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AN EXPERIMENTAL STUDY TO EVALUATE THE EFFECT OF PUTRANJIVA BEEJA IN OVULATION IN FEMALE WISTAR ALBINO RATS

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ABSTRACT

Objective: To evaluate the effect of putranjiva beeja on estrous phase in wistar albino rats. **Method:** The study was been carried out in 5 groups of albino rats with 6 rats in each group. First group was normal control group, second group was positive control group i.e ovral-l group, third group with standard drug, fourth group was treated with putranjiva orally and fifth group with ovral-l followed by putranjiva. The drug was administered orally for 21 days and vaginal smear was taken daily and was observed for estrus phase. **Result:** The study showed the predominance of estrous phase in putranjiva treated rats. **Conclusion:** From the above study it can be concluded that putranjiva can be administered toimprove the estrus phase in wistar albino rats.

KEYWORDS: Putranjiva beeja, Estrus phase, ovulation.

INTRODUCTION

Infertility affects millions of people of reproductive age worldwide. In menstruating women, ovarian dysfunction is one of the main cause for infertility. Ovulatory dysfunction like anovulation results in an alteration in the frequency and duration of menstrual cycle sometimes leading to irregularity in menstruation. In the treatment of infertility, among others ovulation induction therapies with there complications, costly and along with benefits, failure accompanies too. Thus with increasing failure rate, side effects and cost, there is need and scope for the developing and studying of more arthava janaka yogas as mentioned in our

classics, which are safe, effective and economical for the treatment of the diseases like vandyatwa, nashtartava etc. An animal experiment not only benefits humans in there effectiveness but toxicity and teratogenicity effects can be over ruled. In albino rats, estrous cycle consist of 4 phases namely proestrus, estrus, metestrus and diestrus phase.[1] An increase in estrus phase and decrease in diestrus phase indicates the ovulatory period in the Estrus phase of the cycleof wistar albino rats. Bhavapraksha has mentioned about the drug putranjiva in vatadi varga as garbhada, probably corrects the ovulation and regulates the menstrual cycles promoting conception.[2] This study was undertaken to understand the effect of putranjiva on estrus phase in wistar albino rats highlighting about ovulation.

MATERIALS AND METHOD

Ethical Committee Approval Number – SDMCAU/ACA-49/AEC24/2018-19

Drug selection

Group	No of rats	Drugs
1	6	Normal control
2	6	Positive control (ovral-l)
3	6	Standard drug (clomiphene citrate)
4	6	Test group (putranjiva)
5	6	Test drug + ovral-l

Test drug: Putranjiva beeja was taken as a test drug given orally. The reference of putranjiva was obtained from Bhavaprakasha.

Dose Fixation

Dose fixation: Based on the body surface area ratio and by referring to the table of Paget and Barnes(1964)

Dose for rats: Recommended daily intake of putranjiva = 1karsha Human Dose × 0.018 for rat weighing 200g i.e. 12 × 0.018 × 5 = 1.08g/kg

Experimental design and procedure

Wistar albino female rats weighing between 150-250g were used for the study with following conditions. The animals were obtained from the animal house attached to the Pharmacology laboratory, SDM centre for Research in Ayurveda and Allied Science, Udyavara. They were exposed to natural day and night cycles with ideal laboratory condition in terms of ambient temperature, humidity and with maintained hygienic conditions, were fed with rat pellets with

tap water ad libitum. Added labium cleaningand sanitation work were done on alternate days. Paddy husk were provided as bedding materials, which were changed everyday. The experiments were carried out in conformity with guidelines of the Institutional Animal Ethical Committee (IAEC) after obtaining permission. ETICAL CLEARENCE NUMBER– SDMCAU/ACA- 49/AEC24/2018-19.

Animal grouping: 30 wistar albino rats were selected for study which were grouped in to 5 groups with 6 rats in each group.

Route of administration of drug: Test drug were administered for 21 days including experiment day in the morning session between 9-10 am orally followed by cervical smear.

On the 5th group ovral-l was administered orally between 9-10am, 1 hour later with putranjiva and then vaginal smear were observed. The drug was administered through oral route with help of feeding tube attached to syringe.

Duration of the study: 21 days

Daily vaginal smear was observed under 40X objective lens to visualize the estrus phase.

