



ANTIFERTILITY EFFECT OF ALCOHOLIC STALK EXTRACT OF *PIPER BETEL* LINN ON FEMALE ALBINO RATS

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ABSTRACT

To study the antifertility effect of methanolic extract of *Piper betel* Linn (PBL) in female albino rats.

Three months young female albino rats were administered with graded doses of methanolic extract of *Piper betel* Linn for 30 consecutive days and the effect of the extract on the estrus cycle was assessed by observing the changes produced in the vaginal smear, over the 30 days observation period, in comparison to the control.

The rats treated with PBL showed highly significant ($p < 0.05$), dose-dependent decrease in the numbers of estrus phase, in comparison to control rats. Large, cornified cells appeared after proestrus phase and number of cornified cells per field decreased. There was no significant change in the number of proestrus, Metestrus or diestrus phases of the estrus cycle, in the PBL treated group in comparison to control. Anestrus phase appeared in all the rats treated with PBL extract, which was not observed in the control group.

The methanolic extract of *Piper betel* Linn possesses antifertility activity in female albino rats.

Key words: Estrus cycle, vaginal smear, estrus phase, anestrus phase

INTRODUCTION

India within a few years of time span will be the leading country as far as the population growth is concerned. Since the population is rising tremendously, this may affect drastically the economic growth of India. Search for an effective, safe and reversible female antifertility agent with minimum side effects remain a challenge. To date many steroidal and non-steroidal substances are being used as contraceptive agents. Although they act as potent antifertility agents but they are not free from marked side effects¹. The major side effects associated with many potent antifertility drugs are thromboembolism, Irregularity in the menstrual cycle, mammary and other tissue cancers also occur².

The World Health Organization (WHO) has constituted a population control programme, which includes studies on drugs used in traditional medical practices. Medicinal plants¹ products have a long history of indigenous use in India as well as in other countries. Phytotherapy has a very long tradition, although proper scientific explanation is relatively new. In our country as well as in the world, there are several medicinal plants associated with antifertility properties^{3,4}. Fertility regulation with plants or plant preparations has been reported in the ancient literature of indigenous systems of medicine. A large number of plant species with anti-fertility effects have been screened in China and India, since last 50 years and were subsequently fortified by national and international agencies^{5,6}. However, the search for an orally active, safe and effective plant preparation or its compound is yet to be fulfilled for fertility regulation as many of them have incomplete inhibition of fertility or side effects.

Piper betel Linn. (Piperaceae) is a perennial dioecious creeper, probably native of Malaysia but cultivated in India for its leaves, used for chewing⁷. The leaf is carminative, aphrodisiac, tonic, laxative and improves appetite⁸. The alcoholic extract of the leaf-stalk showed significant antifertility effects in both male and female rats^{9,10,11}.

The present study was carried out for observing the antifertility effect of methanolic extract of the petiole (stalk)

of *Piper betel* Linn plant on the oestrous cycle of female albino rats. Interference with oestrous cycle by the plant extract might reflect the antifertility effect of the product as pregnancy requires a normal oestrous cycle. The oestrous cycle of the female albino rat lasts for four to five days and this short length of reproductive cycle can make it feasible for the changes produced by the extract on the reproductive cycle to be observed and compared with the control drug. Hence the oestrous cycle serves as a surrogate marker to evaluate the antifertility effect of the plant extract.

MATERIALS AND METHOD

Collection of Plant materials: The stalks of *Piper betel* Linn (PBL) were collected from the town of Niali in Cuttack District of Odisha, during the month of December 2009 between 4pm to 5pm. One and a half kg of the fresh stalks was collected. The plant material of *Piper betel* Linn were authenticated by the botanist. It was dried in shade for a period of one month. The weight after drying was taken and found to be 330 grams. The dried petioles were ground to powder, after which their weight reduced to 298 grams.

Preparation of Plant extract: The 298 grams of powdered stalk of *Piper betel* Linn was taken in the Soxhlet apparatus. The solvent used for extraction was methanol. The powder was soxhleted for 72 hours at temperature 60°C and concentrated under reduced pressure at 60°C. The final extract obtained amounted to 130 grams.

Experimental animals: Healthy adult female albino rats, three month old, weighing between 120 to 150 grams were selected. The animals were housed in polypropylene cages in groups of six, in the Central Animal House of KIMS Medical College, Bhubaneswar, under 12:12 light dark cycle, at 22°C, fed on germinating grams and allowed water ad libitum. The animals were acclimatized in the laboratory for 2 weeks. Four pre-treatment oestrous cycles were established by vaginal smears as described by Marcondes et al (2002). The female rats that have undergone four successive 4 days cycle were selected for this study. All the plan and procedures were

approved by the Institutional Animal Ethics Committee of S.C.B. Medical College, Cuttack, Odisha.

Stages of Oestrus Cycle of female albino rat¹²

Metestrus – The presence of mixture of leukocytes and epithelial cells.

Diestrus phase – Mainly leukocytes with few epithelial cells.

Proestrus phase – The presence of nucleated or nucleated plus cornified cells.

Estrus phase – The presence of cornified cells only.

