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RESEARCH ARTICLE

Antifertility activity of *Drosera burmannii*

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Abstract

The alcohol and aqueous extracts of the whole plant of *Drosera burmannii* Vahl (Droseraceae) were studied for antifertility activity using an anti-implantation model in female Wistar rats of normal estrus cycle, after overnight cohabitation with males of proven fertility. The day when spermatozoa were detected in the vaginal smear was treated as the first day of pregnancy. The extracts at two dose levels each were administered to female rats from day 1 to day 7 of pregnancy. On day 10, the rats were laprotomized under light ether anesthesia and the number of implantation sites and corpora lutea were noted. Both the extracts produced significant antifertility activity. After subjecting the extracts to phytochemical screening the alcohol extract showed positive tests for quinones, flavonoids, saponins, phytosterols, gums, and mucilage while the aqueous extract showed presence of flavonoids, phytosterols, gums, and mucilage. Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) studies revealed the presence of plumbagin in the alcohol extract.

Keywords: *Antifertility activity;* Drosera burmannii*; anti-implantation model*

Introduction

The population of India in 2006 was estimated at 1,112,187,000. It is expected to increase from 1029 million to 1400 million during 2001–2026, an increase of 36% in 25 years at the rate of 1.2% annually (Census India, 2006). To slow this population rate several programmes have been initiated, one of which is to explore antifertility potential of several herbal drugs. Plants such as *Abroma angusta* L. (Sterculiaceae), *Adhatoda zeylanica* Medic. (Acanthaceae), *Cardiospermum halicacabum* L. (Sapindaceae), *Embelia ribes* Burm.f. (Myrsinaceae), *Hibiscus rosasinensis* L. (Malvaceae), *Momordica charantia* L. (Cucurbitaceae), *Ocimum tenuiflorum* L. (Lamiaceae), *Plumbago zeylanica* L. (Plumbaginaceae), and *Sesamum indicum* L. (Pedaliaceae), have been used as oral contraceptives and abortifacients from time immemorial (Atal & Kapur,1982; Bhatta & Santhakumari, 1971; Goswami & Bokadia, 1979; Dhanwad et al., 2005; Kholkute & Udupa, 1976; Ramesh et al., 1983; Saksena, 1971).

The genus *Drosera* L. is popularly known as the sundews. It is one of the largest genera of carnivorous plants with about 100 species belonging to the family Droseraceae (Santapau & Henry, 1976). Three species of *Drosera* are found in India, viz., *D. burmannii* Vahl, *D. indica* L., and *D. peltata* J. E. Sm. (Anonymous, 1998). Sundews were used as medicinal herbs as early as the twelfth century, when an Italian doctor from the school of Salerno by the name of Matthaeus Platearius described the plant as an herbal remedy for cough under the name "herba sole"; its tea was recommended by herbalists, for dry coughs, bronchitis, whooping cough, asthma, "bronchial cramps" and stomach ulcers (Schilcher & Elzer, 1993). A modern study has shown that *Drosera* L. does exhibit antitussive properties (Oliver-Bever, 1986).

The species of *Drosera* contain several medicinally active compounds including quinones (plumbagin) (Wagner et al., 1984), hydroplumbagin glucoside (Vinkenborg et al., 1969), rossoliside (7-methyl-hydrojuglone-4-glucoside) (Sampara, 1971). *Drosera peltata* contains naphthoquinones such as plumbagin, droserone, hydroserone, besides flavanols quercetin, gossypectin, gossypin, isogossypectin, and proteolytic enzyme of pepsin type (Yoganarasimhan, 2000).

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Drosera burmannii is an acaulescent insectivorous herb with glandular hairs, found throughout the plains and on the hills of India up to 2666 m. It bears rose colored flowers in small racemes (Anonymous, 1998; Keshavamurthy & Yoganarasimhan, 1990). It is reported to contain naphthoquinones, plumbagin, ramanteeon and its glucoside rossoliside (Wagner et al., 1984), flavonoids like quercetin and hyperoside (Wang et al., 1998). The plant possesses rubefacient properties (Anonymous, 1998).

Plumbagin found in species such as *Plumbago zeylanica* L. and *Plumbago indica* L. is reported to have antifertility properties (Gupta et al., 1971; Premakumari et al., 1977; Goswami & Bokadia, 1979). The present investigation was undertaken to investigate the antifertility activity of *Drosera burmannii* in alcohol and aqueous extracts.

