

Original Article

EVALUATION OF *PORTULACA OLERACEA* L FOR ANTI-FERTILITY EFFECT IN FEMALE ALBINO RATS

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ABSTRACT

The objective of the study is to evaluate anti-ovulatory activity, anti-estrogenic activity, effect on uterine muscle weight chloroform extract of *P. oleracea* L in female albino rats. Air-dried aerial part of *P. oleracea* L was extracted using chloroform. Dried chloroform extract was administered orally to albino rats for 14 days. The estrous cycle was monitored every day. After completion of last dose animals were sacrificed under light anesthesia with ether. The number of ova in ovary, weight of uterus and ovary, and Biochemical changes was analyzed using standard methods. Phytochemical studies of chloroform extract were carried out using qualitative methods. *P. oleracea* L extract when administered orally, a strong anti-ovulatory activity (3.8 ± 1.28), and Biochemical changes (131.66 ± 2.88) was observed at the tested dose levels (250 and 500 mg/kg of b w). The extract shows furthermore, significant ($p > 0.05$) increase in uterus and ovary weight (759.33 ± 1.547 and 112.56 ± 0.404) respectively at the high dose. The phytochemical studies of *P. oleracea* L has shown positive test for Alkaloids, Flavonoids, Carbohydrates, Phenols and Proteins. The extract of *P. oleracea* L has anti-fertility effect on ovulation by reducing number of ova in ovary and also Biochemical changes. The significant increase in the uterus weight was indicating that hormonal changes in body of treated animals. All these observations suggest that chloroform extract of *P. oleracea* L has anti-fertility effect.

Keywords: *P. oleracea* L, anti-fertility, Phytomedicine.

INTRODUCTION

Global search for anti-fertility agents is continued to tackle the problem of population explosion that may lead to economic and health impact on the family in particular and the society in general especially in developing countries like china and India where the population growth is very high (Ministry of Health, 2003). Although contraceptives containing estrogen and progesterone are effective and popular, the risks associated to the drugs have triggered the need to develop alternative methods from medicinal plants. Hence, there is a need for a suitable product search from indigenous medicinal plants that could effectively be used in the place of pills [1].

A number of investigations have been carried out on traditionally claimed anti-fertility plants to validate the claim. Recent literature review revealed that 48 out of 72 traditionally employed medical plants for fertility control had anti-fertility potential [2]. The plant *P. oleracea* L was proved to show the muscle relaxant activity [3], anti-inflammatory effect [4], in some middle east countries, it is considered as beneficial for small tumors and inflammation, urinary disorders, liver obstruction, and ulcer of mouth and stomach. Several researchers have shown that *P. oleracea* L is having anti-hyperglycemic activity, anti-tumor activity, and antiulcer activity [5]. This plant has also proved for gastric antiulcer activity [6]. The plant *P. oleracea* L (Porsulane) is commonly known as porsulane an herbaceous weed.

This plant is an annual succulent prostrate herb; stem is about 15.30 cm long, reddish, swollen at the nodes, quite glabrous. Leaves are freshly, sub-sessile, 6.25 mm long alternate or sub-opposite. Flower few together, in sessile terminal heads. Microscopic analysis of the leaf powder invariably shows spherical mineral crystals, sieve plants, tracheas with spiral, annular and scalariform thickening and vessels with bordered pits [7]. An aqueous extract of *P. oleracea* L was shown to have skeletal muscle relaxant effects both *in vitro* and *in vivo*; other studies include: antibacterial and antifungal; wound healing; anti-inflammatory; uterine stimulant and diuretic in rabbits. The aim of the present study to evaluate anti-ovulatory activity, anti-estrogenic activity, effect on uterine muscle weight chloroform extract of *P. oleracea* L in female albino rats.

MATERIALS AND METHODS

Plant material

Collection and identification

The aerial part of *P. oleracea* L was collected around Gulbarga University campus in June 2012. The plant was identified by a taxonomist and a voucher sample representing, Herbarium No. HGUG-5013 was deposited in the Herbarium of Medicinal Plants of the Department of Botany Gulbarga University Gulbarga, Karnataka.

Processing and extraction

The plant material was dried in shade, ground and extracted with Chloroform by soxhlet extraction at 90°C for 12 hrs until the color of elute should be colorless. The extract was taken and solvent was evaporated at room temperature so as to get crude drug and stored at 4°C for further use.

Phytochemical screening

Identification of the chemical constituents was carried out on the powdered aerial part and on the Chloroform extract using chemical methods according to the methodology proposed by [8].

