

POST-COITAL ANTIFERTILITY ACTIVITY OF
CLERODENDRUM PHLOMIDIS LINN.
DICHLOROMETHANE ROOT EXTRACT

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Summary

The present study was carried out to evaluate the claimed antifertility effect of *Clerodendrum phlomidis* Linn. f. (Family: Verbenaceae) dichloromethane root extract in mature female rats. The post-coital antifertility activity of the root extract at dose levels (100, 200, 400 and 600mg/kg, orally) was evaluated in mature female rats by observing no. of implants and estrus cycle. The root extract showed 25.89, 41.97, 93.30 and 100 percent inhibition of implantation at doses of 100, 200, 400 and 600 mg/kg body weight respectively. Among the four doses of extract, dose of 200, 400 and 600 mg/kg were found to be significant ($p < 0.01$) when compared with control. The results suggest that dichloromethane extract of *Clerodendrum phlomidis* roots possess significant post-coital antifertility (antiimplantation) activity in mature female rats which might be due to the presence of steroids.

Keywords: Antiimplantation; *Clerodendrum phlomidis*, root, dichloromethane extract

Introduction

World population is increasing day by day at an alarming rate and this intensifies the need for effective birth control measures. Scientists all over the globe are working to combat this problem. The synthetic agents available today for fertility control produce severe side effects such as hormonal imbalance, hypertension, increased risk of cancer and weight gain (1). Therefore, there is an urgent need to replace these agents by safe and effective alternative antifertility agents such as Plant based contraceptive agents. Many plants have been mentioned in fertility regulation in folklore and traditional medicine (2).

Clerodendrum phlomidis Linn. f. syn. *C. multiflorum* Burm f. (Family: Verbenaceae) commonly known as Arni in Hindi, Agnimantha in Sanskrit and Clerodendrum or wind-killer in English. It is distributed More or less throughout India, Ceylon and Malay Peninsula. The decoction of the root is slightly aromatic and astringent and is used as a demulcent in gonorrhoea. It is also given to children during convalescence of measles (3). Roots have been valued as tonic, febrifuge, diuretic, antidiabetic, anti-inflammatory and anti-tussive (4). Root bark of the plant together with rice wash has been used traditionally to cause sterility in females in ancient India (5). There is no scientific report or evidence published till date on this plant regarding its antifertility activity in female rats. Therefore, the present study was carried out to screen roots of *C. Phlomidis* for preliminary phytochemical investigation and antifertility activity in female rats.

Material and Methods

Procurement and identification of Plant Material

The roots of plant were collected from the campus of Kurukshetra University, Kurukshetra during March 2009 and identified as *Clerodendrum phlomidis* Linn. f. (Family: Verbenaceae) by Dr. H.B. Singh, Scientist Incharge, Raw Materials and Museum, National Institute of Science Communication And Information Resources, New Delhi where a voucher specimen (NISCAIR/RHMD/Consult/-2010-2011/1471/69) has been deposited.

Preparation of Extract

Shade dried roots of the plant were powdered and stored in an air tight container at room temperature and extracted with dichloromethane using soxhlation method. The yield of the extract was found to be 0.6% w/w. A suspension of extract 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.

Phytochemical Screening

The preliminary analysis of all the extracts was performed by the Phytochemical group test for carbohydrates, saponins, catechins, tannins, steroids and triterpenoids, alkaloids, phenols and anthraquinones (6).

Animals

Albino female rats (175-200 g) were selected for experimental study. They were obtained from Haryana Agriculture University, Hisar, Haryana, India. The animals were kept and maintained under laboratory conditions of temperature (21.5 ± 2 °C), humidity ($60 \pm 1\%$) and 12h light/dark cycle; and were allowed free access to food (standard pellet diet) and water *ad libitum*.

Anti-implantation activity

Female albino rats of proven fertility were mated with mature male rats of proven fertility in the ratio of 2:1, in their proestrous or estrous stage. Vaginal smear of each rat 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, 2% v/v) only. Group II, III, IV and V received root extract at the doses of 100, 200, 400 and 600 mg/kg body weight respectively. All the extract doses and vehicle were administered orally to the animals once daily throughout 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. Group VI animals received the extract 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 smears were observed for four weeks after full gestation period and

the female rats were mated with male rats. The number of implants on 10th day of pregnancy was observed (7).

Result & Discussion

Phytochemical screening

Phytochemical screening of *Clerodendrum phlomidis* roots DCM extract showed the presence of carbohydrates, flavonoids, glycosides and steroids as shown in Table 1.

Table 1. Phytochemical screening of *Clerodendrum phlomidis* roots DCM extract

Tests for phytoconstituents	Tests/Reagents	Inference
Alkaloids	Dragendorff's/Mayer's reagent	-
Carbohydrates	Molish's Reagent	+++
Flavonoids	Sodium hydroxide	+
Saponins	Foam test	-
Steroids	Salkowski/Liebermann-Burchard reaction	++
Tannins	Ferric chloride/ Bromine solution	-
Cardiac glycosides	Keller-Kiliani Test	++

Sign '+' indicates the presence whereas sign '-' indicates the absence of phytoconstituents

Antiimplantation study of the extract

For antifertility effect, the dichloromethane extract of *C. phlomidis* root was screened for antiimplantation activity at different dose levels. While screening, DCM extract showed 25.89, 41.97, 93.30 & 100 percentage inhibition of implantation at doses of 100, 200, 400 and 600 mg/kg body weight respectively as shown in table 2. Among the four doses of extract, dose of 200 400 and 600mg/kg were found to be significant ($p < 0.01$) when compared with control. 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, DCM root extract was found to be reversibly effective.

Table 2. Anti-implantation effect of DCM extract of *Clerodendrum phlomidis* roots on female rats

Group s	Treatment (dose)	No. of rats without implantation sites on autopsy day	No. of implants (Mean SEM)	% inhibition of implantation
I	Control (Tween 80,2% v/v)	Nil	11.2± 0.30	Nil
II	DCM (100 mg/kg)	Nil	8.3±0.42	25.89
II	DCM (200 mg/kg)	Nil	6.5±0.3*	41.97
III	DCM (400 mg/kg)	3	0.75± 0.3*	93.30
IV	DCM (600 mg/kg)	5	0*	100

N=6, $P < 0.01$ was considered significant.

Conclusion

In the present study, the dichloromethane extract of *C. phlomidis* root was screened for post-coital antiimplantation effect. The extract at 600 mg/kg body weight, was found more potent in their antiimplantation activity, as 100% of rats failed to show any implantation sites when compared with control rats. However, the extract at 200 and 400 mg/kg dose, also significantly ($p < 0.01$) inhibited the implantation in mature female rats. The preliminary phytochemical screening of the extract showed the presence of carbohydrates, flavonoids, glycosides and steroids. Phytochemical studies include reports of presence of β -sitosterol and γ -sitosterol, cetyl alcohol, clerodin, clerosterol, clerodendrin-A in root of *C. phlomidis* (8). It has also been reported that the presence of isoflavonoids, phytosteroids (9,10,11), alkaloids (12), steroidal saponins (13), saponin glycosides and flavonoids (14) in the herbal extracts are responsible for antifertility activity. Therefore, it can be concluded that the post coital antiimplantation activity of the dichloromethane extract of *C. phlomidis* L. might be due to the presence of Steroid content in the root.

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