Administration of extract: The methanolic extract of *Piper betel* Linn was administered to the female albino rats using gavaging technique. Extract was administered at the same time of the day i.e. 9.00am to 10.00am, everyday, for a period of 30 days. Utmost care was taken while handling the animals and it was ensured that the desired amount of extract reached down to the oesophagus of the animals.

Experimental design

The rats were separated in 4 groups of 6 rats each and treated as follows :

Group I: Animals are treated with distilled water for 30 days. They were called the control group.

Group II: The rats were treated with PBL extract (500 mg/kg body wt.,) for 30 days.

Group III: The rats were treated with PBL extract (1gram/kg body wt.,) for 30 days.

Group III: The rats were treated with PBL extract (1.5 gram/kg body wt.,) for 30 days.

Observation of Oestrous cycle: The effect of the plant extract on the oestrous cycle of the female albino rat was investigated, which is characterized by four phases, Proestrus, Estrus, Metaestrus and Diestrus, occurring in a cycle of 4 to 5 days. All the four phases of the oestrous cycle is determined according to the presence of cell types in the vaginal smear. The vaginal smear was described by Marcondes et al. (2002). The vaginal smear was collected by a sterile cotton swab inserted into the vagina gently¹³, for a continuous period of 30 days after administration of the extract. The vaginal smear was prepared in glass slides using Leishman's stain and observed under microscope under high power. The total length or duration of Proestrus, Estrus, Metaestrus and Diestrus was observed, tabulated and analysed statistically.

Statistical analysis : The observations were analyzed using Chi-square test and Mann-Whitney U test.

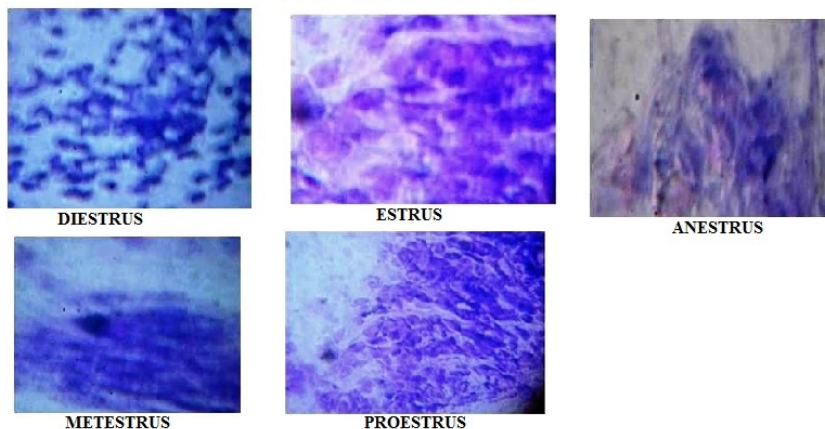
RESULT

Table 1: Effect of methanolic extract of stalk of *Piper betel* Linn. on phases of estrus cycle in rats for 30 days

Group	Drug / Dose (g/kg)	n	Total number of each phase in 30 days			
			Proestrus	Estrus	Metestrus	Diestrus
I	Distilled water	6	32	59	50	31
II	PBL 0.5	6	27	30*	55	35
III	PBL 1	6	29	39*	34*	36
IV	PBL 1.5	6	30	38*	35*	41

Mann Whitney U test, * = p<0.05 in comparison to control group.

STAGES OF RAT ESTRUS CYCLE



As observed in table 1, there was a significant change in the estrus cycle in the rats treated with methanolic extract of *Piper betel* Linn in comparison to the control group. The rats treated with PBL showed highly significant (p<0.05), dose-dependent decrease in the numbers of estrus phase, in comparison to control rats. Not only the occurrence of oestrus cycle decreased but also the histological appearances of the cells were changed. Large, cornified cells appeared after proestrus phase and number of cornified cells per field decreased.

There was no significant change in the number of proestrus, Metestrus or diestrus phases of the estrus cycle, in the PBL treated group in comparison to control.

Anestrus phase appeared in all the rats treated with PBL extract, which was not observed in the control group.

DISCUSSION

The methanolic extract of *Piper betel* Linn was investigated for antifertility activity in experimental model by observing the effects of 3 doses of the extract (0.5, 1, 1.5 mg/kg) for a period of 30 days on the oestrus cycle of female albino rats. Significant alteration in the estrus cycle occurred after 15

days of administration of the extract. The number of estrus phase reduced gradually with appearance of large cornified cells which were few in number. Anestrus phase appeared in all rats of the drug treated groups, but was not seen in control group. No vaginal bleeding was observed in any of the animals. Thus the estrous cycle of rats was significantly altered by the extract.

Normal estrus cycle is necessary for implantation and sustenance of pregnancy, which is in turn dependant upon exact equilibrium of secretion of oestrogen and progesterone. Any imbalance of these hormones can cause disturbance in the estrus cycle of rat^{14,15,16}. Both oestrogen and progesterone rise in proestrus and decline at the end of estrus, a coordinated action of these two hormones may play a part in ovulation and estrous cycle of rats. PBL extract might have interfered with the hormone synthesis which might have brought about changes in the mechanism of estrus cycle and cell cytology during estrus phase. These observations proved presence of antifertility activity of the extract

CONCLUSION

The methanolic extract of *Piper betel* Linn possess antifertility activity in female albino rats.

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