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An Experimental Analysis of Withania somnifera on Estrous Cycle

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ABSTRACT

India, the world's second large country of population of 1.3 billion people after china (1.4 billion) has always fascinated demographers . And now, with the united nations forecasting that India's population will go faster than china's as early as 2022, it looks like the country may well be ready to detonate at its seams. However, few know about an entirely unexpected problem that is currently bedevilling Asia's third largest economy - a considerable decline in its fertility rate. When the fertility rate dips this number, the population is expected to decline. Modern treatment modalities cannot be used continuously due to their side effects.Thus this study was done to evaluate the effect of hydroalcoholic extract and ksheerpaka of Withania somnifera on estrous cycle in female wistar albino rats were orally administered with extract of Withania somnifera 2000 mg/kg body weight /day for 21 days .Results of the present study showed a significance increase in the weight of the uterus and in weight of the ovary was observed in treated group .Further estrous cycle length was observed to be regular and increased .In conclusion , this study suggests that the hydroalcoholic extract of roots of withania somnifera possess ovulatory activity after treating with norethisterone acetate..

KEYWORDS

Withania somnifera, Estrous cycle, Ovary, Uterus, Ovulatory activity

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INTRODUCTION

Withania somnifera is widely used as medicinal plants for several medical conditions. The use of Withania is accelerating due to presence of active constituents found useful for health. According to classics *ashwagandha* root juice is given as *nasya* to achieve conception. *Ashwagandha*. Botanically known as Withania somnifera is unique medicinal plant in *Ayurvedic* system of medicine. A plant emiting horse smell mainly the root. The leaves resemble the ear of pigs. The drug promote sexual potency, complexion and strength .Various formulations are available in the market. Use of *ashwagandha* is increasing day by day due to its number of chemical constituents like steroidal alkaloids, somniferine, withanaloids, withaferine etc mainly present in the roots. In the present study we have observed whether Withania can be used safely in clinical trials for the treatment of ovulatory disorders.

MATERIALS AND METHODS

PLANT MATERIAL

The coarse powder of root of withania somnifera was collected from the Sri Dharmasthala Manjunatheshwara pharmacy, Kuthpady, Udupi, India and hydroalcoholic extract of *ashwagandha* is prepared at the SDM Pharmacology laboratory by Soxhlet extraction method .

ACUTE ORAL TOXICITY STUDY

ACUTE ORAL TOXICITY study of *ashwagandha* extract in Wistar Albino female rats were evaluated in present study. In this study hydroalcholic extract of *ashwagandha* roots has taken and administered to three rats at 2000 mg/kg, orally once in the morning and were observed for 14 days as per study protocol. In this study no toxicity or toxic signs and no any mortality were observed .There were no significant changes in the parameters like body weight, haemato-biocheminal parameters at any dose level. In this study, administration of *ashwagandha* extract did not show any toxicologically significant treatment related changes in body weight, ophthalmic changes, and clinical pathology evaluation. Hematological and serum parameters were within the normal limits, hence from the study we can prove that *ashwagandha* hydroalcoholic extract is nontoxic in Nature.On the basis of previous studies carried the effective dose 2000 mg/kg was being selected for further studies.

EXPERIMENTAL ANIMALS

Thirty six healthy female non-pregnant wistar albino rats were selected randomly and was weighed about 200±50 gm from the animal house of SDM centre for

research in *ayurveda* and allied sciences, kuthpady, Udupi. Rats were marked for identification and housed in polypropylene cages. Animals were maintained under hygienic conditions and they were provided with commercial food pellets and tap water. Added labium cleaning and sanitation work were done on alternate days. Paddy husk had provided as bedding material, which was changed every day.

For experiment designed to determine oral LD_{50} of *ashwagandha* extract study was done on three rats which was administered 2000 mg/kg .Signs of toxicity were observed for 14 days. No toxic effects were observed in this study; hence 2000 mg/kg dose was selected for the main study in 6 rats. Rats were administered the dose of 2000 mg/kg of *ashwagandha* extract and signs and symptoms of toxicity were observed .On day 21, all the animals were euthanized and further examinations done. The experiments would be carried out in conformity with guidelines of the Institutional Ethical committee (IAEC) after obtaining permission

