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Research Article

**EVALUATION OF ANTI-FERTILITY ACTIVITY OF
PAEONIA LACTIFLORA IN WISTAR MALE RAT**Sorabh Kumar Agrawal*¹, N.Abhinaya²

1. Associate professor, Department of Pharmacology, SSJ College of Pharmacy, V.N.Pally, Gandipet, Hyderabad-75, India
2. Department of Pharmacology, SSJ College of Pharmacy, V.N.Pally, Gandipet, Hyderabad-75, India

Abstract

In the present study, antifertility activity was evaluated in methanolic extract of Paeonia Lactiflora. The methanolic extract of Paeonia Lactiflora showed significant antifertility activity. Pre-treatment with methanolic extract showed significant effect on sperm count, motility, testicular histopathology & testosterone at a dose of 500 mg/kg.

Key words: *PaeoniaLactiflora, antifertility activity, Sperm count*

Corresponding author:

Dr. Sorabh Kumar Agrawal,
Associate professor, Department of Pharmacology,
V.N.Pally, Gandipet, Hyderabad-75, India
Email id: doctorsorabh@gmail.com



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INTRODUCTION

India within, few years of time span will be the leading country as far as the population growth is concerned. Since the population rising tremendously, this may affect drastically the economic growth of India. Family planning has been promoted through several methods of contraception, but due to side effect produced by the use steroidal contraceptive and use of abortifaciant drugs [1]. There is a need of drug which is effective with lesser side effects. The plant *Paeonia Lactiflora* are proved and described in Pharmacopoeias & well stabilised documents shown the anti-bacterial, anti-influenza [2], anti-viral [2], anti-inflammatory [3], anti-spasmodic activity. The plant *Paeonia Lactiflora* also used to treat dementia, headache, vertigo, spasm of calf muscle [4, 5]. The dried root of *Paeonia Lactiflora* contains Paeoniflorin, a mono terpene glycoside that is the major active constituent, is present in the range of 0.05–6.01%. Traditionally *Paeonia Lactiflora* plant used in treatment of atopic eczema, boils, and sores; to reduce fevers, induce sterility, and treat burns. The aim of the present study was to evaluate antifertility activity of *Paeonia Lactiflora* in male rats.

MATERIALS AND METHODS

Plant material

The fresh roots of *Paeonia Lactiflora* was collected and authenticated by botanist. Aspecimen sample of the same was preserved in the herbarium section for future reference. The root of *Paeonia Lactiflora* were, chopped into small pieces and dried under shade at room temperature for seven days. The dried roots was powdered and passed through the sieve (coarse10/40). The powder was used for the preparation of extract.

Extraction was done by soxhlet extraction process by using Petroleum ether and 95% w/v methanol [6, 7]. The percentage yield was found to be 14.2% w/w. Preliminary phytochemical studies showed the presence of flavonoids, glycoside, carbohydrates, tannins, amino acid and sterols etc.

Acute toxicity study [8, 9]

Acute toxicity study of methanolic extract of *Paeonia Lactiflora* was carried out in mice according to OECD guidelines. Extract at different doses up to 5000 mg/kg, p.o. was administered and animals were observed for behavioural changes, any toxicity and mortality up to 48 h. There was no toxic reaction or mortality, and found to safe. Based on acute toxicity result we have selected 250 mg/kg and 500 mg/kg for antifertility evaluation.

Experimental Protocol

Test compound: - The Methanolic extract of *Paeonia Lactiflora* (250mg/kg & 500 mg/kg body weight)

Standard drug – Lonidamine 50 mg/kg

Animal-

Male wistar rats (150-200 g) of were obtained from animal house for the study. They were housed under standard condition of temperature (24 ±10 C), relative humidity (65±10%), light and dark cycle (14:10 h) and fed with standard pellet food. The initial body weight of each animal was recorded. All experimental procedures were carried out in strict accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision on Experimentation on Animals and were approved by the Institutional Animal Ethics Committee. (IAEC No: - Pharm/13/06)

Experimental design:

The animals were divided into three groups of 6 animals each.

Group I: Received only (tween 80%) (p.o./ daily) for 60days and served as control.

Group II: Received only standard drug 50 mg/kg body weight (p.o./daily) for 60days and served as standard.

Group III: Received methanolic extract of roots of *Paeonia Lactiflora*, at the dose of 250mg/kg bodyweight(p.o./daily) for 60 days .

Group IV: Received methanolic extract of roots *Paeonia Lactiflora*. At 500 mg/kg body weight (p.o.daily) for 60days.

The methanolic extract of roots of *Paeonia Lactiflora*. Was dissolved in tween80,1% and administered orally by gastric in tubation. Body weights were assessed every alternate day and dose was adjusted accordingly.

Parameters for investigation :

1. SPERM COUNT AND MOTILITY [11]:

The cauda epididymis was chopped in 10ml normal saline, the aliquots of sperm suspension was filled upto 0.5 mark in WBC pipette and diluted with saline upto 11 mark. Sprm count was done in Neuber's chamber in WBC squares. Spermcount/ml was calculated as follows.

