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Antiimplantation activity of petroleum ether extract of leaves of *Cayratia trifolia* Linn. on female Albino rat

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ABSTRACT

Objective: To evaluate the antiimplantation properties of the Pet ether extract of leaves of *Cayratia trifolia* Linn (*C. trifolia*) in rat by observing the implants after dosing the test extract. **Materials:** Pregnant females were separated and divided into thirteen groups each containing six animals and were given Tween–80, 2% v/v. prepared extracts (PEECT) at the dose of 250 mg/kg and 500 mg/kg respectively. All the extracts 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 anaesthesia and number of implants present in both the uterine horns was counted. Each pup was weighed and examined for gross defects. **Result:** Among the two doses of PEECT, a dose of 500 mg/kg was found to be significant ($P < 0.01$) and percentage inhibition of implantations in rats, at doses of 250 and 500 mg/kg, were found to be in PEECT 37.1, 56.7 respectively when compared with control. The highest activity was observed with the 500 mg/kg dose when the implantation were calculated about 10th day after the administration of the test extracts. **Conclusions:** It can be concluded from the study that the Petroleum ether extract of *C. trifolia* Linn. leaves have potent antiimplantation activity.

1. Introduction

The world population, especially which of the developing countries is increasing at an alarming rate and is the root cause of poverty and many other ills of the society. Fertility regulation with plants or plant preparations and medicaments has been mentioned in ancient texts of indigenous systems of medicine of many countries [1]. The use of plants as emmenagogues, abortifacients and as local contraceptives was well known to the ancient physicians of India as is evident from some of the available books, monographs and reviews [2]. *Cayratia trifolia* Linn. Domin syn. (*C. trifolia*) *Vitis trifolia* Linn. (Family: Vitaceae) is a native of India, Asia and Australia [3]. It is a perennial climber, found in the hotter parts of India from Jammu & Rajasthan to Assam, Tripura & West Bengal extending into peninsular India up to 600m [4]. Whole plant of *C. trifolia*

has been reported to contain a yellow waxy oil, steroids/terpenoids, flavonoids, tannins [4]. Leaves are Rubifacient [5] used to stop bleeding of injuries [6,5]. Root bark reduces the muscular pain [5]. The bark extract showed 40–59.9% inhibition of potato virus. The plant is reported to have antibacterial, antifungal, antiprotozoal, hypoglycemic, anticancer and diuretic actions [4]. The herb *Cayratia trifolia* L. is reputedly beneficial for antiimplantation study owing to its ethanomedicinal use by Vanutau's native people [7]. Therefore, the study was undertaken to justify its ethanomedicinal uses. Rational for using the petroleum ether extract: It contains flavonoids and steroids, those responsible for the antiimplantation activity.

2. Materials and methods

2.1 Procurement and identification of plant material

The leaves of plant were collected from the Botanical garden, Kurukshetra University, Kurukshetra during October 2009 and identified as *C. trifolia* Linn. (Family: Vitaceae)

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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/-2010-11/1548/146) has been deposited for further reference.

2.2. Preparation of extracts

Leaves of *C. trifolia* were washed under running tap water and dried in shade for two weeks. The leaves were powdered, sieved and stored in an air tight container at room temperature. 400 g of dried powder was extracted sequentially with petroleum ether and hydro-alcohol (30:70) by using soxhlation method. The extracts were concentrated to dryness using Rotary evaporator (Heidolph, model number- 4011, USA). The extracts were preserved in refrigerator at 4 °C.

2.3. Pharmacological evaluation of the extracts

2.3.1. Animal study

Albino rats (125–140 g) and immature female rats (25–40 g) of either sex were selected for the experimental study. They were obtained from Chaudhary Charan Singh Haryana Agriculture University, Hissar, Haryana; India. The animals were kept and maintained under laboratory conditions of temperature (21.5 ± 22.0 °C), humidity ($60\% \pm 1\%$) and 12 hour light/dark cycle. They were allowed free to food (standard pellets) and water *ad libitum*. Experimental protocols and procedures used in this study were approved by Institutional Animal Ethics Committee of Kurukshetra University, Kurukshetra, India and confirmed to the guidelines of 'Committee for the Purpose of Control and Supervision on Experiments on Animals' [Reg. No. 235/CPCSEA].

