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Study of The Effect of *Cissampelos pareira* Leaf Extract on Estrous Cycle of Albino Mice.

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ABSTRACT

The leaf extract of *Cissampelos pareira* was administered orally at doses of 250mg and 450mg/kg bodyweight/day to normal virgin albino mice for 20 days. The vaginal smear of the mice was collected daily to study the estrous cycle. Estrous cycle was affected by showing a significant decrease in the total number of cycles with a decrease in the duration of proestrus and metestrus phase with concomitant significant increase in the duration of diestrus phase in the treated groups. The duration of diestrus was found to increase significantly in the experimental group which received a dose of 450 mg /kg body weight per day. The changes observed in the other group receiving a lower dose were not significant. Interruption of the estrous cycle may be due to the hormonal imbalance caused by administration of the extract of *Cissampelos pareira* leaves.

Keywords: Estrous cycle; *Cissampelos pareira*; Albino mice; Antifertility effect

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INTRODUCTION

Scientific investigation in search of safe antifertility agents is going on all over the world to tackle the problem of population explosion that may cause economic and health impact in the society especially in developing countries. Though several synthetic contraceptive drugs containing progesterone and estrogen are being popularly used, there are several risks involved in the use of such drugs for long time. Thus, there is a need for suitable products from indigenous medicinal plants that could be used as safe and effective alternative of synthetic drugs. In India, a number of medicinal plants are traditionally used for various ailments without any scientific validation of their efficacy and safety. *Cissampelos pareira* is one of such plants which is used in rural areas of Assam for birth control. This plant, locally known as *Tupuki Lata* is a climber belonging to the family Menispermaceae. There are reports of the use of this herb in other countries as well. It is referred to as midwives herb throughout South America for its long history of use for all types of pregnancy related ailments. It is also used traditionally in many parts of India for the treatment of diseases like asthma, cholera, diarrhoea, dysentery, epilepsy and rabies. Scientific study of the plant in relation to its antifertility properties is still scanty. Therefore, in present piece of work, the estrous cycle of albino mice fed with methanol extract of *Cissampelos pareira* leaves were studied to reveal any possible antifertility effect of the plant.

MATERIALS AND METHODS

Specimen

Laboratory bred adult virgin female albino mice aged 85 to 100 days weighing between 20-25 grams showing regular estrous cycle were selected for the study. The animals were housed in separate polypropylene cages bedded with paddy husk at a room temperature of 25-28^o C. They were fed with nutrient rich food and tap water throughout the study.

Sample collection

The leaves of *Cissampelos pareira* were collected from the surroundings of Guwahati city, Assam, India and authenticated by Botanical Survey of India personnels, Shillong.

Preparation of the extract

The collected leaves were dried in shade and extracted in methanol. The methanol extract was concentrated in a rotary vacuum evaporator to yield semi solid mass, which was further dried under a hot water bath to remove methanol completely.

Plan of the experiment

The experimental animals were divided into three groups (A, B and C) consisting of five animals in each group. The group A served as the control whereas the two groups B and C served as two experimental groups which received dried methanol extract orally at doses of 250 mg and 450 mg/ kg animal body weight per day respectively for 20 days [1]. The estrous cycle of the experimental animals was studied by stained preparation of vaginal smear of the animals daily as described by Cooper *et. al* [4] and compared with the estrous cycle of the control animals.

Study of Estrous cycle

On each day of the study period, vaginal samples were collected two times (morning and evening) to prepare the smear. Smears were analyzed and different cell types were studied using an Olympus CHL microscope with a magnification of 4000X. A strategy of random sampling was used in the counting of cells. For each smear, a total of 10 fields were counted with 10X ocular.

The stages of estrous cycle and their duration were determined by Makonnen *et. al* [6] considering the cytological composition of the vaginal smears and taking into account the relative proportions of the cornified cells (Non nucleated and nucleated), squamous epithelial cells and leucocytes.

Analysis of results

The results were analyzed statistically and presented as mean \pm S.E.M. of n where n equals the number of animals in the sample. Student's "t" test was performed to compare the experimental values with those of respective controls. Differences at $p < 0.05$ were regarded as statistically significant.

RESULTS AND DISCUSSION

Results of the present investigation are presented in the Tables 1 and 2 and also in Figure 2. The control mice exhibited regular estrous cycle (4-5 days) and normal duration of each stage of estrous cycle. However, a significant decrease in the number of estrous cycle and duration of the proestrus and the metestrus stages with significant increase in the duration of the diestrus stage of estrous cycle (Table 1) were observed with the treatment of *Cissampelos pareira* extract.