ETHICAL CLEARANCE NO. SDMCRA/IAEC/PT-11 ON 26/3/18

ESTROUS CYCLE EVALUATION

After grouping the vaginal smear was taken for all 6 groups in morning 9 -10 am daily for 21 days including the day of experimental sacrifice. Followed by drug administration. Vaginal secretion collected with a plastic micropippete filled with normal saline by inserting the tip into the rat vagina but not deeply .Vaginal smear are to be placed on glass slide .A different glass slide has to be used for each animal. One drop is collected with a clean tip from each rat. And stained with methylene blue dye ,stained material was to be observed under a light microscope with 10x and 40x objective lenses .A normal estrous cycle in rats was defined as 4-5 days.Three types of cells to be recognized i.e round and nucleated ones are epithelial cells ,irregular ones without nucleus are the cornified cells and little round ones are the leukocytes .The estrous cycle stages are 1.Proestrus –mainly epithelial cells 2.Estrus –mainly cornified cells 3.Metestrus-cornified and leukocytes and 4.diestrus-mainly leukocytes present. Animals showing normal and regular estrus cycle for 4-5 days. In Group A, only water and normal diet is administered, Group B is treated with Primolut N. Whereas Group C treated with test drug *Ashwagandha* extract and Group D is treated with *Ashwagandha ksheerpaka* Group E is treated with *Ashwagandha* extract and Primolut N at therapeutic dose and Group F is treated with *Ashwagandha ksheerpaka* and Primolut N at therapeutic dose and which has been administered for 21 consecutive days.On 21st day of experiment, the rats are

anesthetised by using chloroform and blood is collected by retrorbital puncture and assigned for biochemical and hormonal investigation.

BODY WEIGHT AND OVARIAN WEIGHT

Body weight was determined before the initiation of experiment and just before killing of each animal .The rats of all the groups were scarificed by administering sodium phenobarbitone , 2000 mg/kg body weight on 21st day .After sacrificing the animal , The rats were anesthetised then incision over the abdomen was taken which was extended above to the neck and below to the vagina , the organs like uterus ,ovary, fallopian tube were excised out from sacrificed animal , weighed and transferred to fixing solution (10% formalin) for histopathological examinations. Ovaries were dissected out , freed from extra deposition and weighed and ovaries from each rat was processed for cholesterol and triglyceride estimation and another for histopathological study.

STATISTICAL ANALYSIS

All the values are expressed as MEAN +_SEM followed by employing one way ANOVA as statical test followed by Dennett's multiple "t" test as post hoc test. Graph pad inst 3 was used for this purpose.P value < 0.05 is considered as statistical significant. Level of significance was observed, noted and interpreted accordingly the duration of study that is of 21 days.

RESULTS

Table 1 Effect of *Ashwagandha* on Estrous Phases

S.NO	GROUPS	ESTROUS PHASES	% CHANGE
1	CONTROL GROUP	7.8±1.241	....
2	PRIMOLUT N	5.5±0.99	29.48↓@
3	*ASHWAGANDHA* EXTRACT	8.2±0.96	5.182↑@
4	*ASHWAGANDHA KSHEERPAKA*	10.4±1.32	33.33↑@
5	*ASHWAGANDHA* EXTRACT +PRIMOLUT N	3.3±0.918	40↓#
6	*ASHWAGANDHA KSHEERPAKA*+PRIMOLUT N	13.4±0.6782**	143.#6↑

Data: MEAN ± SEM, **P<0.01 @-COMPARED WITH NORMAL CONTROL #- COMPARED WITH PROMOLUTE N

Data related to the effect of test drug on complete estrous cycle has been shown in the table 1 and 2. Data shows that there was decrease in the total number of estrous phases in the Primolut group compared to normal control group was found to be statistically non-significant .There is increase in the total number of estrous phases in the *Ashwagandha* extract , *Ashwagandha ksheerpaka* , *Ashwagandha*.Extract + Primolut group was found to be statistically non-significant .There was increase in the total number of estrous phases in the

Ashwagandha.Ksheerpaka + Primolut, groups was found to be statistically very significant when compared to standard control group .

DISCUSSION

Table 2 Consolidated Statement of Estrous Cycle

Parameters	Compared with normal		Compared with primolut –n		
	Primolut	*Ashwagandha Ksheerpaka*	*Ashwaganha* Extract	Primolut *+ashwagandha ksheerpaka*	Primolut+*ashwagandha* extract
PROESTROUS	NSI	NSD	NSD	NSD	NSD
ESTROUS	NSD	NSI	NSI	NSI	VSD
METAESTROUS	SD	NSD	NC	NSI	NSI
DIESTROUS	NSI	NSD	NSD	NSD	NSD

Action of *ashwagandha* on estrous cycle - In *ksheerpaka* group, extract group, and Primolut + *ksheerpaka* group, there is non-significant increase of estrous phases as compared to positive control group this indicates there is increased estrogenic activity . Estrogen secreted by mature graffian follicle or matured secondary follicle might have produced the estrogen hormone and exhibited the estrogenic activity .These changes will promote or prepares for the ovulation. The estrogen produced by graffian follicle or matured secondary follicle which is essential for maturation of the ovarian follicle.

CONCLUSION

The study on the estrous cycle showed significant alteration in the estrous cycle in group III,IV,V. The duration of estrous phase in the test group has increased and there was significant decrease in diestrus phase which shows that the early restoration of next phases and drug has action on anovulation i.e it corrects anovulation and regularizes ovulation.

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