2.3.2. Acute toxicity study of the extracts

Adult albino mice (25–30 g) were divided into sixteen groups each containing 6 mice. The mice were fasted for 6 hours with only access to water *ad libitum* before experimental study. Group II to VI, VII to XI and XII to XVI animals were administered various dose of PEECT, and HAECT extract i.e. 500, 1 000, 2 000, 3 000 and 4 000 mg/kg. Group I received Tween-80 (2%) only. All the doses and vehicle were

administered by oral route. The animals were observed for 72 hours for mortality [8].

2.3.3. Antiimplantation activity

Female albino rats (125–140 g) 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 under the optical microscope, was taken as day 1 of pregnancy. The pregnant females were separated and divided into thirteen groups each containing six animals. Group I animals received only vehicle i.e. Tween-80, 2% v/v. Groups II, IV, VI received all the prepared extracts (PEECT) at the dose of 250 mg/kg; groups III, V, VII received the same extract at the doses of 500 mg/kg respectively. All the extracts 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 anaesthesia and number of implants present in both the uterine horns was counted. Each pup was weighed and examined for gross defects. The vaginal smear was 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. Weight gained by each rat of all the groups was recorded [9–13].

3. Results

Among the two doses of PEECT a dose of 500 mg/kg was found to be significant ($P < 0.01$) and percentage inhibition of implantations in rats, at doses of 250 and 500 mg/kg, were found to be 56.7, 37.1 in PEECT 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, PEECT leaves extract was found to be reversibly effective (Table 1).

Table 1
Antiimplantation effect of extracts on female albino rats ($n = 6$).

Treatment	Body weightgain (g)	No. of rats without implantation sites on day 10	No. of implantation sites	No. of liters born	% inhibition of implantation
Control (Tween 80, 2% v/v)	50.66±0.66	Nil	11.66±0.33	Nil	Nil
PEECT (250 mg/kg)	45.00±2.88	Nil	5.67± 0.67**	3.27±0.12	56.7
PEECT (500 mg/kg)	49.33±1.66	Nil	4.33±0.56**	2.33±0.42	37.1

*Data were expressed as Mean ± SEM, Significant with respect to control: $P < 0.05$; ** Significant with respect to control: $P < 0.01$; Nil – Zero.

4. Discussion

Although a vast amount of synthetic molecules are available or antifertility action but the side effects associated with these agents' demands the research for the plant based drug with fewer side effects. Rats were selected as experimental animals for the antifertility activity, especially antiimplantation. For antifertility effect, the extracts were screened for antiimplantation. In screening, the Petroleum ether extract showed 4.33 ,5.67 percentage inhibition of implantation. It is well known that for implantation, exact equilibrium of estrogen and progesterone is essential and any disturbance in the level of these hormones may cause infertility [14]. The compounds with hormonal value usually disturb the hormonal milieu in the uterus and provoke the antifertility effect [15].

It has been reported that the presence of steroids, [16,17] alkaloids, [18] isoflavonoids, [19] steroidal saponin, [20] saponin glycosides and flavonoids [21] in the plant extracts responsible for antifertility activity.

Although, it is very difficult to pinpoint the exact mechanism of action of antifertility effect of Petroleum ether extract of *C. trifolia* leaves at this time, yet it may be concluded that their effects might probably be due to multiple attributes which are certainly dose dependent.

It can be concluded from the study that the Pet ether extract of *C. trifolia* Linn. leaves have potent antiimplantation activity.

Conflict Of Interest Statement

We declare that we have no conflict of interest.

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