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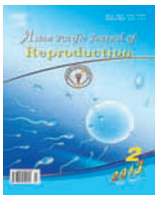

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Antifertility potential of the ethanolic extract of Caesalpinia pulcherrima Linn. leaves

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ABSTRACT

Objective: To assess the antifertility activity of ethanolic extract of *Caesalpinia pulcherrima* Linn (*C. pulcherrima*) leaves in albino female mice. **Methods:** Acute toxicity study of the extract was carried out in adult albino mice. The antifertility activity of the extract at dose levels (200 and 400 mg/kg, orally) was evaluated in two experimental animal models i.e. antiimplantation and esterogenic/antiestrogenic activity in female mice by observing no. of implants, estrus cycle, vaginal cornification, uertus weight and cholesterol content. **Results:** The extract was found to be safe up to a dose of 4 000 mg/kg body weight when administered orally. A good antiimplantation (66.66 %) activity in female mice was observed at the tested dose levels (200 and 400 mg/kg, orally). The extract further showed more significant ($P<0.05$) increase in uterine weight and cholesterol content in immature mice. Simultaneous administration of extract alongwith ethinyl estradiol showed significant estrogenic activity. **Conclusion:** The results suggest that ethanolic extract of *C. pulcherrima* leaves possess significant antifertility activity, therefore, justifying the traditional use of this plant in fertility regulation.

1. Introduction

Caesalpinia pulcherrima Linn. (*C. pulcherrima*) syn. *Poinciana pulcherrima* Linn. (Family: Caesalpiniaceae) commonly known as Peacock flower (English), Guletura (Hindi), is a leguminous, perennial large shrub or small tree that is widely distributed in the tropics. It is native of South America and also cultivated as ornamental plant in India. It is a small tree, 3.7-4.3 m in height. Its prickles are sparse on the branches, bark is grey in colour. Leaves are abruptly bipinnate, leaflets in 13-20 pairs, 1.3-1.9 cm long. Flowers are red or yellow, fragrant. Flowering season of this plant starts from September to November and fruits from March to April. Parts used for medicinal purpose are flowers, leaves, barks and roots. Leaves are traditionally used as purgative, tonic, antipyretic and emmenagogue whereas stem bark also has folkloric use as abortifacient and astringent. Seeds are used in gum troubles and ringworm infections. Flowers are used in bronchitis, asthma, intestinal worms and malaria whereas roots in convulsions, intermittent fevers, lungs and skin diseases[1-2].

2. Materials and methods

2.1. Procurement and identification of plant materials

The leaves of plant were collected from University College, Kurukshetra University, Kurukshetra during October 2008 and authentified by Dr. B.D. Vashistha, Reader, Department of Botany, Kurukshetra University, Kurukshetra as *C.pulcherrima* Linn. (Family: Caesalpiniaceae). A voucher specimen (No. IPS/KUK/C-1/2008) of the plant is preserved in the herbarium of the Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra for further reference.

2.2. Preparation of extract

Leaves of *C. pulcherrima* were dried in shade, powdered and stored in an air tight container at room temperature. 1 690 g of dried powder was extracted with ethanol (95%) using soxhlation method. The extract was concentrated to

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dryness using Rotary evaporator. The yield of the extract was found to be 14.12% w/w. The extract was preserved in a refrigerator. A suspension of ethanolic extract of C. pulcherrima leaves (CPEE) was prepared in Tween 80 (2% v/v). The suspension was freshly prepared on the day of experiment by dissolving a given quantity of the dried extract in an appropriate volume of Tween 80. Doses of the extract were prepared according to body weight of the animal.

2.3. Phytocemical screening

Phytochemical screening of ethanolic extract was carried out as per reported methods[3,4].

2.4. Acute toxicity study of the extract

Adult albino mice (25-30 g) were divided into five groups each containing six mice. The mice were fasted for 6 hours with only access to water ad libitum before experimental study. Group I, II, III and IV animals were administered various dose of CPEE i.e. 500, 1 000, 2 000, 3 000 and 4 000 mg/kg. Group V received Tween 80 only. All the doses and vehicle were administered by oral route. The animals were observed for 72 hours for mortality [5,6].