Experimental Animals

All anti-fertility experiments were performed on in-bred adult, cyclic virgin female albino rats (2-months-old and weighing 190–230 g body weight, obtained from Ragvendra Enterprise Bangalore. All the animals used for this experiment were bred in a standard animal house. The animals were housed in polypropylene cages and maintained under 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 pellets diet (Amrut laboratory animal feed diet Pune, Maharashtra India) and tap water *ad libitum*. The animals were allowed to acclimatize to the laboratory environment for 1 h before being subject to the experiments. All experimental procedure were carried out in strict accordance with the guidelines prescribed by the committee for the purpose of control and supervisor on experimentation on animals (CPCSEA Reg. No-34800/2001) and were approved by the institutional animal ethical committee.

Acute toxicity studies

The acute toxicity study was performed as described by [9]. Adult albino mice of either sex were divided into thirteen groups containing six animals in each group. The mice were fasted for 18 h with water ad libitum. The three suspensions prepared as above were administered orally at four different doses of 250, 500, 1000 and 1500 mg/kg body weight, respectively to different groups of rats separately. Control rats received the vehicle (Tween-80, 1%, p.o.) only. The animals were observed for 72 h for behavioral changes and mortality.

Test material administration

Administration of the extract was done with intra-gastric tube on the basis of the animal's body weight. The dose 250 and 500 mg/kg body weight for each animal was calculated considering the human dose (dry weight equivalent approximately 4 g/kg aqueous macerate employed as vaginal douche in divided doses) based on ethno medical use of the plant part [10].

Anti-fertility studies

Study of vaginal opening and 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 14 days. The other two groups received dried chloroform extract of *P. oleracea* L orally at a dose of 250 and 500 mg/kg animal body

weight/day respectively for 14 days. The estrous cycle was studied by stained preparation of vaginal smear of the animals. The stages of estrous cycle and its duration were determined as described by [11].

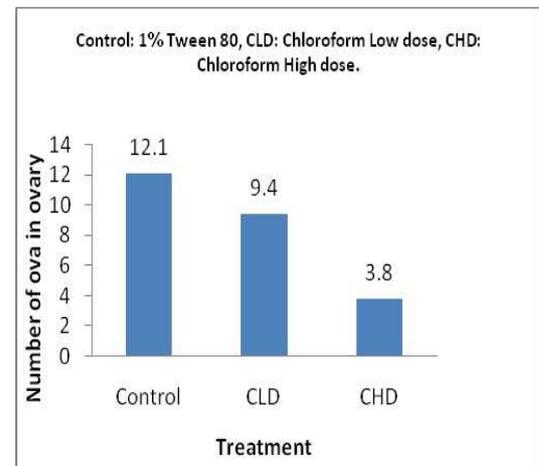


Fig. 1: Effect of Chloroform extract of *P. oleracea* L on number of ova in ovary.

Table 1: Effect of chloroform extract of *P. oleracea* L on the estrus cycle of mice treated for 14 days

Groups and Treatments	Duration of estrous cycle (Days)	Duration of different stages of estrus cycle (days)			
		Proestrus	Estrous	Metestrus	Diestrus
Control (1% Tween 80)	4.62±0.20	0.84±0.12	0.98±0.16	0.86±0.14	1.94±0.25
CLD (250 mg/kg b. w)	5.86±0.48	0.62±0.17	1.36±0.23	0.68±0.13	3.44±0.46*
CHD (500 mg/kg b. w)	6.10±0.54	0.53±0.20	1.86±0.34	0.56±0.09	3.65±0.52**

CLD: Chloroform low dose, CHD: Chloroform high dose. Data are Mean ± SEM, n=6, $p < 0.05$.

Table 2: Effect of Chloroform extract of *P. oleracea* L on ovary and uterine weight

Group No.	Treatment	Dose	Dosing days (mg/kg of b. w)	ovarian weight (mg/100g)	uterine weight (mg/100g)
I	Control	0.5 ml	1-15	31.33 ±1.5275	222.66±2.5166
II	CLD	250	1-15	80.33±1.5275	354.43±3.7859
III	CHD	500	1-15	112.56±0.4041*	759.33±1.547*

Control: 1% Tween 80, CLD: Chloroform extracts Low Dose and CHD: Chloroform extracts High Dose. Values are Mean ± SD, number of animals in each group was triplicate (n=3), * $p < 0.05$. b. w: body weight.

Table 3: Effect of Chloroform extract of *P. oleracea* L on Biochemical change in ovary and uterus.