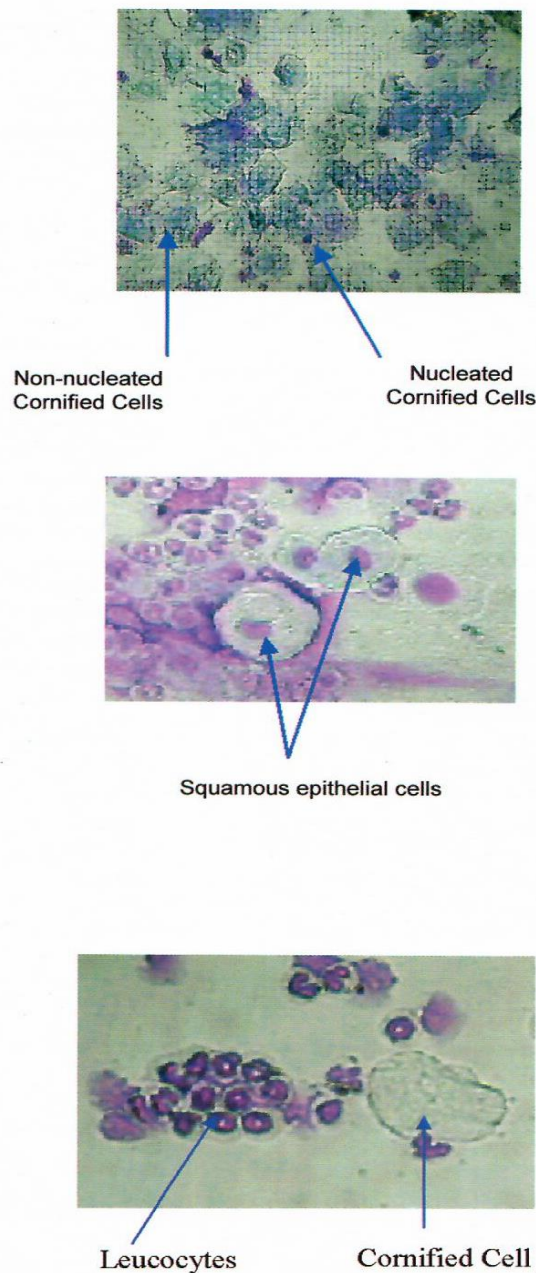


Figure 1: Different cell types of the estrous cycle.

Table 1: Effects of methanolic extract of *Cissampelos pareira* leaves on the estrous cycle of mice

Groups	Treatment (mg.kg ⁻¹ day ⁻¹)	No of cycles	Duration in days (mean ± S.E.M)			
			Proestrus	Estrus	Metestrus	Diestrus
A	Control	3.80±0.20	3.20±0.32	4.30±0.35	3.08±0.31	9.42±0.52
B	250	3.08±0.34*	2.04±0.22*	4.42±0.48 ^{NS}	2.19±0.36*	11.12±0.82*
C	450	2.91±0.31*	1.92±0.18**	5.14±0.32*	1.82±0.28*	11.35±0.76*

N=5, Data are mean±S.E.M, significant at * p<0.05, ** p<0.01; NS-non significant

Table 2: Percent deviation of the duration of estrous cycle over control in the experimental groups of mice

Groups	Treatment (mg.kg ⁻¹ day ⁻¹)	No of cycles	Duration in days (mean ± S.E.M)			
			Proestrus	Estrus	Metestrus	Diestrus
A	Control (No treatment)					
B	250	-18.94% *	-36.25% *	+2.79% ^{NS}	-28.89% *	+18.05% *
C	450	-23.42% *	-40.00% **	+19.5% *	-40.91% *	+ 20.49% *

There was no significant difference in the length of estrus phase from that of control in 250 mg/kg body weight /day administered group (B). However, significant increase in the duration of diestrus stage was observed in 450mg/kg body weight/day administered group (C).

No significant differences were observed in the number of different cellular types of control and treated groups during the study (Table 3). Structural changes in the different cell types of vaginal smear were not observed in the animals of the treated groups (Figure 1).

Table 3: Distribution of the cellular types in different stages of the estrous cycle in control and treated groups of albino mice (Values expressed in percentage)

Cellular types	Groups	Proestrus	Estrus	Metestrus	Diestrus
Squamous epithelial	A	8.04	3.65	17.5	4.5
	B	8.30	4.02	12.8	5.2
	C	7.2	2.8	15.2	5.6
Cornified (Non nucleated)	A	30.5	60.97	5.2	---
	B	28.4	63.2	6.3	
	C	31.6	62.5	4.1	
Cornified (Nucleated)	A	2.29	4.88	0.9	1.2
	B	3.1	3.45	0.7	1.4
	C	4.5	4.52	0.82	1.0
Leucocytes	A	59.17	30.48	76.4	94.3
	B	60.20	29.33	80.2	93.4
	C	56.7	30.18	79.88	93.4

Group A: Control group; Group B & C are Experimental groups receiving doses 250 and 450 mg.kg⁻¹ day⁻¹ respectively.

