emmenaaaaagogue*, as general tonic and hematinic (in anemia, chronic fevers and for improving intellect). Applied locally on muscular pain and inflammations.[3]
Maricha is a katu, penetrating, augments digestive fire, mitigates kapha and vata, usna virya, increases pitta, causes dryness, and cures dyspnea, pain in the abdomen and intestinal worms.[4] & it acts as stimulant, carminative, diuretic, *anticholerin*, sialagogue, bechic, anti asthamatic, used in fever, dyspepsia, flatulence,indigestion and as mucus membrane & gastro intestinal stimulant. [5]
Shunti choorna is Laghu, snigdha in guna, Katu in rasa so it acts as a kaphaghna (Reduces the kapha) & Usna virya so it is vrishya uttejaka. It is having Madhura Vipaka, is suitable for body and it increases the rasa

helps in formation of Shukra, Dipana, Bhedana in nature. It is VataKaphahara, are the properties[6]. It acts as an antiemetic, anti-flatulent, hypocholesterolemic, expectorant, circulatory stimulant, diaphoretic, increases bioavailability of prescription drugs. Used for irritable bowel and diarrhea, colds and influenza.[7]

[3]Nagakeshara is Snigdha Laghu, Ruksha in guna, Kashaya, Tikta in Rasa, Ushna virya in nature, Katu Vipaka, Pramathi, Grahi, Pachana, Sothahara are the karma, Dosha: Kapha Pittashamaka.[8] [&] used as an astringent, haemostatic, particularly in uterine bleeding and renal diseases.[9]

According Acharya charaka therapeutic effect of drug depends on its rasa, guna, virya, vipaka and prabhava.[10] Based on this concept Pippalyadi yoga ingredients are Katu rasa, Laghu, Snigdha, Tikshna guna, Ushna virya, Madhura and Katu vipaka. Due to its Laghu guna it is easily absorbable and it can enters every part of the cells easily. Madhura vipaka is suitable for body and it increases the rasa and also helps in formation of Shukra. It has vataghna property having Tarpana and Brimhana karma. So by these qualities, it nourishes the body. It has also regeneration capacity. Due to proper nutrition and vatanulomana follicle starts to get mature as we observed increase in size of follicle and after getting maturity rupture of follicle also occurs.

In this regard to assess their efficacy on Ovulation and Hormones related to Reproductive system on Infertility is a global phenomenon in modern age as well.

MATERIALS AND METHODS:

Materials

1) Drugs

a. Letrozole (1 mg/kg Propylene glycol to induce PCOS (oral)

b. Clomiphene citrate (oral) (standard drug)

c. Pippalyadi yoga (oral) (test drug)

2) Animals

Female albino rat's sixteen female albino rats were taken, 8 in each group and Maintained under standard laboratory conditions.

Method

1) **Method of preparation of Pippalyadi Yoga:** Pippalyadi Yoga contains Shunti, Maricha, Pippali, Nagakesara, which were Prepared As per the reference in Chakradatta Chapter shloka number. "Chapter 62, *Shloka* 27, Page number 273.
2) **Experimental study:** IAEC approval was taken to conduct study on animals before to start the study with letter reference no BLDEA/AVS/AMV/407 21-22 DATED 14/8/2020 from BLDEA's AVS Ayurveda mahavidyalaya vijayapur. Which is CCSEA Approved with registration number 533/CCSEA

Selection of animals

Inclusion criteria : Healthy female albino rats, aged 6 weeks (42 days) and weight between 150 and 200 g Were Included in the study.

Exclusion criteria: Unhealthy y female and male albino rats, aged below 6 weeks and weight below 150 g and Above 200 g were e excluded from the study.

Method of preparation of drug and administration

Group I: 25µg of clomiphene citrate/200 g body weight was taken with 1 ml of Propylene Glycol and fed to female albino rats.

Group II: 180 mg of pippalyadi yoga/200 g body weight was taken with 1 ml of Propylene Glycol and fed to female albino rats.

Experimental study protocol:

Induction after inducing PCOS, four albino rats were included in each Group for drug (Standard, test group) administration.

Induction of polycystic ovaries

- As a first step, PCOS was induced by the administration of letrozole suspension at the Concentration of 1 mg/kg dissolved in 1 ml Propylene glycol once daily for 21 days to all the rats in both groups[11.]
- After 21 days, after 24 hr of last dose of letrozole and 18 h of fasting period, half of the animals, i.e., 4 from each group were sacrificed.
- Ovaries and uterus were excised and sent for histomorphometry and blood sample was sent For hormonal assay
- Then, the remaining animals, i.e., 4 in each group were subjected for the drug administration, i.e., standard drug (clomiphene citrate), test drug (Pippalyadi yoga), for a period of 21 days.
- Later they were sacrificed, ovaries and uterus sent for histomorphometry and blood sample was Sent for hormonal assay. Testosterone, FSH, LH, Oestrogen, and Progesterone were assessed.

RESULTS:

Table No 1: Showing Mean ± SD values of Histomorphometric values After Inducing PCOS and after the treatment in all the groups.

