# Antiimplantation activity of polar and nonpolar Fruit extract of *Cayratiatrifolia Linn.*

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## *ABSTRACT:*

*CayratiatrifoliaLinn.Dominsyn.(C. trifolia) Vitistrifolia Linn. (Family:Vitaceae) is a plant species grown in India, Asia and Australia. It iseternal climber, found in the tropical region of India from Jammu &Rajasthan to Assam, Tripura & West Bengal extending into peninsular India.Female albino rats (125-140 gm) of proven fertility were mated with mature male rats of proven fertility in the ratio of 2:1, in their proestrous or estrous stage.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 $10^{th}$ day of pregnancy was observed. Weight gained by each rat of all the groups was recorded.Among the two doses of PEECT and HAECT, 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, 37.7in PEECT and 64.3,40.3 in HAECT respectively when compared with control.For antifertility effect, the extracts were screened for antiimplantation and estrogenic/antiestrogenic activity. In screening, the Petroleum ether extract showed 5.67, 4.33 and hydro alcoholic extract showed 7. 50, 4.70 percentage inhibition of implantation.*

# 1.INTRODUCTION:

The ancient system of medications of many countries has mentioned the use of plants and their preparations as medicaments in fertility regulation. There are evidences of the use of plants as abortifacients, emmnagogues and local contraceptives by the ancient Indian physicians available in books, monographs and reviews [1].CayratiatrifoliaLinn. Dominsyn.(C. trifolia) Vitistrifolia Linn. (Family:Vitaceae) is aplant species grown in India, Asia and Australia [2]. It iseternalclimber, found in the tropical region of India from Jammu &Rajasthan to Assam, Tripura & West Bengal extending intopeninsular India. The plant of C. trifoliacontainsyellow waxy oil, steroids/terpenoids, flavonoids, tannins [3].This plant is also known as Fox grape in English [4]. The Leaves of C.trifolia are Rubifacientwhich are used to stop bleeding of injuriesthe root bark decreases the muscular pain [5, 6]. The bark extract has been reported to show 40-59.9%inhibition of potato virus. The plant also possesseshypoglycemic,antibacterial,anticancer, antifungal, antiprotozoaland diuretic actions [3]. This plant also possesses Anthocyanins group, which act as natural dye and used as an alternative to phenolphthalein indicator in titration processes [7]. A study reported that theethanolic extract of plant show the antioxidant properties [8]. According to the popular belief of natives the herb Cayratiatrifolia L. is very helpful for antiimplantation by its ethanol medicinal use [9]. Therefore, we have taken up this study to evaluate the anti-implantation activity of ethanolic extract ofCayratiatrifolia*Linn* in rats.

# 2.MATERIAL AND METHOD

## 2.1 Procurement and identification of Plant Material

The fruits of plant were collected from the Botanical Garden, Kurukshetra University, Kurukshetra during October 2009 and identified as *CayaratiaTrifolia*Linn. (Family: Vitaceae) byDr.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:

Fruits of *CayratiaTrifolia*Linn. were washed under running tap water and dried in shade for two weeks. The fruits were then powdered, sieved and stored in an air tight container at room temperature. 400 gm 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 [10].

## 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 National institute of pharmaceutical education and research (NIPER) Punjab. India. The animals were kept and maintained under laboratory conditions of temperature (21.5-22.0 $^{0}$C), humidity (60%) 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 IEC University, Baddi (H.P), India and confirmed to the guidelines of 'Committee for the Purpose of Control and Supervision on Experimemtson Animals' [Reg. No. ].

***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, 1000, 2000, 3000 and 4000 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 [12].

**2.4*Antiimplantation activity***

Female albino rats (125-140 gm) 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 (HAECT) 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 $10^{th}$ 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 $10^{th}$ day of pregnancy was observed. Weight gained by each rat of all the groups was recorded (Gupta et al., 2004; Gebrieet al., 2005; Koneriet al., 2007) [12-16].

# 3.RESULT

## 3.1 Pharmacological evaluation:

### 3.1.1Acute toxicity study of the extracts

All the extracts were found to be safe at both the doses used and no mortality was observed up to the dose of 4000 mg/kg, orally.

### 3.1.2Antifertility activity

#### 3.1.2.1*Antiimplantation activity*

Among the two doses of PEECT and HAECT, 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, 37.7in PEECT and 64.3,40.3 in HAECT 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 $10^{th}$ 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 5.2 and in figure 5.11 and 5.12.