Materials and methods

Plant material

The whole plant of *Drosera burmannii* was collected from the forests of Savanadurga, Bangalore during February, 2006 and authenticated by S.N. Yoganarasimhan (Taxonomist and Research Co-ordinator, Department of Pharmacognosy, M.S. Ramaiah College of Pharmacy, Bangalore). A voucher herbarium specimen (*Hema Basnett* 005) in flowering and fruiting condition along with a voucher sample of the crude drug material was deposited at the herbarium and museum of M.S. Ramaiah College of Pharmacy (MSRCP), Bangalore. The material was washed, shade-dried, powdered, passed through sieve no. 60 and stored in airtight containers in daylight for three months at room temperature (± 20°C).

Preparation of the extracts

Alcohol extract

A weighed quantity (500 g) of the air-dried powdered drug was taken and extracted for 48 h at 50–60°C with 200 ml of ethanol (90%) in a Soxhlet extractor. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50°C (yield 32.21% w/w, dry weight basis). The ethanol extract was prepared in distilled water containing 2% v/v Tween 80 (as a suspending agent).

Aqueous extract

A weighed quantity (500 g) of the air-dried powdered drug was taken and macerated with hot water at 80°C. The maceration process was carried out for 24 h. The macerate was filtered through Whatman No.1 filter paper. The filtrate was concentrated in a rotary flash evaporator at a temperature not exceeding 50°C (yield 11.36% w/w, dry weight basis) and extract was prepared in distilled water for experimental purpose.

Phytochemical screening

Phytochemical tests of the alcohol and aqueous extract of *Drosera burmannii* were carried out following the methods of Kokate (1999). The standard plumbagin was obtained from Hi Media, Mumbai. TLC studies were carried out following Wagner et al. (1984). HPTLC were carried out using a Camag HPTLC system equipped with Linomat V sample applicator, Camag TLC Scanner 3 and CATS 4 software for interpretation of the data was used. An aluminum plate (20 x 10 cm) precoated with silica gel $60F_{254}$ (E. Merck) was used as adsorbent. The plates were developed using toluene:glacial acetic acid (55:1) and toluene:chloroform:glacial acetic acid (1:1:0.1) as mobile phase for the alcohol and aqueous extracts, respectively, in a Camag twin trough chamber to a distance of 8 cm each.

Animals

Swiss albino mice weighing 18–25 g and albino rats (Wistar strain) weighing 180–190 g of either sex were used for the study. The animals were procured and housed in the animal house of M.S. Ramaiah College Pharmacy, Bangalore at least two weeks prior to the study, so that they could adapt to the new environment. The animal house was well maintained under standard hygienic conditions, at 20 ± 2°C, humidity (60 ± 10%) with 12 h day and night cycle, with food and water *ad libitum*. Rats were housed in groups of four and mice in groups of six per cage. Cleaning and sanitation were done on alternate days. Paddy husk was provided as bedding material, which was cleaned every day. The study was carried out as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) norms after obtaining approval from the Institutional Animal Ethical Committee of MSRCP.

Acute toxicity studies

Acute toxicity studies were carried out following Ghosh (2005) to study the acute toxic effects and to determine the minimum lethal dose of the drug extracts. Swiss albino mice of either sex weighing 18–25 g were used for the study. The alcohol and aqueous extracts were administered orally to overnight fasted animals at doses of 30, 100, 300, 1000, and 3000 mg/kg of body weight. After administration of the extracts, the

animals were observed continuously for the first three hours for any toxic manifestation. Thereafter, observations were made at regular intervals for 24 h. Further, the animals were under investigation up to a period of 1 week.

Experimental details

The animals were divided into 6 groups of 6 animals each. Group I served as control. Group II served as a standard group, and was treated with ethinyl estradiol (0.45 mg/kg) orally for 7 days. Group III and IV were treated with alcohol extract (250 and 450 mg/kg body weight, respectively) orally for 7 days. Group V and Group VI were treated with aqueous extract (250 and 450 mg/kg body weight, respectively) orally for 7 days.

Anti-implantation activity was determined following the method of Khanna and Chaudhary (1968). Female rats showing regular estrous cycles were selected on the basis of two consecutive cycles. They were then paired with males of proven fertility in the ratio of 3:1, until the vaginal smear indicated positive mating. The day of mating was considered as day 1 of pregnancy for each female rat. This was indicated by the presence of thick clumps of spermatozoa in female vaginal smear. Mated female rats were assigned to control and experimental groups randomly until each group consisted of 6 animals. The animals were administered alcohol and aqueous extracts of the drug at two dose levels each. The standard group was administered ethinyl estradiol 0.45 mg/kg body weight. All the drugs were administered orally using a feeding needle. The rats were administered the dose for 7 consecutive days and autopsied on the 10th day of pregnancy. During autopsy, the number of implantation sites on both uterine horns and the number of corpora lutea on the two ovaries was recorded for each rat.

