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Pharmacological evaluation of anti-fertility activity of ethanolic extract of *Jatropha gossypifolia* leaf in female albino mice

Sachin Jain[*1], Gajendra Pratap Choudhary[2], Dinesh Kumar Jain[1]

[1]*College of Pharmacy, IPS Academy, Rajendra Nagar, AB Road, Indore MP INDIA 452012*
[2]*School of Pharmacy, DAVV Indore MP INDIA 452012*

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ABSTRACT

Objective: Anti-fertility activity of ethanolic extract of *Jatropha gossypifolia* leaf in female albino mice. **Methods:** *Jatropha gossypifolia* leaf extract, when administered orally, altered the estrous cycle pattern in female mice, prolong the length of estrous cycle with significant increase in the duration of diestrus stage and reduced significantly the number of litters in albino mice. Treatment of mice with extract of 250 and 450 mg/kg body weight/day for 21 days caused a prolonged estrous 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/day). **Results:** The analysis of the principal hormones involved in estrous cycle regulation showed that the plant extracts altered gonadrotrophin release (LH, FSH and prolactinn) and estradiol secretion. **Conclusions:** The results indicated the anti-fertility effect of *Jatropha gossypifolia* leaf extract in female albino mice.

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 [1, 2] are available, 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 part of this research programme, we present in this paper antifertility efficacy of leaves of the plant *Jatropha gossypifolia*. An 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 strangury, 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, [7]. Flower contains flavonoid aglycones, flavonol glycosides; isovoacristine, N-methyl-voaphylline (hecubine). Kaempferol, and leaves contains Dregamine, and a dimeric alkaloid, conophylline 17- β oestrdiol. It contains compounds other than diterpenoids. The chief compounds reported are triterpenoid, sterol, alcohol and hydrocarbon. The phenolic compounds include flavonoid lignans, coumarin tannin, phenanthrenes, quiones, phenolic acid, alkaloids, cyanogenic glucosides and glucosinolates [8].

The present study was therefore carried out to evaluate the claimed antifertility effect of *Jatropha gossypifolia* leaves using different aspects of reproductive physiology in albino mice.

2. Material and Methods

*Corresponding author: Sachin Jain Assistant Professor Sachin Jain Assistant Professor College of Pharmacy, IPS Academy, Rajendra Nagar, AB Road Indore India
E-mail: sachin225819@rediffmail.com
Tel: +91942444386
Fax: 07314041627

2.1 Collection of plant material and extraction

Leaves of *Jatropha gossypifolia* were collected from Indore (Madhya Pradesh) and authenticated by Prof. S. R. Upadhyaya (Botanist) Government P.G. College, Indore MP India. The leaves of *Jatropha gossypifolia* were collected and shade dried. The dried leaves were coarse powdered and the powder was packed in to soxhlet column and extracted with ethanol (64.5 – 65.5℃). The extract was concentrated under reduced pressure (bath temp 50℃). The dried extract was stored in airtight container in refrigerator.

2.2 Phytochemical studies of the extract

Phytochemical studies of the ethanolic leaf extract were carried out by qualitative and TLC methods produced by [9, 10].

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/day and 450 mg/kg body weight/day 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 [11]. The hepatotoxicity of the extract was tested by carrying out liver function tests after regular intervals. The Serum Glutamate Oxaloacetate Transaminase (SGOT) Serum Glutamate Pyruvate Transaminase (SGPT) level in the mice administered with doe of 250 mg/kg body weight/days showed no significant change ($P > 0.05$). Initially, the other dose 500 mg/kg body weight/days (which was the double of the first dose) was selected. However, 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/days 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 & 450 mg/kg animal body weight/day. The estrous cycle was studied by stained preparation of vaginal smear of the animals. The stage of estrous cycle and its duration were determined [12].

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/group) were selected as mentioned above. Two groups received leaf extract for 8 days 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 [13]. 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 sample were collected from the caudal vein of the animal in all the stages of estrous cycle. Serum FSH, LH, prolactin, 17 β estradiol and 17 0H progesterone concentrations were measured by ELISA micro well kits.

2.5.4 Determination of oral LD_{50}

LD_{50} of the leaf extract was determined as described by [14]. The extract was administered orally into six groups of mice (10 mice/group) in six different doses. i.e., 3, 4, 5, 7 and 8 g/kg body weight/day. Based on mortality rat, the oral LD50 value for 24 h was calculated.

2.5.5 Statistical analysis

The data were statistically analyzed and expressed as mean ±S.E.M. Statistical analysis of the variance between control and experimental values was done student's t-test [15].

