



Evaluation of *Cissampelos pareira* root for antifertility activity

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ABSTRACT

Background: The practice of traditional medicine for the control of fertility in most parts of India is based on the uses of plant medicines for many years. *Cissampelos pareira* Linn. is one of the folk medicinal plants commonly used as antifertility agent in some places of India.

Objective: The aim of the present study was to evaluate the antifertility effect of the methanolic extract of *Cissampelos pareira* root with mechanism of action. **Methods:** The methanolic extract of *Cissampelos pareira* root were investigated for its effect on estrous cycle, effect on implantation and reproductive hormones in female rats at two oral dose level: 250 mg/kg and 500 mg/kg. Serum estradiol and progesterone level were estimated by ELISA method. Serums were isolated from blood samples collected on 12th, 19th, and 21st day of pregnancy. **Results:** No significant effect on extending the estrous cycle has been found with methanolic extracts of *Cissampelos pareira* root [CPR] treated group as compared to control group. Reduction in the number of implants in CPR treated group is significant. Furthermore, there is no significant change in serum estradiol and progesterone level from day 12th to 19th in CPR treated groups as compared to control group. **Conclusion:** All these observations suggest that CPR neither has any effect on extending the estrous cycle nor on implantation. We may conclude that alkaloids, the chief chemical constituent, present in CPR has no role in regulating the fertility.

KEYWORDS: Antifertility effect; Estrus cycle; Female rats; *Cissampelos pareira* root methanolic extract [CPR];

1. INTRODUCTION

At present the total population of the world is 7 billion 2 crore¹. Current population of India is about 1.27². The ever increasing population is the concern for having a small family for which contraceptive becomes essential part of our life. There are different kinds of contraceptives available in market that act at different stages of fertilization. Each type of synthetic contraceptive has some side effects. Antifertility agents are capable of reducing or eliminating fertility. Safer antifertility drugs are the main motto of all research on anti-fertility projects. The various literature surveys have been suggested that synthetic antifertility agents have severe side effect like breast cancer, cervical cancer etc^{3:4:5}. Traditional medicines are practiced worldwide for regulation fertility since ancient times. Natural birth control includes the use of herbs which may be taken orally, to extend the duration of estrous cycle or to interfere with ovulation or implantation or abortifacient or inserted vaginally, to act as natural spermicidal. Examples of these include lemon juice, wild yam, wild carrot and neem.

Some herbal contraceptives have a cumulative effect in the body; they need to be taken regularly to maintain the contraceptive effect. Examples are wild yam and neem. A comprehensive summary of medicinal flora inhabiting throughout the world regarding their traditional usage by various tribes/ethnic groups for fertility regulation in females is as follows: 577 plant species belonging to 122 families traditionally used in fertility regulation in females⁶. *Cissampelos pareira* Linn var. is used to control fertility temporarily from different parts of Assam, India has been reported. It is thought to be an excellent remedy to alleviate and help with symptoms associated with menstruation and balances hormones in women. *Cissampelos pareria* leaf extract, when administered orally, altered the oestrous cycle pattern in female mice, prolonged the length of oestrous cycle with significant increase in the duration of diestrus stage and reduced significantly the number of litters in albino mice. Investigation on reproductive hormones (estrogen and progesterone) involved in estrous cycle regulation revealed that the *Cissampelos pareira* leaf extract altered gonadotropin release (LH, FSH and prolactin) and estradiol secretion⁷. The methanolic extract of *Cissampelos pareira* stem has significant ($p < 0.001$) antifertility effect and is safe at the effective doses (250mg/kg and 500mg/kg). Methanolic extract of *Cissampelos pareira*

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stem had been shown its anti-implantation activity by decreasing the progesterone level. Flavonoids present in methanolic extract of *Cissampelos pareira* stem is one of the responsible chemical constituent for the antifertility activity of the extract⁸.

The present study was, therefore, carried out to evaluate the *Cissampelos pareira* root for antifertility effect using different parameters like effect on estrous cycle, effect on implantation and effect on reproductive hormones.