OBSERVATION PARAMETER

- In vivo changes – Oestrous cycle monitoring.
- Hormonal assay – Serum Estradiol and Progesterone.

RESULTS

Table 1: Effect of Putranjiva on Proestrous Phases.

Groups	Proestrous Phases	% Change
Control	3.83 ± 0.54	
Ovral-L	6.16 ± 1.62	60.83↑@
Standard m	1 ± 0.36	73.89↓@
Putranjiva	2.16 ± 0.54	43.60↓@
Putranjiva + Ovral-L	5 ± 1.84	18.83↓#

Data: MEAN ± SEM,

@-Compared with normal control#-Compared with ovral-l

The data related to the effect of test drug on number of proestrus phase in complete estrous cycle has been shown in the table. The data shows there was a decrease in the number of proestrus phase in the standard group and putranjiva beeja group when compared with the normal control group. The observed decrease was found to be statistically not significant. The

data shows there was a increase in the number of proestrus phase in the ovral L group when compared with the normal control group. The observed increase was found to be statistically not significant. The data shows there was a decrease in the number of proestrus phase in the putranjiva+ ovral L group when compared with the ovral L group. The observed decrease wasfound to be statistically not significant.

Table 2: Effect of Putranjiva on Estrous Phases.

Groups	Estrous phase	% change
Control	5.33 ± 0.80	
Ovral-l	4.3 ± 1.58	18.76↓@
Standard	7.5 ± 2.06	40.71↑@
Putranjiva	9.33 ± 1.52	75↑@
Putranjiva + ovral-l	10.16 ± 1.90	57.38↓#

Data: MEAN ± SEM,

@- compared with normal control#- compared with ovral-l

The data related to the effect of test drug on number of estrous phase in complete estrous cycle has been shown in the table. The data shows there was a increase in the number of estrous phase in the standard group and putranjiva beeja group when compared with the normal control group. The observed increase was found to be statistically not significant. The data shows there was a decrease in the number of estrous phase in the ovral L group when compared with the normal control group. The observed decrease was found to be statistically not significant. The data shows there was a decrease in the number of estrous phase in the putranjiva + ovral L group when compared with the ovral L group. The observed decrease was found to be statistically not significant.

Table 3: Effect of Putranjiva on Metestrous Phases.

Groups	Metaestrous Phase	% Change
Control	7.83 ± 0.60	
Ovral-l	9 ± 1.82	14.94↑@
Standard	4.5 ± 1.05	42.52↓@
Putranjiva	3.33 ± 0.71*	57.47↓@
Putranjiva + Ovral-l	5.6 ± 1.03	37.77↓#

Data: MEAN ± SEM,

@ - Compared with normal control#- compared with ovral-l

The data related to the effect of test drug on number of metestrus phase in complete estrous cycle has been shown in the table. The data shows there was a decrease in the number of metestrus phase in the standard group and putranjiva beeja group when compared with the

normal control group. The observed decrease was found to be statistically significant. The data shows there was a increase in the number of metestrus phase in the ovral L group when compared with the normal control group. The observed increase was found to be statistically not significant. The data shows there was a decrease in the number of metaestrous phase in the putranjiva+ ovral L group when compared with the ovral L group. The observed decrease was found to be statistically not significant.

Table 4: Effect of Putranjiva on Diestrous Phases.

Groups	Diestrous Phase	% Change
Control	4 ± 0.89	
Ovral-l	2.5 ± 0.50	37.5↓@
Standard	7.83 ± 2.56	95.75↑@
Putranjiva	5.5 ± 1.60	37.5↑@
Putranjiva + Ovral-L	0.66± 0.33	73.6↓#

Data: MEAN ± SEM,

@ - Compared with normal control#- compared with ovral-l

The data related to the effect of test drug on number of diestrous phase in complete estrous cycle has been shown in the table. The data shows there was a increase in the number of diestrous phase in the standard group and putranjiva beeja group when compared with the normal control group. The observed increase was found to be statistically not significant. The data shows there was a decrease in the number of diestrous phase in the ovral L group when compared with the normal control group. The observed decrease was found to be statistically not significant. The data shows there was a decrease in the number of diestrous phase in the putranjiva+ ovral L group when compared with the ovral L group. The observed decrease was found to be statistically not significant.