**Table3.2: Antiimplantation effect of extracts on female albino rats.**

| Treatment | Body weight gain (gm) (Mean ± S.E.M) | No. of rats without implantation sites on day 10 | No. of implantation sites (Mean ± SEM) | No. of liters born (Mean ± SEM) | % 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 |
| HAECT (250 mg/kg) | 50.66±0.66 | Nil | 7.50±0.62* | 4.00± 1.00 | 64.3 |
| HAECT (500 mg/kg) | 50.66±1.66 | Nil | 4.70±1.42*** | 0.00± 0.00 | 40.3 |

**N = 6. *(Significant with respect to control: $p<0.05$); ** (Significant with respect to control: $p<0.01$);***(Significantwith respect to control: $p<0.001$); Nil - Zero.**

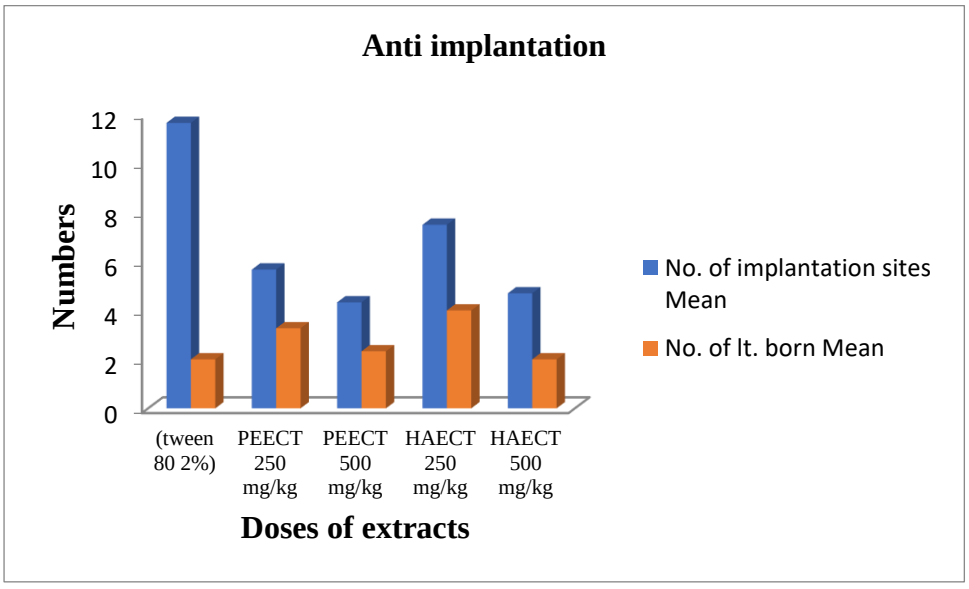


**Fig 5.11:Antiimplantation effects of *Cayratiatrifolia* L. extracts on female albino rats**

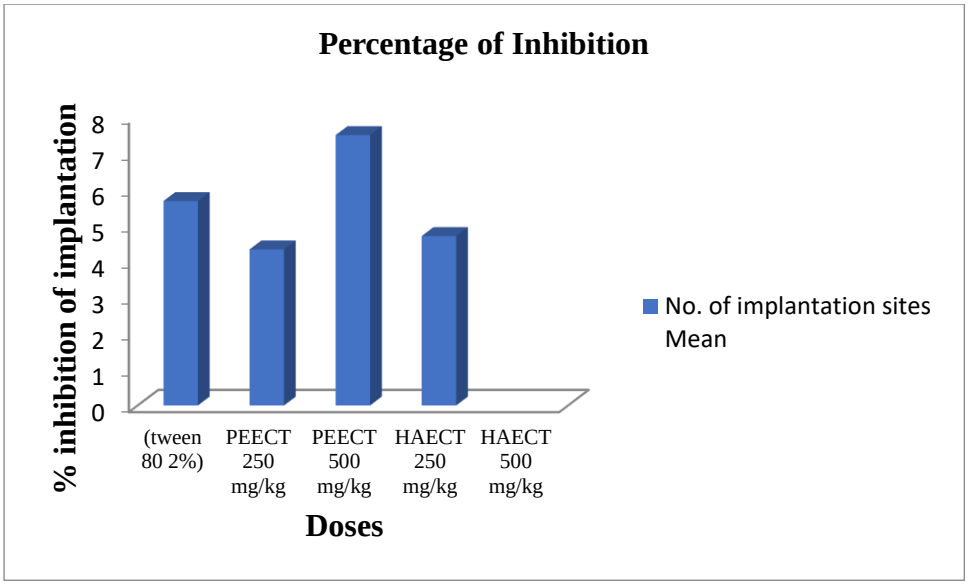


**Fig 5.12:Percentage of inhibition of implantation of *Cayratiatrifolia* L. extracts on female albino rats.**

## Discussion:

In the present study, *Cayratiatrifolia* L. antifertility study owing to its ethanomedicinal use by Vanutau' s native people [9]. Therefore, the study was undertaken to justify its ethanomedicinal uses. As a result, extracts of *Cayratiatrifolia* leaves viz. Petroleum ether and hydroalcohol were successively prepared by Soxhlet method and stored in refrigerator at 4º C.

Isolated chemical constitutentsStimasterol and β Sitosterol might be responsible for antifertility activity. Both the active constitutents in *Allium cepa* and *Helicotropiumindicum* (Boraginaceae) was responsible for antifertility activity [1, 13].

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 and estrogenic/antiestrogenic effects. For antifertility effect, the extracts were screened for antiimplantation and estrogenic/antiestrogenic activity. In screening, the Petroleum ether extract showed 5.67, 4.33 and hydro alcoholic extract showed 7. 50, 4.70 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 [17, 18]. The compounds with hormonal value usually disturb the hormonal milieu in the uterus and provoke the antifertility effect [19, 20].

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