2. MATERIALS AND METHODS

2.1. Collection and extraction

The *Cissampelos pareira* root was collected in the month of August, 2010 from Panchkula (30.74°N, 76.80°E) district of Haryana, India. Provisional identification was performed in Botany Department of Panjab University, Chandigarh, India, in comparison with the existing specimen number PAN/6954. Further identification has been performed by NISCAIR, India with reference No. NISCAIR/ RHMD/ Consult/ 2014/ 2534/ 113-2. Collected *Cissampelos pareira* roots were properly cleaned, chopped into small pieces and dried under room temperature without exposure to sunlight. Air dried *Cissampelos pareira* roots (100 g) were then powdered and passed through 80 mesh sieve. Powdered roots were extracted using methanol by maceration at room temperature for 48 hrs. The methanolic extract was concentrated in rotary evaporator to yield dark brown semi solid mass.

2.2. Phytochemical screening

The chemical constituents of CPR were identified by using chemical methods⁹ and thin layer chromatography (TLC)¹⁰.

2.3. Pharmacological Screening

2.3.1. Animals

All antifertility experiments were performed on adult, virgin female Sprague dawley (SD) rats weighing between 150–200 gm. Female SD rats (250-300g body weight) were used for acute toxicity study. All the animals used for this experiment were bred in animal house of National Institute for Pharmaceutical Education and Research (NIPER), Mohali, Punjab, India. Polypropylene cages were used to house experimental animals. Animals were kept in environmentally controlled room provided with a 12:12 h light and dark cycle for each 24 h period at a temperature of approximately 25°C. They were fed on laboratory chow and tap water. Animal study was performed in the animal house of Rayat Institute of Pharmacy, Ropar, Punjab, India with due permis-

sion from the Institutional Animal Ethics Committee (approval no. is RIP/IAEC/2010–11/25). All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by Committee for the purpose of control and supervision on experiments on animals (CPCSEA).

2.3.2. Test material administration

CPR was reconstituted in distilled water and 1% w/v carboxyl methyl cellulose (CMC) to get the desired concentration for all pharmacological tests. Administration of the extract was done orally.

2.3.3. Acute toxicity study of CPR

Three groups (1a, 1b, and 1c) of female SD rats (each group of five rats) were used for acute toxicity study as per Organization for Economic Co-operation and Development guide lines 425, 2001 for testing of chemicals. All animals were kept in fasting over night before dosing. For acute toxicity study limit test at dose of 2000mg/kg had been performed. One animal of each subgroup was administered the test dose (2000mg/kg). As animals of all subgroup survived without any abnormality, four additional animals of each group were also treated with same dose of each extract sequentially. The treated animals were kept under observation for 14 days for mortality and general behaviour.

2.3.4. Effect of CPR on the estrous cycle

To study the effects of CPR on the estrous cycle three groups (2a, 2b and 2c) of female SD rats (n = 6) were employed. Vaginal smear from each animal was examined under a microscope every morning at 9.00 a.m. for 21 days (about 5 cycles). The smears were evaluated by vaginal smear method¹¹. The duration of the estrous cycle together with that of the various phases was determined for 21 days. Vaginal smear from each animal was placed on individual glass slide and examined under a microscope every morning at 9:00 a.m. for 21 days. Images of each stage of the estrous cycle were taken by a high resolution camera. Determination of the proportion of leucocytes, epithelial cells and cornified cells present in the vaginal smear was the criteria for determination of different phase¹². The Control group (2a) received the vehicle (1% w/v CMC in distilled water) orally for 21 days. The CPR treated groups (2b and 2c) received 250 mg/kg and 500 mg/kg of the crude extract respectively orally for 21 days and the same parameters were determined.

2.3.5. Effect of CPR on the implantation

Three groups of matured female rats (n = 6) were selected for this experiment. All the experimental animals were then allowed to mate with matured proven fertility male rats (one male for two females)¹³⁻¹⁴.

One group served as a control and received the vehicle orally for 8 days from day 1(D₁) pregnancy. The other two groups received 250 mg/kg and 500 mg/kg of the crude extract daily by the same route of administration for 8 days from D₁ pregnancy. On day 12th day (D₁₂) all the rats of control and treated group were anaesthetized with normal dose of ketamine-xylazine anaesthesia¹⁵ and laparotomized to count the no. of implants of each rat.

