

ANTIFERTILITY EFFECT OF ALCOHOLIC AND AQUEOUS EXTRACTS OF *DOLICHANDRONE FALCATA* LEAVES ON ESTROUS CYCLE OF FEMALE ALBINO RATS.

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ABSTRACT

Ancient literature mentions the use of a number of plant preparations for fertility regulation. Documented experiments or clinical data are however lacking. Therefore the present study was undertaken to explore the abortifacient activity of *Dolichandrone falcata* and its effect on the estrous cycle. A strong abortifacient activity (100%) was observed at the dose level of 200 mg/kg and 400 mg/kg body weight of alcoholic and aqueous leaf extract of *Dolichandrone falcata*. The *Dolichandrone falcata* leaf extract was also found to prolong the estrous cycle significantly, particularly the diestrous phase.

Keywords: Antifertility activity, Estrous cycle, *Dolichandrone falcata*, Female albino rat.

INTRODUCTION

Ancient Indian literature abounds with information on a large number of plants reported to have abortifacient, contraceptive and sterilizing properties. Numerous plants have been reportedly used historically by women to aid child delivery, stimulate menstrual flow or reduce fertility^{1, 2}. Modern scientific studies in experimental animals have confirmed the effect of some of these herbs on the reproductive system.³⁻⁶ Scientific validity of the plants used by the tribal's for the purpose of contraception such as abortion, may generate greater confidence and wider acceptance of herbal contraceptives.

Dolichandrone falcata L. Bignoniaceae, is a deciduous tree commonly known as Medshingi in local areas of Melghat region of Maharashtra, India. It occurs as a small to medium sized tree, 6 to 15 meters in height. Different parts of this plant are reported to possess medicinal properties. Its bark paste is applied on fractured or dislocated bones⁷. It is also used as a fish poison. A decoction of the fruits is being used to procure abortion. Bark juice is used in cases of menorrhagia and leucorrhoea⁸. The leaves are used as anti-oxidant, antiallergic, anti-inflammatory, anti cancer, antiestrogenic and for anxiolytic activities⁹. Despite these traditional claims and successful isolation of the bioactive compounds, no in depth scientific study has been performed regarding its abortifacient properties and its effect on estrous cycle.

Therefore, the present work was undertaken to validate scientifically the abortifacient role of *Dolichandrone falcata* leaves as acclaimed by the traditional tribal user of Melghat region.

MATERIALS AND METHODS

Collection of plant material

The plant *Dolichandrone falcata* was collected from Melghat region and identified and authenticated by experts from Botanical Survey of India, Pune (Accession No. MA WD OF₁). A voucher specimen of the sample was deposited in the herbarium collection at department.

Preparation of extract

The leaves of *Dolichandrone falcata* were collected, shade dried, powdered and subjected to soxhlet extraction successively with distilled water and alcohol. The extract was evaporated to near dryness on a water bath and kept at 4°C in refrigerator until the experimental testing.

Phytochemical screening

The presence of various plant constituents in the plant extract was determined by preliminary phytochemical screening as described by Thimmaiah¹⁰.

Procurement and rearing of experimental animal

Albino rats (Wistar strain) used in the present investigation were procured from Sudhakarrao Naik Institute of Pharmacy, Pusad (M.S). The rats were acclimatized for 15 days to the best laboratory conditions (prior to experiment), and maintained on balanced diet (Trimurti lab feeds, Nagpur) and water was provided *ad libitum*.

Acute toxicity study

The animals were divided in four groups of six rats each. The extract was administered orally at the dose of 1000, 2000 and 4000 mg/kg body weight to the first three groups respectively. The fourth group was treated as control and received the vehicle only. The rats of both experimental and control groups were observed for 72 hr. for behavioral changes and mortality¹¹.

Abortifacient activity

The plant extract were tested in female albino rats for abortifacient activity by the method described by Khanna and Chaudhary¹². The vaginal smear of caged female rats of known fertility was monitored daily. Unstained material was observed under a light microscope. The proportion among the cells observed was used for determination of the estrous cycle phase. The female rats were caged with males of proven fertility in the ratio of 2:1 in the evening and examined the following day for the evidence of copulation. Rats exhibiting thick clump of spermatozoa in their vaginal smear were separated and that day was designated as 1st day of pregnancy. These rats were randomly distributed into four groups, a control group and three experimental groups of six animals each. On the 10th day of pregnancy animals were laprotomised under light anesthetic ether using sterile conditions. The two horns of uteri were examined to determine the implantation sites, thereafter the abdominal wound was sutured in layers. Post operational care was taken to avoid any infection.

The alcoholic and aqueous leaf extract of *Dolichandrone falcata* at a dose of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight was fed to operated pregnant rats daily by an intragastric soft rubber catheter from day 11 upto the 15th day of pregnancy. The animals were allowed to go full term. After delivery the pups were counted and the abortifacient activity of extract was evaluated. Litters were examined for malformations if any.