$X \times 20 \times 10^4 / \text{cm}^2 / \text{ml} / \text{epididymis}$. Where 'X' is the average mean of spermatozoa of all 4 squares. (Table no. 1)

2. BODY AND ORGAN WEIGHT:

The initial and final body weights of the animals were recorded. The testes, epididymis, seminal vesicle and ventral prostate were dissected out, freed from adhering tissue, blotted on a filter paper and weighed on a sensitive balance (Precisa, XB series) to the nearest milligram. (Table no. 2&3)

3. RADIO IMMUNO ASSAY OF TESTOSTERONE:

Blood samples were collected by retro orbital and allowed to clot at room temperature for about 1h and these rum was separated by centrifuging at 3000-4000 rpm for 15min. Serum levels of testosterone were assayed in duplicate

using DSL-4000 active Testosterone Coated-Tube Radio immune assay kit. (Table no. 4)

4. TISSUE BIOCHEMISTRY:

The other testes (left) were kept at -20°C until assayed for cholesterol and ALP. The testis was homogenized with ice-cold distilled water in a pre-cooled mortar and pestle to contain 10mg/ml. The homogenate was centrifuged at 3000 rpm for 15 minutes and the supernatant was used for the estimation of cholesterol Content and alkaline phosphates activity (ALP) using (Auto span, Spandiagnostics Ltd) diagnostic kits. (Table no. 5)

5. STATISTICAL ANALYSIS:

Analysis of each data set was performed by

student-t test, and one-way analysis of variance (ANOVA). Statistically significant effects were further evaluated with Newman-Keuls tests. Differences were considered significant at $P < 0.01$. Results were expressed as means \pm SEM.

RESULT AND DISCUSSION

Preliminary phytochemical studies showed the presence of flavonoids, glycoside, carbohydrates, tannins, amino acid and sterols. Methanolic extract of root of *Paeonia Lactiflora* significant effect on sperm count, motility, weight of reproductive organ & serum testosterone level in male rats at high dose 500mg/kg.

Table No.1

Effects of Methanolic extract of *Paeonia Lactiflora* on Epididymal sperm density and motility

Group	Sperm Density(count/ml)	Sperm Motility(%)
Control	58.91 \pm 2.84	74.0 \pm 0.85
Standard	18.08 \pm 2.38	22.66 \pm 0.80
Test Group -I	50.08 \pm 2.38	62.06 \pm 0.80
Test Group- II	32.53 \pm 0.81	44.5 \pm 0.76

Values are represented as mean \pm S.E.M (n=6)

One-way ANOVA followed by Student-Newman-Keuls post test ($P < 0.001$)

Table2: Effect of Methanolic Extract of *Paeonia Lactiflora* on Body Weight(g)

Treatment	Day1	Day61
Control	244.0 \pm 5.85	246.0 \pm 7.17
Standard	241 \pm 5.85	245 \pm 5.85
Test Group -I	251.63 \pm 2.27	254.6 \pm 6.12
Test Group - II	235.66 \pm 4.01	238.66 \pm 16.92

Values are represented as mean \pm S.E.M (n=6)

One-way ANOVA followed by Student-Newman-Keuls post test ($P < 0.001$)

Table 3: Effect of Methanolic Extract of *Paeonia Lactiflora* on Reproductive Organ Weights (g/kg bodyweight)

Treatment	Testis	Epididymis	Seminal vesicle	Prostategland
Control (Tween80,1%)	7.60±0.16	3.08±0.22	4.93±0.07	2.30±0.07
Standard	4.60±0.16	2.01±0.11	2.60±0.01	1.80±0.16
Test Group -I	6.83±0.17	2.65±0.07	3.06±0.06	2.90±0.13
Test Group -II	5.02±0.15	2.38±0.08	2.90±0.13	1.95±0.02

Values are represented as mean ± S.E.M (n=6)

One-way ANOVA followed by Student-Newman-Keulspost test (P< 0.001)

Table 4: Effect of Methanolic Extract of *Paeonia Lactiflora* on Serum Testosterone level in ng/ml

Treatment	Testosterone Level(ng/ml)
Control	4.23±0.04
Standard	1.80±0.16
Test Group -I	3.23±0.24
Test Group -I	2.41±0.04 *

Values are represented as mean ± S.E.M (n=6)

One-way ANOVA followed by Student-Newman-Keulspost test (P< 0.001)

Table 5: Effects of Methanolic Extract of *PaeoniaLactiflora* on Biochemical parameters

Treatment	Cholesterol mg/gin Testes	ALP ActivityIU/Lof Testes
Control	148.8±0.14	70.07±0.09
Standard	115±0.16	97.6±0.16
Test Group -I	125±0.28 ^b	62.08±0.28 ^a
Test Group -II	110±0.28	74.75±0.38 ^a

Values are represented as mean ± S.E.M (n=6)

One-way ANOVA followed by Student-Newman-Keulspost-test (P< 0.001)

CONCLUSION [12-15]

The methanolic extract of *Paeonia Lactiflora* at the dose of 500 mg/kg showed a significant increase in the serum testosterone level, increased ALP and cholesterol levels and a mild hyper cellularity of leydigcells in the histopathological observation. Hence further studies need to be carried out to investigate the effect of higher doses of Methanolic extract on the androgenic activity in male rats. The male antifertility study showed that the methanolic extract of root have male antifertility activity at the dose of 500mg/kg.

Hence further studies need to be carried out to find out the cytotoxic effect and interaction at the receptor level to find out the most probable mechanisms of anti-fertility activity.

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