Table 5: Effect of Putranjiva on Serum Estrogen.

Groups	Serum Estrogen (NG/DL)	Percentage (%)
Control	33.33 ± 0.66	
Ovral-l	28.66 ± 0.33	14.01↓@
Standard	38.33 ± 4.09	15.00↑@
Putranjiva	42.66 ± 4.84	27.99↑@
Putranjiva + Ovral-l	33.66 ± 2.72	17.44↑#

Data: MEAN ± SEM,

@ - Compared with normal control# - Compared with ovral-l

The data related to the effect of test drug on serum estrogen in complete estrous cycle has been

shown in the table. The data shows there was a increase in serum estrogen in the standard group and putranjiva group when compared with the normal control group. The observed increase was found to be statistically not significant. The data shows there was a decrease in serum estrogen in the ovral L group when compared with the normal control group. The observed decrease was found to be statistically not significant. The data shows there was a increase in serum estrogen in the putranjiva+ ovral L group when compared with the ovral L group. The observed increase was found to be statistically not significant.

Table 6: Effect of Putranjiva on Serum Progesterone.

Groups	Serum Progesterone (NG/DL)	Percentage (%)
Control	15.15 ± 4.44	
Ovral-L	12.61 ± 2.48	16.76↓@
Standard	17.1 ± 4.84	12.87↑@
Putranjiva	20.66 ± 5.25	36.36↑@
Putranjiva + Ovral-L	19.2 ± 10.00	52.26↑#

Data: MEAN ± SEM,

@ - Compared with normal control# - Compared with ovral-l

The data related to the effect of test drug on serum progesterone in complete estrous cycle has been shown in the table. The data shows there was a increase in serum progesterone in the standard group and putranjiva group when compared with the normal control group. The observed increase was found to be statistically not significant. The data shows there was a decrease in serum progesterone in the ovral L group when compared with the normal control group. The observed decrease was found to be statistically not significant. The data shows there was a increase in serum progesterone in the putranjiva+ ovral L group when compared with the ovral L group. The observed increase was found to be statistically not significant.

DISCUSSION

Consolidation Statement – Estrous Phase

	Compared With Normal Control			Compared with Positive Control
Parameters	**Positivecontrol (ovral-l)**	**Standard**	**Testdrug**	**Test drug + ovral-l**
Proestrus	NSI	NSD	NSD	NSD
Estrus	NSD	NSI	NSI	NSI
Metestrus	NSI	NSD	SD	NSD
Diestrus	NSD	NSI	NSI	NSD

Proestrus phase

The data shows that, proestrus phase was increased in positive control group i.e ovral-l group when compared to normal control group which was statistically non-significant. There was decrease in standard group and test group i.e. putranjiva group when compared to normal control group which was statistically non-significant. There was decrease in proestrus phase in combined group when compared to positive control group which was statistically non-significant. Ovral-l it contains progestin and estrogen in a constant value thus preventing ovulation. The drug clomiphene citrate and the test drug probably has more of estrogen which prepares the reproductive organs indicating towards ovulation. It work mainly by preventing the release of an egg (ovulation). Concluding that in ovral-l, there might be an increase in proestrus phase when compared to the standard and test drug.

Estrus phase

The data shows that, estrus phase was decreased in positive control group i.e. ovral-l group when compared to normal control group which was statistically non-significant. There was increase in estrus phase in both standard and putranjiva group when compared to normal control group which was statistically non-significant. There was increase in estrus phase in combined group which was statistically non-significant. Estrous phase is second stage in the estrous cycle immediately before metestrus characterized by a receptivity to a male and to mating. Often referred to as 'heat'. Pheromones may also be secreted only at this stage of her cycle. This further emphasis the fact that in the standard and in the test drug the period for fertilization is prolonged under the influence of estrogen. The estrogen in humans is also responsible for the peak of LH seen prior to ovulation.

Metestrus phase

The data shows that, metestrus phase was increased in positive control i.e. ovral-l group when compared with normal control group which was statistically non-significant. There was decrease in metestrus phase in standard group which was statistically non-significant and in putranjiva group when compared to normal control group which was statistically significant. There was decrease in metestrus phase in combined group which was statistically non-significant.