The anti-implantation percentage was calculated by using the formula:

$$I_C\text{-}I_T \times 100/I_C$$

where I_C and I_T = number of implantation sites in the uterine horns for the control and drug treated female rats, respectively.

Statistical analysis

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. All values are expressed as mean ± SEM.

Results

Phytochemical studies revealed the presence of flavonoids, saponins, phytosterols, gums, and mucilage for both extracts. TLC and HPTLC of the alcohol extract revealed the presence of 10 spots at R_f 0.03, 0.05, 0.08, 0.12, 0.18, 0.21, 0.24, 0.29, 0.56, 0.81, with a most pronounced spot of maximum area at R_f 0.56, corresponding to that of plumbagin reference standard. The aqueous extracts revealed the presence of four spots at R_f 0.01, 0.06, 0.15, and 0.24 with no spot corresponding to that of plumbagin reference standard (R_f 0.66). This is due to the hydrophobic nature of plumbagin (Nayana et al., 2005). The most prominent spot is at R_f 0.01.

Acute toxicity studies were carried out to evaluate toxicity and to determine the minimum lethal dose of the drug extracts, using Swiss albino mice. It was found that alcohol and aqueous extracts up to a dose of 3000 mg/kg body weight did not show any toxic manifestations or death.

Antifertility effect of *Drosera burmannii* was carried out with an anti-implantation model in female rats. Alcohol and aqueous extracts at dose levels of 250 and 450 mg/kg body weight were found to have significant antifertility activity as indicated by the significant reduction in the number of implants. The alcohol extract at 250 and 450 mg/kg body weight reduced the implantation up to 78.74% ($p < 0.001$) and 85.23% ($p < 0.001$), respectively. Similarly, the aqueous extract, at doses of 250 and 450 mg/kg body weight, reduced the implantation up to 67.22% ($p < 0.01$) and 83.66% ($p < 0.001$), respectively (Table 1).

Table 1. Antifertility activity of *Drosera burmannii.*

Treatment	Dose (mg/kg)	Implantation	Corpora lutea	% Inhibition of Implantation
Control	-	10.16 ± 0.83	0.16 ± 0.16	-
Standard (Ethinyl estradiol)	0.45	0.5 ± 0.50***	0.00 ± 0.00	95.07
Aqueous extract	250	3.33 ± 0.98**	1.0 ± 0.51	67.22
Aqueous extract	450	1.66 ± 0.80***	0.83 ± 0.40	83.66
Alcohol extract	250	2.16 ± 1.97***	1.0 ± 0.51	78.74
Alcohol extract	450	1.5 ± 0.957***	2.1 ± 1.10	85.23

Values are expressed as Mean ± SEM.
$X = (I_C - I_T) \times 100/ I_C$ where I_C and I_T = number of implantation sites in the uterine horns for the control and drug treated female rats, respectively.
Tukey-Kramer multiple comparison test.
***$p < 0.001$, **$p < 0.01$ when compared to control.

Discussion

Anti-implantation activity may be caused due to the imbalance in endogenous estrogen and progesterone levels (Dhanwad et al., 2005). The loss of implantation caused by the extracts may be due to their antizygotic, blastocytotoxic or anti-implantation activity (Hafez, 1970).

The roots of *Plumbago zeylanica* and *Plumbago indica* are traditionally reported to have antifertility activity (Gupta et al., 1971; Goswami & Bokadia, 1979). Plumbagin found in these species has been proved to possess significant anti-implantation activity in rats (Premakumari et al., 1977).

The phytochemical studies on *Drosera burmannii* revealed the presence of naphthoquinones such as plumbagin, along with flavonoids and phytosterols. In addition to plumbagin, sterols and flavonoids also have been reported to possess antifertility activity (Miksicek, 1993; Evans, 2001). The alcohol extract at both the dose levels showed highly significant antifertility activity ($p < 0.001$) while aqueous extract showed higher significance with the higher dose at $p < 0.001$, in comparison with the lower dose at $p < 0.01$. The phytochemical tests and HPTLC studies revealed the absence of quinones and plumbagin, respectively, in the aqueous extract. However, the aqueous extract also exhibited significant antifertility activity, which may be due to other phytoconstituents like sterols and flavonoids.

In conclusion, the results of this study confirm the antifertility potential of *Drosera burmannii*.

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