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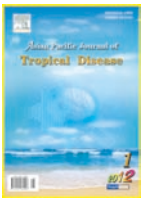

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Antifertility effect of chronically administered *Tabernaemontana divaricata* leaf extract on female albino mice

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ABSTRACT

Objective: To study the antifertility activity of *Tabernaemontana divaricata* leaf extract on female albino mice. **Methods:** Animals were divided into three groups consisting of five animals in each group. One group served as control and received vehicle orally for 21 days. The other two groups received dried ethanolic extract of leaves orally at a dose of 250 and 450 mg/kg animal body weight per day. After 21 days of treatment, extract was withdrawn from the mice and estrous cycle was studied for another 21 days. **Results:** The results of this study revealed that treatment of mice with extract of 250 and 450 mg/kg body weight for 21 days caused a prolonged eastrous cycle with significant increase in the duration of diestrus phase and elongation of estrus stage in treatment with higher dose (450 mg/kg body weight) and simultaneously decrease in the luteinizing hormone (LH) in both the treated groups and follicle stimulating hormone (FSH) in higher dose of treatment compared to the control animals may indicate the disturbance of estrous cycle and ovulation through suppression of FSH. **Conclusions:** It concludes that disturbance on the estradiol secretion with significant decrease during estrous stage of the cycle observed with the extract treatment may be due to impairment of LH and FSH.

1. Introduction

The quest for the oral contraceptive agent that can control human fertility is as old as recorded history. Although a wide variety of synthetic contraceptive agents are available[1, 2], these cannot be used continuously due to their severe side effects[3,4]. Hence people are looking back to age old tradition of using herbal medicines, which have minimum side effects. India in general and Western Ghats region in particular has enormous wealth of medicinal plants. Presently, a major programme on systematic investigation of medicinal plants for their phytochemical, biological and pharmacological properties, including antifertility properties, was undertaken in our laboratory [5, 6]. As a part of this research programme, we present in this paper antifertility efficacy of leaves of the plant *Tabernaemontana divaricata* (*T. divaricata*), a glabrous, evergreen shrub 1.8–2.4 m in height with silvery grey bark and milky latex. Leaves are simple, opposite, elliptic or elliptic–lanceolate, smooth, glossy green, acuminate and wavy margins; flowers are white, sweetly fragrant in 1–8 flowered cymes at the bifurcations of the branches, lobes of corolla overlapping to right in the bud. It is used as thermogenic, anodyne, astringent, vermifuge, odonalgia and in treatment of paralysis, arthralgia and melalgia. Flower juice mixed with oil alleviates burning sensation, cures eye sore and skin diseases, leaves juice applied to wounds to prevent inflammation and used in opthalmia[4]. Flower contains Dregamine, 20–epiervatamine, tabernaemontanine, vobasine, voacangine, voacamine, flavonoid aglycones, flavonol glycosides; isovoacristine, voaphylline–hydroxyindolenine, janetine (tetrahydrolivadine), N–methyl–voaphylline (hecubine),Kaempferol, and leaves contains Dregamine, 20–epiervatamine, tabernaemontanine, vobasine, voacangine, voacamine, flavonoid aglycones, flavonol glycosides, isovoacristine, α–amyrin, lupeol and their acetates, β–sitosterol, coronaridine, apparicine, ervaticine (2–acyl indole derivative), ervatinine, hyderabadine, lahoricine, mehranine, stapfinine, voacristine, voharine and a dimeric alkaloid, conophylline 17–β oestrdiol. The present study was therefore carried out to evaluate the claimed antifertility effect of *T. divaricata*

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leaves using different aspects of reproductive physiology in albino mice.

2. Materials and Methods

2.1. Collection of plant material and extraction

Leaves of *T. divaricata* (Linn.) were collected from Indore Madhya Pradesh India. The leaves were shade dried, the dried leaves were coarse powdered and the powder was packed into soxhlet column and extracted successively with petroleum ether (60-80 ℃), ethanol (64.5-65.5 ℃) and distilled water. The extracts were concentrated under reduced pressure (Bath Temp 50 ℃). The dried extracts were stored in airtight container.

2.2. Phytochemical studies of the extract

Phytochemical studies of the ethanolic leaf extract were carried out by qualitative and thin layer chromatography (TLC) methods produced by Harbone, 1884[7].