Diestrus phase

The data shows that, diestrus phase was decreased in positive control i.e ovral-l group when compared to normal control group which was statistically non-significant. There was increase

in diestrus phase in both standard and putranjiva group when compared to normal control group which was statistically non-significant. There was decrease in diestrus phase in combined group which was statistically non-significant. The role of metestrus phase and diestrus phase is not of significance in the study of ovulation.

Consolidation Statement – Biochemical Parameters

	Compared with NormalControl			**Compared withPositive Control**
Parameters	**Positive control (ovral -l)**	**Standarddrug**	**Test drug**	**Test drug + ovral -l**
Estrogen	NSD	NSI	NSI	NSI
Progesterone	NSD	NSI	NSI	NSI

Serum Estrogen

The data showed that serum estrogen level was found to be increased in test drug and standard group which indicates that the drug is having estrogenic effect but which is statistically non-significant when compared to normal control group. In positive control group there was decrease in estrogen level this decrease is statistically non-significant. This probably indicates the progesterone activity of the drug. In test drug + ovral-l group there was increase in estrogen level which is non-significant, hence it can be understood that the drug putranjiva is having estrogenic activity when administered along with ovral-l which has combined effect of estrogen and progesterone in it. Estrogen level help in the maturation of the follicle with endometrial priming, this folliculogenesis helps in the ovulation. Ovral L is a combined oral contraceptive preparation, it acts mainly through the mechanism of gonadotropin suppression due to the estrogenic and progestational activity of the constituent. The predominant effect of estrogen is to inhibit secretion of FSH, while continued action of progesterone is to inhibit LH. There is a constant variable of estrogen and progesterone present which otherwise hampers the physiological steroid hormones and thus act as a contraceptive. Estrogen inhibits FSH rise and prevents follicular growth.

SERUM PROGESTERONE

The data showed that serum progesterone level was decreased in positive control group when compared to normal control group which is non-significant. There was increase in serum progesterone level in test group and standard group when compared to normal control group the increase was found to be non-significant. There was non-significant increase in serum progesterone in test drug + ovral-l group when compared to positive control group. In

steroidogenesis, progesterone is having important role. Pregnenolone is a hormone naturally produced in the body by adrenal gland and is also made from cholesterol, and is starting material in the production of testosterone, progesterone, cortisol, estrogen and other hormones.

After ovulation there will be increase in serum progesterone. An increase in serum progesterone level indicates proper timely functioning of corpus luteum. By administration of putranjiva beeja choorna it was observed that there was increase in serum progesterone level which was non-significant statistically. Thus even though there was no drastic increase in the serum progesterone concentration, drug helped to maintain the level of progesterone indicating the positive effect over progesterone concentration and ovulation.

CONCLUSION

The drug putranjiva contains madhura and katu rasa, guru guna and sheeta virya. Madhura rasa which is predominant of prithvi and ap mahabhuta and with guru guna, does anulomana which is classically understood in the physiology of menstruation, i.e. with the proper release of the ovum followed by menstruation. The function of garbhastapana indicates towards a normal ovulation procedure with a receptive endometrium ending in a normal pregnancy. Putranjiva has madhura rasa and katu rasa, this katu rasa is predominant of agni mahabhuta resulting in pitta vardhana probable in endometrial regeneration and proliferation following ovulation. The main chemical constituents of Putranjiva is saponins which is a steroid present in plant and plant products, affects the growth and reproduction of animal. Flavonoids seen in this plant, depending on estrogen concentration, it act as agonist or antagonist. These two constituents might help in estrogen activity prior to ovulation. The estrus phase indicates the ovulatory period in the albino rats. the increase in the estrous phase indicates prolonged ovulatory period with decrease in the diestrous phase. (chance or opening for conception probably due to extended ovulatory period). Concludingly, test drug putranjiva showed more number of estrus phase when compared to normal control group which indicates ovulatory phase in rats. But the results did not show statistically significance. The vaginal smear of group 5 i.e. test drug + combined oral contraceptive group also showed increase number of estrus phase which infers ovulatory phase of rats proving the drug putranjiva effective in conception in the presence of controlled estrogen and progesterone.

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