Effect on estrous cycle

The alcoholic and aqueous extract of *Dolichandrone falcata* leaves at a dose of 200 mg/kg and 400mg/kg was found to be active amongst the three treatments in antifertility testing. Hence it was subjected to a detailed investigation for study of estrous cycle. To

study the effect of *Dolichandrone falcata* leaves extract on the estrous cycle, the selected animals were divided into three groups of 6 rats each. Group 1 served as control group, group 2 received alcoholic extract and group 3 received aqueous extract at a dose of 200 mg/kg and 400 mg/kg respectively for 30 days. Vaginal smear using saline solution were taken twice daily during the entire treatment period to determine the cell type. The duration of estrous cycle together with that of various phases was determined^{13,14}.

All experimental protocols were subjected to the scrutinization and approval of institutional Animal Ethics Committee registration number 1060/ac/07/CPCSEA (IAEC/4/2009).

RESULTS

Phytochemical study

Preliminary phytochemical screening of the *Dolichandrone falcata* leaves extract revealed the presence of steroids, tannins, alkaloids, flavonoids, and saponins. (Table 1)

Acute toxicity

Acute toxicity test on the animals did not show any change in general behaviour, skin effects, defecation, loss of hair or other physiological activities. No mortality was observed in the treated and control group of the rats up to a dose of 4000 mg/kg body weight. Hence one-tenth of this dose was used for abortifacient testing.

Abortifacient activity

The alcoholic and aqueous extract of *Dolichandrone falcata* leaves when evaluated for their antifertility activity, were found to exhibit significant pregnancy interceptive activity. A dose level of 200 mg/kg and 400mg/kg body weight of alcoholic and aqueous extract respectively showed 100% abortifacient activity. The alcoholic extract at a dose of 100 mg/kg body weight showed 86.48% abortion, while 100 mg/kg and 200 mg/kg body wt. of aqueous extract showed 33.33% and 50.98% abortion respectively. The percent resorption index increased from zero in the control group to 100% in the 200 mg/kg body wt and 400 mg/kg body weight of alcoholic and aqueous extract treated animals. (Table 2, 3)

Table 1: Phytochemical profile of *Dolichandrone falcata* leaves extract

Name of the plant	Alkaloids	Simple Phenolics	Steroids	Anthraquinone	Flavonoids	Tannins	Saponins
<i>Dolichandrone falcata</i>	+	+	+	-	+	+	+

+ Present, - Absent

Table 2: Effect of aqueous extract of *Dolichandrone falcata* (leaves) on fertility of female rats when fed orally from day 11 to 15 of pregnancy

Treatment Groups	Body Wt. (gm)	Drug Dose (mg/kg of body wt)	Sample Size	No. of foetus in individual rats on day 10	No. of litter delivered	No. of resorption in individual rats	No. of resorption in Mean + S. E.	% abortifacient activity
Group - 1 Control (Vehicle)	150 -200	-	6	7,7,9,8,7,4	6(7,7,9,8,7,4)	0,0,0,0,0,0	0	Nil
Group - 2 Aqueous Extract	150 -200	100	6	11,8,8,8,7,9	6(8,5,6,6,4,5)	3,3,2,2,3,4	2.83 + 0.30***	33.33%
Group - 3 Aqueous Extract	150 -200	200	6	6,9,9,9,10,8	6(3,4,5,4,5,4)	3,5,5,4,5,4	4.33 +0.33***	50.98%
Group- 4 Aqueous Extract	150 -200	400	6	8,2,6,6,8,4	6(0,0,0,0,0,0)	8,2,6,6,8,4	5.66 + 0.95***	100%

Values in Means + S. E. (Standard error), n=6, *P<0.05, **P<0.01, ***P<0.001, When compared between group

Table 3: Effect of alcoholic extract of *Dolichandrone falcata* (leaves) on fertility of female rats when fed orally from day 11 to 15 of pregnancy

Treatment Groups	BodyWt.(gm)	Drug Dose(mg/kg of body wt)	Sample Size	No. of foetus in individual rats on day 10	No. of litter delivered	No. of resorption in individual rats	No. of resorption in Mean + S. E.	% abortifacient activity
Group - 1 Control (Vehicle)	150 -200	-	6	7,7,9,8,7,4	6(7,7,9,8,7,4)	0,0,0,0,0,0	0	Nil
Group - 2 alcoholic Extract	160 -200	100	6	5,7,8,6,6,5	6(0,1,0,2,1,1)	5,6,8,4,5,4	5.33+0.61***	86.48%
Group - 3 alcoholic Extract	150 -190	200	6	8,6,6,4,2,3	6(0,0,0,0,0,0)	8,6,6,4,2,3	4.83+0.91***	100%
Group- 4 alcoholic Extract	140 -190	400	6	9,8,11,8,9,14	6(0,0,0,0,0,0)	9,8,11,8,9,14	9.83+0.95***	100%

Values in Means + S. E. (Standard error), n=6, *P<0.05, **P<0.01, ***P<0.001, When compared between group.

Effect of extract on the estrus cycle of rats

The present study revealed that the alcoholic and aqueous extract of *Dolichandrone falcata* leaves showed an abortifacient effect.