2.5. Anti-implantation activity

Female albino mice of proven fertility were mated with mature males of proven fertility in the ratio of 2:1, in their proestrous or estrous stage. Vaginal smear of each mice was taken daily between 9:00 a.m. to 10:00 a.m. The day on which spermatozoa appeared in the vaginal smear was taken as day 1 of pregnancy. The pregnant females were separated and divided into four groups each containing six animals. Group I animals received vehicle (Twin-80) only. Group II and III received CPEE at the doses of 200 and 400 mg/kg respectively. All the extract doses and vehicle were administered orally to the animals once daily throughout 7 days of pregnancy. On 10th day of pregnancy, the animals were laparotomized under light ether anesthesia and number of implants present in both the uterine horns was counted[7]. Group IV received CPEE at a dose of 400 mg/kg orally from day 1 to day 7 of pregnancy and were allowed to deliver after full term. Each pup was weighed and examined for gross defects. The vaginal smear was observed for four weeks after full gestation period and the female mice were mated with fertile males. The number of implants on 10th day of pregnancy was observed.

2.6. Estrogenic and antiestrogenic activity

Immature female albino mice (20-24 days old) weighing between 10 to 12 g were divided into six groups each containing six animals. Group I (control) was administered with vehicle i.e. Tween 80. Group II and III received 200 and 400 mg/kg of CPEE, respectively. 17- α -ethinyl estradiol (0.03 mg/kg) suspended in vehicle, was administered to Group IV animals to induce estrus. Group V and VI animals received CPEE at the doses of 200 and 400 mg/kg respectively, along with ethinyl estradiol (0.03 mg/kg). All the doses and vehicle were administered orally for seven days. On the 8th day, all the animals were sacrificed under light ether anesthesia. The vaginal opening and vaginal cornification of all the mice were observed before anesthesia and uterus of all the animals were weighed quickly on a sensitive balance. Further, uteri were processed for estimation of cholesterol contents[7-10].

3. Results

3.1. Phytochemical screening

Phytochemical screening of leaves extract showed the presence of glycosides, flavonoids and tannins.

3.2. Acute toxicity study of the extract

The CPEE was found to be safe at the doses used and there was no mortality up to the dose of 4 000 mg/kg of extract administered orally.

3.3. Anti-implantation activity

Among the two doses of CPEE, dose of 400 mg/kg was found to be significant ($P<0.01$) and percentage inhibition of implantations in mice, at doses 200 and 400 mg/kg, were found to be 42.85% and 66.66 % respectively when compared with control. No toxic effects were observed in the animals and their pups either by gross visual examination or in the weight of animals. All the animals in reversible effect study group exhibited the normal estrous cycle after gestation period and the number of implantations on 10th day of pregnancy was found to be normal as compared to control. Hence, ethanolic leaves extract was found to be reversibly effective. The results of antiimplantation study are shown in Table 1.

Table 1
Anti-implantation effect of *C. pulcherrima* leaves extract in female mice.

Groups	Treatments (dose)	No. of rats without implantation sites on 10th day of pregnancy	No. of implants (Mean ± SEM)	% inhibition of implantation
I	Control	Nill	11.2 ± 0.23	Nill
II	CPEE (200 mg/kg)	Nill	6.4 ± 1.39*	42.85
III	CPEE (400 mg/kg)	4	0.5*	66.66

n=6.* Significance in relation to control:$P<0.01$.

3.4. Estrogenic and antiestrogenic activity

The result of the C. pulcherrima leaves ethanolic extract (CPEE) on the immature mice uterus is shown in Table 2. Oral administration of the extract (200 and 400 mg/kg) caused significant increase in uterine weight in immature mice. The uterotrophic potency was less than that of ethinyl estradiol. CPEE at dose of 400 mg/kg, significantly increased uterotrophic response ($P<0.01$) when compared with control animals. The extract at the higher dose induced vaginal opening and the presence of cornified cells in the vaginal smear indicated proestrus phase. Simultaneous administration of CPEE with ethinyl estradiol at a dose of 400 mg/kg caused a significant increase ($P<0.01$) in uterine weight as well as cholesterol contents in the uteri of mice when compared with ethinyl estradiol alone. The extract, therefore, has estrogenic activity at both dose levels when given alone. Moreover, along with ethinyl estradiol, it exhibited strong estrogenic property.