	Groups	Mean ± SD of values	
		After Inducing PCOS	After treatment
Cortex	I	135.125 ± 8.323	160.75 ± 6.386
	II	132.5 ± 6.845	163.375 ± 6.675
Medulla	I	70.625 ± 3.815	86.5 ± 6.188
	II	72 ± 5.014	82.75 ±5.625
Endometrial thickness	I	85.375 ±6.368	110.75 ±6.409
	II	69.5± 6.302	91.5 ±4.309
Myometrial thickness	I	85.375 ±6.368	110.75 ±6.409
	II	69.5± 6.302	91.5 ±4.309
Primary follicle size	I	85.375 ±6.368	110.75 ±6.409
	II	69.5± 6.302	91.5 ±4.309

Table no 2: Showing Mean ± SD values of Hormonal values After Inducing PCOS and After The treatment in all the groups.

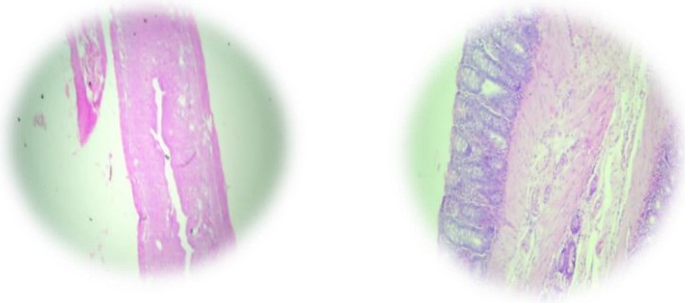
	Groups	Mean ±SD of values	
		After Inducing PCOS	After treatment
Testosterone	I	1.065 ± 1.464	0.155 ± 0.172
	II	0.662 ± 0.536	0.237 ± 0.056
Progesterone	I	7.79 ± 6.627	28.232 ± 32.301
	II	3.13 ± 2.594	36.21 ± 31.179
Oestrogen	I	1.65 ± 0238	2.375 ± 0.377
	II	1.4 ± 0.294	7.615 ± 10.366
FSH	I	0.515 ± 0.79	0.48 ± 0.568
	II	0.077 ± 0.036	0.245 ± 0.47
LH	I	0.603 ± 0.875	0.545 ± 0.762
	II	0.043 ± 0.059	1.07 ± 1.499

Figure 1: Rat ovaries showing cystic follicles in letrozole-induced PCOS – before Treatment.

A B

(A) Group I (standard) (B) Group II (test drug).

Figure 2 Rat ovaries showing cystic follicles in letrozole-induced PCOS - before Treatment.

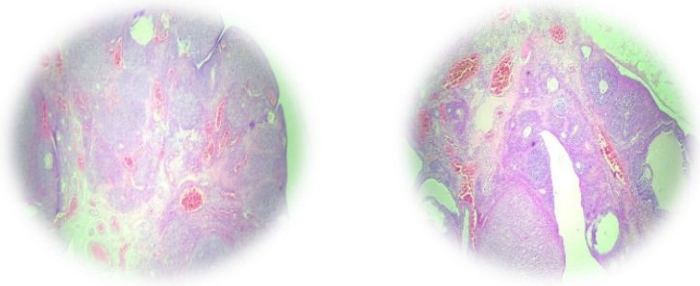


A B

(A) Group I (standard) (B) Group II (test drug)

Hormonal assay due to drug administration

Figure3: Rat ovaries showing ovarian follicles and corpus leuteum in letrozole-induced PCOS - after treatment.



A B

(A) Group I (standard) (B) Group II (test drug)

In standard drug (Group I)

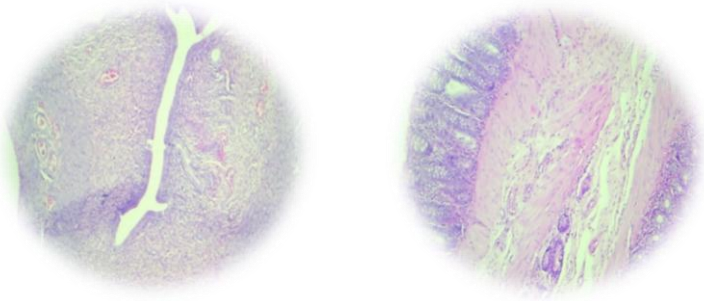
1. The testosterone level decreased after treatment from 1.065 ±1.464 pg/ml to 0.155 ± 0.172.
2. The Oestrogen level increased from 1.65 ± 0238 pg/ml to 2.375 ± 0.377.
3. The progesterone increased from 7.79 ± 6.627 ng/ml to 28.232 ± 32.301.
4. The FSH level decreased from 0.515 ± 0.79 pg/ml to 0.077 ± 0.036.
5. The LH level decreased from 0.603 ± 0.875 Mu/Ml to 0.545 ± 0.762.

