

Antiestrogenic Activity of Petroleum Ether Extract of the Leaves of *Catharanthus roseus* (Vinca rosea) in Female Albino Mice



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Abstract : Petroleum ether extract of the leaves of *Catharanthus roseus* when subjected to mouse uterotrophic test proved to be antiestrogenic when administered alone. It also inhibits the estrogen induced gain in the uterine weight when administered along with estradiol 17 β . Keeping these results in view the leaf extract was injected intramuscularly at the daily dose of 2, 5, 10 mg/day/mouse from day 7 to 9 postcoitum to mated females. The 10 mg dose proved to be highly effective in suppressing pregnancy, while the 2 mg and 5 mg doses proved to be subeffective.

Key words : Petroleum ether, Uterine weight, Corpora lutea, Plants, Uterotrophic.

Introduction

The use of plant preparations and medicaments for pregnancy interruption was known to ancient physicians of India (Chopra et al. 1946; Satevati *et al.* 1987). During the last few decades sporadic attempts have been made by Indian investigators to develop contraceptive agents from substances of plant origin. Crude extracts of various parts of a large number of plants, screened for their antifertility activity in laboratory animals have shown promising results (Joshi & Ambuja, 1968; Sharma and Jacob, 2001; Shivalingappa *et al.*, 2001) The changes in the biochemical milieu of the uterus have also been reported. (Gupta and Joshi, 2005)

Catharanthus roseus or periwinkle or sadabahar is a member of family Apocyanaceae. It is well known for its alkaloids vincristine and vinblastine which possess anti - cancer properties. Considerable antifertility activity of *Catharanthus roseus* has been reported in male rats and mice (Murugund *et*

al. 1989; Murugund and Akbarsha, 1995; Chauhan and Mathur 1992). The present investigation, therefore attempts to investigate the hormonal profile and to evaluate the pregnancy efficacy of this interesting plant.

Materials and Methods

Swiss albino mice (18-24 gm) of proven fertility, maintained under laboratory conditions, were used for this study. Leaves of *Catharanthus roseus* were locally obtained from agricultural farms and nurseries near Jaipur. The leaves were dried and ground. The leaf extract was prepared by Soxhlet extraction with petroleum ether (b.p. 60-80°C), concentrated and dried under vacuum. The daily dose of leaf extract (2, 5, or 10mg) was dissolved in 0.1 ml olive oil vehicle and injected intramuscularly (im). Sets of suitable vehicle treated animals were maintained as controls.

Uterotrophic effect

Immature female mice (18-21 days old) were divided into 2 groups of 5 animals each.

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The mice were injected (im) for 3 consecutive days at the following dose regimens :

- (i) Controls, olive oil vehicle treated (0.1 ml/day/mouse)
- (ii) Leaf extract treated (10mg/day/mouse)
- (iii) Estradiol - 17 β treated (0.01 mg/day/mouse)
- (iv) Leaf extract (10mg) + Estradiol 17 β (0.01mg/day/mouse)

Animals were autopsied 24 hours after the last injection. The uterine horns were dissected out and cleared of adherent tissue and their wet weights were recorded.

Effect on Nidation

Parous, non-pregnant female were mated with adult males of proven fertility. Mating was confirmed by the presence of vaginal plug and/or by the presence of spermatozoa in the vaginal smear. The day of mating was taken as day 0. To the mated females, the daily dose of petroleum ether extract of *Catharanthus* leaves was administered from day 7-9 postcoitum. The control and experimental animal were autopsied on day 12 postcoitum and the number of corpora lutea and

implantation sites if any were counted in each ovary and uterine horn, respectively. Body weights were obtained before and after the experimentation.

Results and Discussion

Effect on uterine weight

The leaf extract when subjected to uterotrophic assay proved to be antiestrogenic when administrated alone as is evident by the decline in the uterine weights of the experimental mice (Table-I). The extract further showed significant antiestrogenic activity when administrated in combination with estradiol- 17- β (Table 1).

Effect on Nidation

Intramuscular injection of petroleum ether extract of leaves of *Catharanthus roseus* from day 7 to 9 postcoitum (pc) effectively inhibits nidation (Table 2). In this experimentation, the minimum cent percent effective dose obtained was 10 mg/day / mouse. However, the doses 2 mg/day/mouse and 5 mg/day/mouse were subeffective and the percentage of implantation sites in relation to the number of corpora lutea dropped to 48.27 and 20.83 respectively on day 12 pc (Table 2).

Table 1 : Uterotrophic Effect of Petroleum Ether Extract of leaves of *Catharanthus roseus* in Prepubertal mice

	Group	Treatment mg/dose/day/nice for 3 days	Uterine Weight (mg) Mean \pm SEM
1	Control	-	1.5 \pm 0.05
2	EDP (Estradioldipropionate 17 β)	0.1	6.00 \pm 0.3***
3	<i>Catharanthus roseus</i>	5	0.72 \pm 0.1*
4	<i>Catharanthus roseus</i> + EDP	5.0+0.1	2.22 \pm 0.7**

Significant Difference at:

* $P < 0.05$ (Almost significant),

** $P < 0.01$ (Significant)

*** $P < 0.001$ (highly significant)

Table 2 : Effect of administration of various extracts of leaves of *Catharanthus roseus* from day 7 to 9 postcoitum (pc) on the body and uterine weight of mice.

Group	Extract	Treatment mg dose/day/mice	Autopsy on Day 12 Postcoitum		
			Corpora	Implantation	Percentage Implantation
			lutea	Sites	
		-	61	52	85.24
		2	48	10	20.83
Control	Petroleum	5	58	28	48.27
	ether	10	45	0	0

Many plants possessing antiestrogenic property have been reported to possess significant antifertility activity. An active antiestrogen has been reported to decrease the wet uterine weight (Drill 1966, Edgren & Calhoun, 1961). Moreover, antiestrogenic nature may be designated to those compounds which interfere with any action of the estrogen. (Sankaran and Prasad, 1973). The pregnancy interceptive effect of *Catharanthus roseus* leaf extract can be interpreted due to this antiestrogenic nature of the plant. During pregnancy the uterus enlarges in part due to the action of hormones and also due to the stimulus of the concepts (Psychoyos, 1963). It can be predicted that the estrogen antagonism leads to the development of an inadequate progesterational endometrium incapable of supporting pregnancy. Thus, it can be argued that the antiestrogenic nature of the extracts could be embryotoxic and the conceptus undergoes resorption with a consequent decrease in the wet uterine weight.

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