2.3. Animals

Laboratory bred virgin female Swiss albino mice aged 85-100 days weighing between 22-25 g, showing regular estrous cycle were used and were allowed free access to water and food (bread, gram, salted corn powder, etc.) throughout the study.

2.4. Test material administration

The leaf extract was administered orally in two different doses of 250 mg/kg body weight and 450 mg/kg body weight to two experimental groups of albino mice. The dose for each group was calculated considering the human dose based on ethnomadical uses of the plant for birth control[8]. The hepatotoxicity of the extract was tested by carrying out liver function tests after regular intervals. The serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruveta transaminase (SGPT) levels in the mice administered with dose of 250 mg/kg body weight showed no significant change (P>0.05). Initially, the other dose 500 mg/kg body weight (double of the first dose) was selected. But this dose was observed to cause a significant elevation in SGOT and SGPT levels of the mice after 10 days of extract administration. Hence a lesser dose of 450 mg/kg body weight was selected, as this dose caused no significant change in transaminase activity during the study period.

2.5. Pharmacological screening

2.5.1. Study of estrous cycle

Animals were divided into three groups consisting of five animals in each group. One group served as control and received vehicle orally for 21 days. The other two groups received dried ethanolic extract of leaves orally at a dose of 250 and 450 mg/kg body weight. The estrous cycle was studied by stained preparation of vaginal smear of the animals. The stage of estrous cycle and its duration were determined as described by Mokonnen, 1999[9].

After 21 days of treatment, extract was withdrawn from the mice and estrous cycle was studied for another 21 days, i.e., post-extract period.

2.5.2. Study of reproductive outcome in mice

Three groups of mature female mice (five mice each group) were selected as mentioned above. Two groups received leaf extract for 8 days and control group received vehicle for the same period. All the experimental mice were then allowed to mate with mature fertile mate mice and the treatment was continued for 21 days. The number of litter was determined after the completion of one gestation period in all experimental groups. The litters were allowed to grow and the growth of litters produced from the extract-administered group was compared with those of control group.

The reversibility of antifertility effect of the extract was also studied in the treated groups according to the method of Edwin S *et al*[10]. For this study, the extract was administered continuously for 21 days and then the extract was withdrawn. After 21 days of extract withdrawal, animals were allowed to mate with male mice. The number of litter was determined after the completion of one gestation period.

2.5.3. Study of reproductive hormones

Blood samples were collected from the caudal vein of the animal in all the stages of estrous cycle. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, 17 β-estradiol and 17 OH progesterone concentrations were measured by ELISA micro well kits.

2.5.4. Determination of oral LD50

LD_{50} of the leaf extract was determined as described by Salhad, 1997[11]. The extract was administered orally into six groups of mice (10 mice each group) in six different doses. i.e., 3, 4, 5, 7 and 8 g/kg body weight. Based on mortality mice, the oral LD50 value for 24 h was calculated.

2.6. Statistical analysis

The data were statistically analyzed and expressed as mean ±SEM. Statistical analysis of the variance between control and experimental values was done student's t-test[12].

3. Results

3.1. Phytochemical studies

Table 1

Effect of ethanolic extract of *T. divaricata* on the estrous cycle of mice for 21 days and number of litters produced in different groups of mice

Groups	Duration of estrous cycle (days)	Duration of different stages of estrous cycle (days)				No. of litters
		Proestrus	Estrus	Metestrus	Diestrus	
Group 1(control)	4.63±0.14	0.97±0.07	0.99±0.19	0.87±0.15	1.98±0.21	7.90±0.12
Group 2 (250 mg/kg body weight)	4.87±0.52	0.56±0.13	1.25±0.23	0.63±0.25	3.72±0.45	3.80±0.34
Group 3: (post- treatment of 250 mg/kg body weight))	4.62±0.42	0.86±0.56	1.23±0.23	0.86±0.13	1.85±0.21	7.40±0.42
Group 4 (450 mg/kg body weight))	6.21±0.52	0.49±0.32	1.78±0.12	0.49±0.56	3.50±0.46	2.50±0.23
Group 5 (post- treatment of 450 mg/kg body weight))	5.00±0.54	0.47±0.23	1.35±0.48	0.78±0.16	2.38±0.25	5.90±0.38