In test drug (Group II)

1. The testosterone level decreased from 0.662 ± 0.5369 Ng/ml to 0.237 ± 0.0568.
2. The oestrogen level increased from 1.4 ± 0.29 pg/ml to 7.615 ± 10.366.
3. The progesterone level increased from 3.13 ± 2.594 ng/ml to 36.21 ± 31.179.
4. The FSH level increased from 0.077 ± 0.036 pg/ml to 0.245 ± 0.47.
5. The LH level increase from 0.043 ± 0.059 Mu/ml to 1.07 ± 1.499.

Histomorphometric changes due to drug administration.

Figure 4: Rat ovaries showing endometrial thickness in letrozole-induced polycystic Ovarian syndrome - before treatment.



A B

(A) Group I (standard) (B) Group II (test drug)

In standard group (I)

1. Cortex thickness increase after treatment from 135.125 ± 8.323 to 160.75 ± 6.386.
2. Medulla thickness level increase from 70.625 ± 3.815 to 86.5 ± 6.188.
3. Endometrial thickness level increases from 85.375±6.368 to 110.75±6.409.
4. Myometrium thickness Increases from 148.625±4.779 to 194.5±10.057.
5. Primary follicle size increases from 178.875±3.482 to 205.625±8.798.

In Test group (Group II)

1. Cortex thickness increase from 132.5±6.845 to 163.375±6.675.
2. Medulla thickness level increase from 72±5.014 to 82.75±5.625.
3. Endometrial thickness increase from 69.5±6.302 to 91.5±4.309.
4. Myometrium thickness decrease from 170.75±6.964 to 166.875±6.6.
5. Primary follicle size increase from 178.5±4.811 to 210.5±7.29.

DISCUSSION:

Pippalyadi yoga is of the herbal formulation mentioned in "Chakradatta". It is indicated in Vandhya (disorders of female Infertility)' so this formulation is expected to contribute positive and better result in PCOS. Hence Pippalyadi yoga was administered in letrozole induced infertility Wister strain albino rats to evaluate its efficacy.

PCOS rats to evaluate its effect on PCOS. PCOS was induced in rats by the administration of letrozole suspension for 21 days. Then treated with standard (Clomiphene citrate), both test drugs for 21 days. The changes in the hormonal level and the histomorphometric changes were noted and assessed.

The standard drug Clomiphene citrate has both estrogenic and anti- estrogenic properties. Clomiphene appears to stimulate the release of gonadotropins, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), which leads to the development and maturation of ovarian follicle, ovulation, and subsequent development and function of the corpus luteum, thus resulting in pregnancy.[12]

In Hormonal values changes After Inducing PCOS and after the Treatment In all the Groups. (Ref Table no 1)

Progesterone: Level of progesterone which was low after inducing PCOS was increased after completion treatment course of Standard drug Clomiphene citrate, test drug Pippalyadi yoga

Oestrogen: Level of Oestrogen which was low after inducing PCOS was increased after completion treatment course of Standard drug Clomiphene citrate, test drug Pippalyadi yoga.

FSH: Level of FSH, which was high after inducing PCOS was decreased after completion treatment course in Clomiphene citrate, which was low after inducing PCOS was increased after completion treatment course in Pippalyadi yoga & of Standard drug Clomiphene citrate, test drug Pippalyadi yoga.

LH: Level of LH, which was high after inducing PCOS was decreased after completion treatment course in Clomiphene citrate which was low after inducing PCOS was increased after completion treatment course in Pippalyadi yoga of Standard drug Clomiphene citrate, test drug Pippalyadi yoga.

Testosterone: Level of Testosterone which was high after inducing PCOS was decreased after

Completion treatment course of Standard drug Clomiphene citrate, test drug Pippalyadi Yoga.

In Histomorphometric changes After Inducing PCOS and after the Treatment In all the Groups. (Ref table no 2)

Cortex: Thickness of cortex, which was low after inducing PCOS was increased after Completion treatment course of Standard drug Clomiphene citrate, test drug Pippalyadi yoga.

Medulla: Thickness Medulla, which was low after inducing PCOS was increased after Completion treatment course of Standard drug Clomiphene citrate, test drug Pippalyadi yoga.

Endometrium: In Endometrial thickness, which was low after inducing PCOS was Increased After completion treatment course of Standard drug Clomiphene citrate, test Drug Pippalyadi Yoga.

Myometrium: In Myometrial thickness, which was low after inducing PCOS was Increased after completion treatment course in Clomiphene citrate, which was high After inducing PCOS was decreased after completion treatment course in Pippalyadi Yoga of Standard Drug Clomiphene citrate, test drug Pippalyadi yoga.

Primary follicle size: In Primary follicle size, which was low after inducing PCOS Was increased after completion treatment course of Standard drug Clomiphene citrate, Test drug Pippalyadi yoga. The test drug Pippalyadi yoga has shown a significant effect

Based on above results of harmonal essay as well as histomorphometric values of pippalyadi yoga has significant result in comparison with clomifin citrate in letrozol induced PCOS model of animal experimental study, with this this study gives evidence to the classical medicine indicated in vandya disease which may be correlated to the PCOS

CONCLUSION:

The test drug Pippalyadi yoga has shown a significant and similar to that of Clomiphene citrate result in letrozole-induced PCOS.

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