Treatment of rats with alcoholic and aqueous extract prolonged the estrous cycle significantly (Table 4) characterized by a prolongation of the diestrus phase. The four phases of estrous cycle observed under the microscope reveal that a positive estrous

smear is one in which only large, irregular cornified cells are seen indicating maximum growth of the vaginal mucosa. A metaestrus smear will have many cornified cells, but also some leukocytes and epithelial cells, indicating the post ovulatory stage and desquamation of the vaginal mucosa. A diestrus smear will not only show few epithelial cells, mucous cells and few leukocytes,

indicating a quiescent uterus and resting vaginal epithelium. A proestrus smear will have many epithelial cells with granular cytoplasm, indicating a rapidly growing vaginal epithelium and also the preovulatory stage. Withdrawal of the treatment did not indicate any significant change either in the four phases of the estrous cycle, or in the duration of the cycle.

Table 4: Effect on estrous cycle of female albino rats after the administration of 200 mg/kg alcoholic extract and 400 mg/kg aqueous extract of *Dolichandrone falcata*

Phases	Proestrous phase (days)	Estrous phase (days)	Metaestrous phase (days)	Diestrous phase (days)	Estrous Cycle (Days)
Vaginal opening/ Cell type obtained in a vaginal smear	25% to 40% Epithelial cells only	Above 70% Few cornified cells	50% to 70% Cornified cells plus many leukocyte	50% to 70 % Leukocytes plus epithelial cells	
Group I Control	0.59 +0.04	0.65+0.08	0.81 + 0.14	2.27 +0.21	4.32+0.18
Group II Alcoholic extract 200 mg/kg	0.47+0.02***	.060 +0.01*	0.77 +0.02**	3.49+0.04***	5.33+0.21***
Group III Aqueous extract 400mg / kg	0.45+0.03***	0.56+0.02*	0.70+0.03***	4.18+0.02***	5.89+0.06***

Values in Mean + S. E. (Standard error), n = 6, *P<0.05, **P<0.01, ***P<0.001, When compared with control.

DISCUSSION

Termination of pregnancy has been practiced since antiquity. Although synthetic abortifacients of known mechanisms are effective and popular, but the risks associated with these drugs have triggered the need to develop new molecules from medicinal plants, which stimulated our interest in *Dolichandrone falcata* leaves.

Preliminary phytochemical studies of the extract indicated the presence of alkaloids, tannin, flavanoids saponins, steroids and phenolics. The extract of *Dolichandrone falcata* showed similar constituents like alkaloids and flavanoids which are reported to have contraceptive activity¹⁵.

Steroidal saponins, one of the active principles of most antifertility agent are also detected in *Dolichandrone falcata* leaves. The antifertility activities observed in the present study might be attributed to these active principles and it can be presumed that these may individually or synergistically affect the uterus leading to abortion. This finding agrees with that of Tafesse¹⁶ for causing abortifacient activity due to *Asparagus africanus* extract in rats.

The absence of clinical toxicity symptoms such as tremors, weakness, and refusal of feeds, diarrhea, weight loss, hair loss, coma and death suggests that the *Dolichandrone falcata* leaves extract was not clinically toxic to the female rats.

The pregnancy interceptive effect of the alcoholic and aqueous extract ranging from 86.48%, to 100% for alcoholic extract and from 33.33% to 100% for aqueous extract of *Dolichandrone falcata* leaves can be interpreted as due to the estrogenic nature of the plant. Regular development of all the events leading to nidation, at least in rats and mice, is chiefly under the direct command of estrogen-progesterone interplay at the cellular level¹⁷ and a slight disturbance in this hormonal balance may result in an unfavorable endometrial environment. Therefore, the increase in the number of dead fetus as well as reduced survival ratio is an indication of the post coital antifertility activity of the *Dolichandrone falcata* leaves. This finding agrees with that of the antifertility effect of *Senna alata* leaves extract in rats¹⁸ and methanolic extract of *Achyranthes aspera* leaves to pregnant rats¹⁹.

The rat has a characteristic short estrus cycle of 4 to 5 days in phases²⁰ which make them ideal for reproductive studies²¹. The presence and absence of four cell types and the relative proportion of each cell type, determine the stages of the estrous cycle. An estrous cycle is a rhythmic reproductive cycle in sexually matured female mammals²² and is influenced by the release of gonadotropin releasing hormone from the hypothalamus, gonadotropins from the pituitary gland and six hormones from the gonads. While female

cyclicity characterized by vaginal changes as observed in estrous cycle is an index of good functioning of the neuroendocrine reproductive system and ovarian activity, loss of normal estrus cycle indicates the disruption of ovarian progesterone and estrogen balance²³. An irregular pattern of estrous with a prolonged diestrus and consequently a reduced number of ova in the ovary was attributed to administration of *Gareenia Kola* seed extract²⁴. The present observation of irregular estrous cycle which correlates well with antifertility effect of *Dolichandrone Falcata* is consistent with the finding on neem flower extract²⁶.

In conclusion, the present study suggests that the alcoholic and aqueous extract of *Dolichandrone falcata* leaves show antifertility activity causing cessation of estrous cycle at the diestrus phase in albino rats.

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