N = 6, data are mean±SEM

Table 2

Hormone levels in various groups of animals during the study

Different hormones	Groups	Stages of estrous cycle (days)			
		Proestrus	Estrus	Metestrus	Diestrus
LH mlU/mL	Control	9.68±0.21	4.48±0.13	0.78±0.23	0.87±0.12
	250 mg/kg body weight/day	7.02±0.43	3.58±0.65	0.79±0.12	1.08±0.42
	450 mg/kg body weight/day	6.02±0.21	2.23±0.15	0.68±0.18	0.48±0.34
FSH mlU/mL	Control	7.18±0.67	8.30±0.23	3.37±0.24	4.35±0.57
	250 mg/kg body weight/day	8.38±0.18	10.56±0.26	3.05±0.12	6.12±0.24
	450 mg/kg body weight/day	5.86±0.14	4.47±0.34	3.24±0.56	2.64±0.34
Prolactin ng/mL	Control	30.60±0.21	24.74±0.23	12.10±0.76	15.30±0.15
	250 mg/kg body weight/day	34.12±0.12	27.15±0.12	10.18±0.59	19.48±0.25
	450 mg/kg body weight/day	43.46±0.23	26.08±0.56	10.47±0.56	19.37±0.75
Estradiol ng/ml	Control	806.12±0.53	712.04±0.34	274.00±0.16	283.54±0.45
	250 mg/kg body weight/day	667.41±0.57	461.75±0.45	264.19±0.23	265.19±0.87
	450 mg/kg body weight/day	944.05±0.23	512.25±0.56	215.08±0.34	200.52±0.62
17 OH Progesterone ng/mL	Control	10.45±0.23	11.70±0.58	16.61±0.25	21.84±0.68
	450 mg/kg body weight/day	11.42±0.21	13.25±0.45	15.47±0.56	23.12±0.53

N=6, data are mean±SEM

Qualitative TLC studies of the extract revealed the presence of sterol, steroids, alkaloids and flavonoids.

3.2. Effect of the extract on the estrous cycle and the reproductive hormones

The result from the cytological, hormonal and reproductive screening (Table 1 and 2) in the present study revealed that the ethanolic extract of *T. divaricata* leaves could be responsible for the antifertility effect. Treatment of mice with extract of 250 and 450 mg/kg body weight for 21 days caused a prolonged eastrous cycle with significant increase in the duration of diestrus phase (Table 1) and elongation of estrus stage in treatment with higher dose (450 mg/kg body weight).

Treatment of mice with leaf extract decreased the mean number of litters (Table 1) suggesting the antifertility effect of the extract. The number of litters appeared to decrease more with higher dose of treatment, which may suggest dose dependant antifertility effect. All the litters of treated mice grew up normally without showing any physical abnormality indicating that the plant is not abortifacient and teratogenic in albino mice. Absence of toxicity and any of the doses administered justifies the safe nature of the leaf extract. The LD50 of leaf extract was found to be 6.9 g/kg in mice. The increase in the number of litters observed in both the post-treatment groups may suggest reversibility of the antifertility effect.

4. Discussion

In the present study the decrease in the LH in both the treated groups and FSH in higher dose of treatment compared to the control animals may indicate the disturbance of estrous cycle and ovulation through suppression of FSH. In the present study an increase in prolactin level was observed which was more pronounced during proestrus stage with higher dose of extract. These observations are comparable with the studies made by Aprioku JS *et al*[14], who reported that a combination of enhanced prolactin and suppressed LH secretion in adult mice is due to prolongation of estrus cycle.

The present study is comparable with the studies made by Gbotolorun SC *et al* and Aprioku JS *et al*[9, 14], who had reported antifertility effect with similar observation in guinea pig and rats on treatment with seed extract of Ricinus communis and root extract of Rumex steudelii, respectively. However, significant decrease in the duration of proestrus and metestrus stage in experiment group was recorded than those of control animals. These changes were found to revert back after withdrawal of the treatment except proestrus stage in groups with higher dose of treatment. The prolongation

of diestrus phase may lower the chance of pregnancy in animals.

In our study, no detectable change was observed in the level of progesterone with treatment of leaf extract. Disturbance on the estradiol secretion with significant decrease during estrous stage of the cycle observed with the extract treatment may be due to impairment in the release of LH and FSH causing hormonal imbalance. These observations could also suggest the antifertility effect of *T. divaricata* leaves.

Conflict of interest statement

We declare that we have no conflict of interest.

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