Table 2
Estrogenic activity of *C. pulcherrima* leaves extract in female mice.

Groups	Treatments (Dose)	Uterus weight (mg) (Mean ± SEM)	Vaginal cornification	Cholesterol content (mg/100 mg of uterus) (Mean ± SEM)
I	Control	15.17 ± 0.88	Nill	2.98 ± 0.22
II	CPEE (200 mg/kg)	17.33 ± 1.20*	Nill	3.18 ± 1.07
III	CPEE (400 mg/kg)	37.83 ± 2.08**	Open (4/6) $^{+}$	5.18 ± 0.237**
IV	Ethinyl estradiol (0.03mg/ kg)	56.33 ± 2.60**	Open (6/6) $^{++}$	6.87 ±1.58**
V	CPEE (200 mg/kg) + Ethinyl estradiol (0.03 mg/kg)	66.33 ± 4.80$^{\Delta}$	Open(6/6) $^{++}$	7.10 ± 0.60
VI	CPEE (400 mg/kg) + Ethinyl estradiol (0.03 mg/kg)	87.83 ± 4.63$^{\Delta\Delta}$	Open (6/6) $^{++}$	8.44 ± 0.55$^{\Delta}$

n=6, + = nucleated epithelial cells; ++ = cornified cells. Ethinyl estradiol was taken as standard, * Significance in relation to control: P<0.05, ** Significance in relation to control: P<0.01. $^{\Delta}$ Significance in relation to standard: P<0.05, $^{\Delta\Delta}$ Significance in relation to standard: P<0.01.

4. Discussion

In the present study, the ethanolic leaves extract of plant was screened for antiimplantation and estrogenic/ antiestrogenic effects. The extract at 400 mg/kg body weight, was found more potent in their antiimplantation activity, as 66.66% of mice failed to show any implantation sites when compared with control group animals. It is well known that exact equilibrium of estrogen and progesterone is an essential condition for implantation and any disturbance in the level of these hormones may result in infertility[11]. The compounds having hormonal value usually disturb the hormonal milieu in the uterus and provoke the infertility effect. Therefore, keeping these facts in mind, the extract was further screened for estrogenic and antiestrogenic potential.

Estrogens cause an increase in uterine weight, retention of fluid leading to ballooning of the uterus, vaginal opening and increase in cholesterol content of uterus, therefore creating non receptive condition and changing the uterine milieu in the uterus[12]. Presence of cornified cells in vaginal smear indicated the estrogenic activity. Administration of extract at higher dose (400 mg/kg) to immature mice showed significant increase in uterine weight. The estrogenic effect of the extract was also evidenced by vaginal opening and presence of cornified cells in the vaginal smear. The treatment also showed increase in cholesterol content of uterus when compared with the control group. Simultaneous administration of the extract with ethinyl estradiol increased the uterine weight and cholesterol content in the uterus when compared with 17 α -ethinyl estradiol when administered alone. Hence, antiimplantation effect of ethanolic leaf extract of *C. pulcherrima* might be due to the estrogenic activity. However, it did not show any antiestrogenic activity when given along with ethinyl estradiol.

It can be concluded from the study that the ethanolic extract of *C. pulcherrima* Linn. leaves have potent antiimplantation activity. The inhibition of implantations in mice might be due to the estrogenic nature of the extract. The preliminary phytochemical screening of the extract showed the presence of glycosides, flavonoids and tannins. Literature shows that several flavonoids have been reported to possess antifertility activity[11,13]. Therefore, antiimplantation activity of leaves extract of this plant might be due to the presence of flavonoid contents in the extract. The results of the present study indicate that *C. pulcherrima* leaves possess antifertility activity, and thus, this study supports the claimed folkloric, ethnomedicinal use of this plant in fertility regulation. Further studies are recommended for isolation, characterization and understanding the exact mechanism(s) of active constituent(s) responsible of these constituents for antifertility effects.

Confcict of interest statement

We declare that we have no conflict of interest.

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