3. Results

3.1 Phytochemical studies

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

Table 1

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

S. No.	Groups	Duration of estrous cycle (days)	Duration of different phages of estrous cycle (days)				No. of litters
			Proestrus (days)	Estrus (days)	Metestrus (days)	Diestrus (days)	
1	Group 1: Control	4.63 ±0.14	0.97 ±0.07	0.99 ±0.19	0.87 ±0.15	1.98 ±0.21	7.9 ±0.12
2	Group 2: 250 mg/kg bw/d	4.87 ±0.52	0.56 ±0.13	1.25 ±0.23	0.63 ±0.25	3.72 ±0.45	3.8 ±0.34
3	Group 3: Post- treatment of 250 mg/kg bw/d	4.62 ±0.42	0.86 ±0.56	1.23 ±0.23	0.86 ±0.13	1.85 ±0.21	7.4 ±0.42
4	Group 4: 450 mg/kg bw/d	6.21 ±0.52	0.49 ±0.32	178 ±0.12	0.49 ±0.56	3.50 ±0.46	2.5 ±0.23
5	Group 5: Post- treatment of 450 mg/kg bw/d	5.00 ±0.54	0.47 ±0.23	1.35 ±0.48	0.78 ±0.16	2.38 ±0.25	5.9 ±0.38

N = 6 data are Mean±SEM

Group 2 and group 4 compared with group 1: **= highly significant ($P \leqslant 0.001$)

Table 2

Hormone levels in various groups of animals during the study

S. No.	Different hormones	Groups	Stages of estrous cycle			
			Proestrus	Estrus	Metestrus	Diestrus
1	LH mlU/ml	Control	9.68 ±0.21	4.48 ±0.13	0.78 ±0.23	0.87 ±0.12
2		250 mg/kg bw/d	7.02 ±0.43	3.58 ±0.65	0.79 ±0.12	1.08 ±0.42
3		450 mg/kg bw/d	6.02 ±0.21	2.23 ±0.15	0.68 ±0.18	0.48 ±0.34
4	FSH mlU/ml	Control	7.18 ±0.67	8.3 ±0.23	3.37 ±0.24	4.35 ±0.57
5		250 mg/kg bw/d	8.38 ±0.18	10.56 ±0.26	3.05 ±0.12	6.12 ±0.24
6		450 mg/kg bw/d	5.86 ±0.14	4.47 ±0.34	3.24 ±0.56	2.64 ±0.34
7	Prolactin ng/ml	Control	30.60 ±0.21	24.74 ±0.23	12.10 ±0.76	15.30 ±0.15
8		250 mg/kg bw/d	34.12 ±0.12	27.15 ±0.12	10.18 ±0.59	19.48 ±0.25
9		450 mg/kg bw/d	43.46 ±0.23	26.08 ±0.56	10.47 ±0.56	19.37 ±0.75
10	Estradiol pg/ml	Control	806.12 ±0.53	712.04 ±0.34	274.00 ±0.16	283.54 ±0.45
11		250 mg/kg bw/d	667.41 ±0.57	461.75 ±0.45	264.19 ±0.23	265.19 ±0.87
12		450 mg/kg bw/d	944.05 ±0.23	512.25 ±0.56	215.08 ±0.34	200.52 ±0,62
13	17 OH Progesterone ng/ml	Control	10.45 ±0.23	11.70 ±0.58	16.61 ±0.25	21.84 ±0.68
14		450 mg/kg bw/d	11.42 ±0.21	13.25 ±0.45	15.47 ±0.56	23.12 ±0.53

N = 6 data are Mean±SEM

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 *Jatropha gossypifolia* leaves could be responsible for the antifertility effect. Treatment of mice with extract of 250 and 450 mg/kg body weight/day for 21 days caused a prolonged estrous 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/day).

4. Discussion

The present study is comparable with the studies made by [7], 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.

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 LD_{50} 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.

In the present study the decrease in the LH in both the treated groups and FSH in higher dose of treatment (Table 2) 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 [8], who reported that a combination of enhanced prolactin and

suppressed LH secretion in adult mice is due to prolongation of estrus cycle.

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 *Jatropha gossypifolia* leaves.

5. Conclusion

The present study demonstrated that an ethanolic extract of *Jatropha gossypifolia* leaves has the antifertility activity. Enhancement of prolactin and suppressed of LH secretion in adult mice which increase the prolongation of estrus cycle. The analysis of the principal hormones which involved in estrous cycle regulation shows the alteration in gonadrotrophin release (LH, FSH and prolactinn) and estradiol secretion.

Indication of tables

Table 1 Effect of ethanolic extract of *Jatropha gossypifolia* on the estrous cycle of mice for 21 days and number of litters produced in different groups of mice.

Table 2 Hormone levels in various groups of animals during the study.

Conflict of interest statement

We declare that we have no conflict of interest.

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