The cyclic changes of the vaginal smear observed in the estrous cycle gives a reasonable index of ovarian activity and hormonal synthesis of estrogen and progesterone [9]. The data obtained in the present study reveals that mice treated with different doses of *C. pareira* leaf extract causes a significant decrease in the number of estrous cycle with shortening of proestrus and metestrus stage and significant increase in the duration of diestrus and estrus stages. Gebrie *et.al* [5] reported a significant increase in the duration estrous cycle and diestrus phase after administration of the root extract of *Rumex steudelii* in wistar albino rats. Valsala [12] reported the marked inhibition of estrous cycle by root powder of *Mimosa pudica*. Disturbances in estrous cycle with prolonged diestrus phase in albino rats with treatment of edifenphos was also reported by Math and Kaliwal [7]. Budreau and Singh [3] also reported significant increase in the time span in estrus and

diestrus phase after administration of dimethoate in mouse which can be correlated with the findings of the present study.

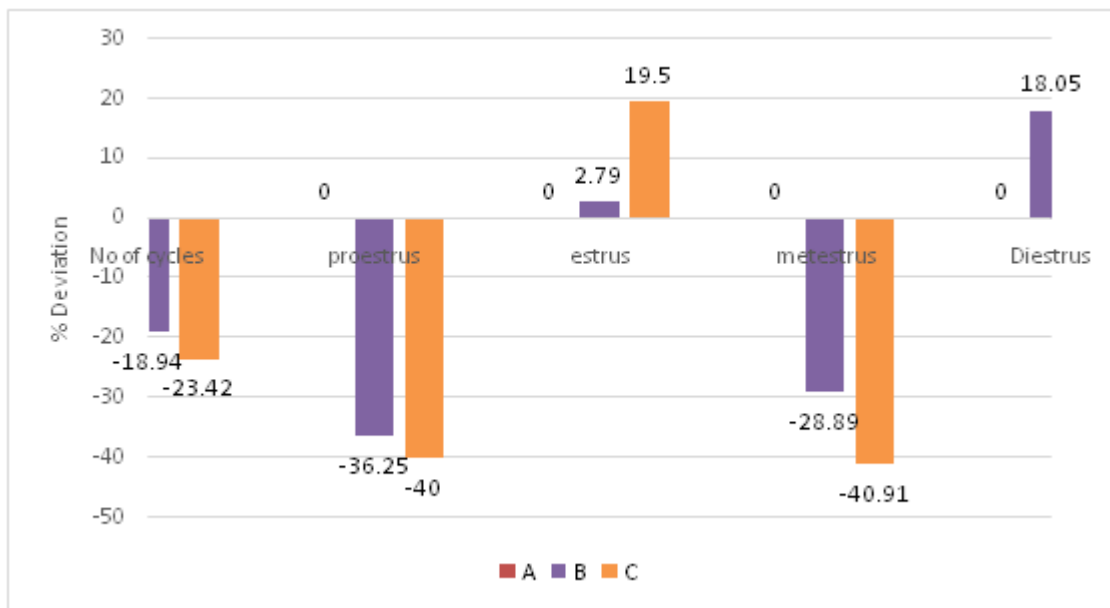


Figure 2: Percent deviation of the duration of estrus cycle over control in the experimental groups of mice.

The appearance of prolonged diestrus phase and estrous cycle observed in the present study indicates the suppression of secretion of estrogen from the ovary, which might be due to less release of gonadotropins thereby causing hormonal imbalance. The observed long duration of estrous cycle with prolonged diestrus phase (safe period) on treatment with *C. pareira* leaf extract suggests antifertility action of the leaves. The increased duration of diestrus may explain the lowering in the chances of conception.

The prolongation of estrus and diestrus phase observed in the study with reduction in the number of reproductive cycle in stipulated time may be due to hormonal imbalance which coincide with some other reports of similar observations on animals treated with different compounds [3,12-13].

However, unaltered cellular structure and number of different cell types as observed in the vaginal smear of the experimental group of mice suggest that the extract of the leaves of *Cissampelos pareira* did not cause any noticeable abnormalities in the structure of the cells present in the vaginal smear.

CONCLUSION

The present study shows that *Cissampelos pareira* leaf extract causes prolongation of estrous cycle in mice without producing any abnormalities in the cellular structures. This observation corroborates with the folklore information about the antifertility effect of the plant. However, more detailed investigation is required to reveal the mode of action of the extract.

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