2.3.6. Effects of CPR on reproductive hormones:

Three groups of animals (n = 6) were taken for this study. These were marked as control, CPR₁ and CPR₂ treated group¹⁶. Control group received vehicle, CPR₁ group received *Cissampelos pareira* root methanolic extract (250 mg / kg), CPR₂ treated group received *Cissampelos pareira* root methanolic extract (500 mg / kg) from D₁ to D₈ (day 8) of pregnancy. Blood samples were collected by retro orbital puncture of the animal in D₁₂, D₁₉ (day 19) and D₂₁ (day 21) of pregnancy. The serums were isolated by centrifugation. The procedures outlined in the manufacturer’s protocol were adopted for the quantitative determination of serum 17β estradiol and progesterone concentration. These hormone were measured by enzyme linked immuno sorbent assay (ELISA) using rat kit and ELISA micro plate reader.

2.4. Statistical analysis

The data were statistically analyzed using statistical packages (GraphPad InStat) and the level of significance was tested with paired t-test for estrous cycle and unpaired t-test for the rest of the tests. Data were expressed as mean ± S.E.M.

3. RESULTS

3.1. Phytochemical screening

the result of phytochemical investigation (Chemical test and TLC) of methanolic extract of *Cissampelose pareira* root indicated that it contain alkaloids, essential oils (Table 1and Table 2) which was reported in our previous study¹⁷.

Table 1. Phytochemical investigation of *Cissampelos pareira* (root, stem, leaves, flower) methanolic extract¹⁷

Test for active constituents	Results* Root
Triterpenes	+
Saponine	—
Alkaloids	+
Tannins	+
Flavonoids	—
Glycosides	—

Table 2. TLC of *Cissampelos pareira* root methanolic extract¹⁷

Solvent medium	No. of spots	R _f * (×100)	Inference
Methanol:			
Conc. ammonia (200:3)	1	50 ± 0.3	Alkaloid [#]
BAW (3:1:1)	1	77.0 ± 0.1	Alkaloid [#]
BAW (4:1:5)	—	—	No flavonoid ^Δ
Benzene:Et. acetate :formic acid (9:7:4)	—	—	No chalcones ^Δ No auronos ^Δ
Chloroform	—	—	—
Benzene	1	38.0 ± 0.1	Essential oil [§]
Chloroform:benzene (1:1)	1	58.0 ± 0.5	Essential oil [§]
Ether:benzene (1:1)	1	38.0 ± 0.3	Essential oil [§]

N.B: conc.=concentrated; Et. =Ethyl,* Mean value of three counts taken for determining R_f; # Spray reagent used for identification of alkaloid is Dragendroff’s reagent; ^Δ Colour development had been seen under UV light; § Spray reagent used for identification of essential oil is Vanillin-Sulphuric acid and then heated at 100^o - 105^oC for development of colour

3.2. Pharmacological Screening

3.2.1. Acute toxicity study of CPR

All 5 rats of 1a, 1b, 1c were alive without any kind of unwanted symptom after treatment with methanolic extract of *Cissampelose pareira* root orally at a dose of 2000mg/kg body weight respectively.

3.2.2. Effect of CPR on the estrous cycle

Treatment of rat with the methanolic extract of *Cissampelos pareira* root orally at dose of 250mg/kg and 500mg/kg for 21 days has not extend estrous cycle significantly as indicated in Table 3. There was no significant increase in the length of the diestrous phase and decrease in the duration of proestrous and metaestrous stage in treated group than those control animals. There was no significant difference in the length of both the estrous cycle and diestrous phase from that of the control after withdrawing the extract.

Table 3. Effect of CPR (250 mg/kg and 500 mg/kg for 21 days) on the estrous cycle of rat

Group	Duration				
	Estrous Cycle	Proestrous	Estrous	Metaestrous	Diestrous
2a[Control]	4.12 ± 0.2	0.94±0.12	0.98±0.16	0.96 ±0.14	1.24±0.24
2b[Extract]	4.21 ± 0.35*	0.88±0.16**	1.09±0.24**	0.87 ±0.12*	1.36±0.28*
2b ₁ [Extract]	4.26 ± 0.18**	0.83±0.19**	1.17±0.29*	0.84 ±0.13*	1.50±0.32*
2b[Post Extract]	4.15 ± 0.29	0.91±0.26	1.01±0.17	0.90 ±0.17	1.28±0.19
2b ₁ [Post extract]	4.20 ± 0.41	0.88±0.17	1.05±0.19	0.87 ±0.23	1.23±0.33

N= 6, Data are mean ±S.E.M. * p < 0.05; ** p < 0.01

3.2.3. Effect of CPR on the implantation

The decrease in no. of implants for CPR treated groups (250 mg/kg

and 500 mg/kg) and control group are not significantly different (Table 4).