Groups	Treatment	Dose	Dosing day's	Organ type	Protein (mg/100g)	Cholesterol (mg/100g)
I	Control	0.5 ml	1-15	Uterus	239.33±0.5773	504.66±4.1633
				Ovary	91.55±2.4165	241.33±1.5275
II	CLD	250	1-15	Uterus	131.66±2.8867*	300.15±0.2156
				Ovary	92.66±2.5166*	181.43±1.2503
III	CHD	500	1-15	Uterus	131.66±2.8867	301.15±1.6270
				Ovary	88.33±2.5166	181.43±1.2503

Control: 1% Tween 80, CLD: Chloroform extracts Low Dose and CHD: Chloroform extracts High Dose, b. w: body weight. Values are Mean ± SD, number of animals in each group was triplicate (n=3), * $p < 0.05$.

Anti-ovulatory activity

Female albino rats are divided into 3 groups each group containing 6 animals (n=6), fastened overnight and allowed free access to water ad libitum. Different groups of female rats were treated with test drug at 500 and 250 mg/kg of b.w as high and low dose respectively, vaginal smear from each rat was examined daily for 15 days and those rats exhibited three regular cycles were used. The vaginal smear was observed; drugs and vehicle were started in the estrous phase and administered orally, daily for 15 days. Group first

received vehicle only (1% Tween 80) and served as control. Group second and third received Chloroform extract of *P. oleracea* L at the dose of 500 and 250 mg/kg of b.w as high and low doses respectively for 15 days treatment to cover 3 regular estrous cycles. The vaginal smear and body weight of each animal was observed every morning between 9 and 10 am on the 16th day, 24 hrs after last dose, the rats from each group were anesthetized and sacrificed. Ovaries and uteri were dissected out, freed from extra deposition and weighed on a sensitive balance. Fimbriated part of the oviduct was dissected out from the rats, suspended in normal saline placed

on microscopic slide with cover slip to count number of ova in the oviduct. Ovary and uterus were processed for biochemical analysis [12].

Effect of the extract on the weight of genital organ and body weight

Fifteen matured female colony breed Wister strain albino rats were employed. The animals were divided in three groups (five each). The first and second groups received the extract 250 and 500mg/kg of body weight for 10 days by intra-gastric route respectively. The second group received the extract 500 mg/kg of body weight. The third group receives vehicle (1% Tween 80) for the same number of days by the same route. On the 11th day, all the animals were weighed and sacrificed under diethyl ether anesthesia. The ovaries and uteri were dissected out, freed from surrounding tissues, blotted on filter paper and weighed quickly on a balance sensitive to 0.0001 g [1]. The ovary and uterine ratios were then calculated by dividing the ovary and uterine weight in milligrams by body weight in grams as described by [13].

Biochemical studies

Freshly removed ovary and uterus tissues were weighed to required milligram for biochemical analysis. The biochemical analysis in ovary and uterus of the treated rats were carried out to know the effect of chloroform extract on the total protein content and total cholesterol content of both organs. The total protein and cholesterol content of ovary and uterus were estimated by the method as described in Refs [14, 15] respectively.

Statistical analysis

Results are expressed as Mean \pm SEM. The statistical analysis was carried out using one way ANOVA analysis. The *p*-value of 0.05 or less was considered significant for all experiment.

RESULTS

Phytochemical screening

The chloroform extract of *P. oleracea* L subject to Preliminary phytochemical screening using chemical method revealed the presence of Alkaloids, Glycosides, Proteins, Saponins, Flavones, Tannins, Gum and mucilage. The test for Steroids, Phenols, Oils and fats however, showed negative results.

Acute toxicity studies

No mortality and changes in the behavior were observed in all the treated and control groups of mice up to a dose of 1500 mg/kg body weight. Hence, 250 and 500 mg/kg body weight was used for anti-fertility testing.

Effect of the extract on the estrous cycle

The Treatment of rats with the extract of 250 and 500 mg/kg body weight/day for 15 days caused a prolonged estrous cycle with significant increase in the duration of diestrous phase (Table 1) and elongation of estrus stage in treatment with higher dose (500 mg/kg body weight/day). The prolonged estrous cycle and diestrous phase (safe period) observed with the chloroform extract could also suggest the anti-fertility effect.

Anti-ovulatory activity

A significant decrease in number of ova (3.8 ± 1.28) in the ovary of high dose (500 mg/kg of b w) treated group was observed when compared with control group. However, there is no decrease in the number of ova (9.4 ± 0.86) in the ovary of low dose (250 mg/kg of b w) treated group. There is no change in the body weight and estrous cycle of the animals treated with both low and high dose when compared with control group (Fig 1).

The flavonoids Such as Apigenin, Luteolin and Quercetin are rich in the ethanol extract of *P. oleracea* L. these flavonoids inhibit the activity of cyclooxygenase and consequently ovulation [18].

Effect of the extract on the weight of genital organ and body weight

The weights of the ovary and uterus of both low and high doses increased significantly (112.56 ± 0.040 and 759.33 ± 1.47) when compared to control group as shown in Table 2. In the present study

the increase in the wet weight of the ovary in the extract treated animals compared to the control animals may indicate inhibition of ovulation through suppression of follicular stimulating hormone.

Biochemical studies

The response of the uterus and ovary for total protein and Cholesterol contents in low dose (250mg/kg b. w) were significantly decreased when compared with control group. However, there was a significant increase in the levels of uterine and ovary total protein and Cholesterol of rats treated with 500 mg/kg/ of b. w (Table 3).

DISCUSSION

The prolongation of diestrous phase may explain the remote chance of the rats to get pregnant. The observation that there was no significant change in the diestrous phase and estrous cycle after withdrawing the extract from those of the control could explain the reversible nature of the anti-fertility effect of the extract which has also been observed from the preliminary studies as mentioned above. The present study is comparable with the studies described in Refs [16, 17], who had reported anti-fertility effect with similar observations in guinea pigs and rats on treatment with seed extract of *Ricinus communis* and root extract of *Rumex steudellii*, respectively. However, significant decrease in the duration of proestrus and metestrus stage in experimental group was recorded than those of control animals. These changes were found to revert back after withdrawal of the treatment except the proestrus stage in groups with higher dose of treatment. The prolongation of diestrous phase may lower the chance of pregnancy in animals. The anti-ovulatory results of this report indicate the extract of *P. oleracea* L has potential anti-ovulatory activity with reference to *P. oleracea* L has been reported to have an anti-inflammatory activity [4]. Studies have revealed that the process of ovulation is comparable to an inflammatory process [19]. Anti-inflammatory drug have been employed in blocking ovulation [20]. The anti-inflammatory activity of medicinal plants may be responsible for its observed effect in blocking ovulation. The anti-inflammatory property of flavonoids is believed to result from inhibition of cyclooxygenase enzyme [21]. Cyclooxygenase, which converts arachidonic acid derived from cell membrane to prostaglandins (PG), as two isomers, Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) [22]. Cyclooxygenase-1 is endogenous form of the enzyme necessary for the production of PG while COX-2 is thought of as being an inducible enzyme associated with inflammation. The latter is thought to be essential for ovulation mechanism. It was revealed that all traditional non-steroidal anti-inflammatory drugs affect the action of both COX-1 and COX-2 but produces the most of their effect by blocking COX-2 [23]. COX-2 is induced in various cells by stimulation of cytokines and/or growth factors. It is expressed in many condition and organs such as in acute inflammation, bone resumption, kidneys and brain, female reproductive organs [24]. COX-2 deficient mice suffer from defect in reproductive function such as ovulation and fertilization [25], implying that COX-2 is important in ovulation. These studies indicate that COX-2 enzyme is essential for follicular rupture through the metabolites of arachidonic acid, which play important role in follicular rupture by activating protease. The results of present study suggest that Chloroform extract may block ovulation by inhibiting cyclooxygenase activity (perhaps COX-2) and PG synthesis. The present study shown that, increase in the wet weight of the uterus may also explain for the effect of the extract on hormonal changes that take place in the non-pregnant rats via the effect of the extract on the ovary. Furthermore, these results are suggestive for further studies to observe the histological changes of the genital organs after extract treatment [26]. The absence of change in body weight after 14 days treatment with the extract revealed that there is no major negative impact on the general metabolic status of the animals. The biochemical study of present investigation revolved the role of cholesterol as an obligatory precursor in progesterin biosynthesis in rat, rabbit and bovine luteal tissues has been reported earlier [27, 28]. Thus, in present study, the significant decrease in cholesterol content of ovarian tissue of chloroform extract-treated rats suggests the insufficient of cholesterol level towards biosynthesis of hormone in ovaries. Thereby it results the hypo-functioning of steroidogenic activity of the ovary of the chloroform extract treated rats [29, 30].

The steroidogenesis in ovaries is under the physiological control of two dehydrogenases namely Glucose-6-phosphate dehydrogenase (G-6-PDH) and Δ^5 - 3β -hydroxysteroid dehydrogenase (Δ^5 - 3β -HSD) [31]. These are two protein enzymes present in the ovary. Chloroform extract treated in both low and high doses inhibited the activity of these two enzymes significantly in dose-dependent manner.

In Conclusion, The present study indicates that the chloroform extract of *P. oleracea* L has anti-fertility effect and is safe at the effective anti-fertility doses used in this study. Further work on the mechanism(s) of anti-fertility actions and on isolation of the active component(s) responsible for the anti-fertility effect are underway. Chronic toxicity study, however, should also be performed in parallel.

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