Table 4. Effect of CPR (250 mg/kg and 500 mg/kg for 8 days from D₁ pregnancy) on implantation of rat

Groups	No. of Implants
Control	9.3 ± 0.30
3b (250 mg/kg)	8.5±0.49**
3b ₁ (500 mg/kg)	7.8±0.57**

**p<0.01 compared with control

3.2.4. Effects of CPR on reproductive hormones

Estradiol increased throughout gestation in control group. Estradiol levels for *Cissampelos pareira* root (CPR₁ and CPR₂) methanolic extract (at a dose of 250mg/kg and 500mg/kg respectively) treated group also increased. However, at day19 the mean estradiol level of treated groups was little above the control group's value. The rise in estradiol observed in CPR₁ and CPR₂ treated group as like as control group from D₁₉ to D₂₁ (Table 5). The progesterone level was increased in D₁₉ from D₁₂ for control group and then declined in D₂₁. On D₁₂ the progesterone level was identical in CPR₁ and CPR₂ treated group and on D₁₉ progesterone level became little lower in these groups.

Table 5. Effects of CPR-Me on Reproductive Hormones.

Groups	Hormone	D ₁₂	D ₁₉	D ₂₁
Control	Estradiol(pg/ml)	22.44 ±0.41	34.43±1.12	47.39±1.60
	Progesterone(ng/ml)	64.17 ±1.86	73.40±2.06	37.95±2.10
CPR1(250 mg/kg)	Estradiol (pg/ml)	22.64±1.9**	35.36±3.8*	47.12±2.1**
	Progesterone(ng/ml)	63.26±5.4*	69.59±6.8*	36.59±2.9*
CPR2(500 mg/kg)	Estradiol(pg/ml)	22.86±1.3**	36.01±4.3*	47.02±1.7
	Progesterone(ng/ml)	62.63±6.5*	69.09±7.8*	36.19±0.9

*p<0.05, **p<0.01 compared with control

4. DISCUSSION

In ethno-medicinal practices, the roots of *Cissampelos pareira* are used in the treatment of various ailments related to urinary problems and skin infections, and in tumor inhibitor activity, antibacterial, anti-malarial, diuretic activity, anticonvulsant activity and anti diarrhoeal activity etc¹⁸⁻¹⁹. North east Indian tribal people use the plant to prevent pregnancy²⁰⁻²². Scientifically it had been proved that methanolic extract of *Cissampelos pareira* leaf and stem are safe and effective antifertility agents. Phytochemical investigation revealed that *Cissampelos pareira* root was a rich source of alkaloid. The presence of indole alkaloids extend the estrous cycle²³, ergocronine and vinblastin cause interference with ovum implantation²⁴ and the alkaloids from *S. alata* leaves²⁵ exhibited anti-implantation, anti-gonadotropic, anti-progesteronic, embryonic resorptive, feto-maternal toxic

activities. Therefore, *Cissampelos pareira* root was evaluated for antifertility properties. In the present study all animals survived without any side effect after administration of CPR (2000 mg/kg) therefore, the LD₅₀ of this extract is higher than 2000 mg/kg and the maximum dose (500 mg/kg) used for antifertility evaluations is safe. The observations suggest that there was no significant change in the diestrous phase and estrous cycle after treatment with CPR from those of the control. There is a less chance of rats to get pregnant if the diestrous phase is prolonged. It could explain that CPR has no significant effect on estrous cycle to prevent fertility. It is noted that after stopping administrating the extract the normal diestrous phase and estrous cycle resumed. CPR does not have any significant effect on ovulation and implantation also. Increased level of estrogen and progesterone inhibit further fertilization during pregnancy. Higher progesterone level also supports the pregnancy. Little lower progesterone level in CPR treated group as compared to control group rationalizes the no significant anti-implantation activity of CPR. Though *Cissampelos pareira* root is a rich source of alkaloids, it has no significant effect on extending the estrous cycle and implantation.

CONCLUSION:

The present study indicates that the methanolic extract of *Cissampelos pareira* root has no significant antifertility effect. Alkaloids present in methanolic extract of *Cissampelos pareira* root have no antifertility effect.

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