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# BIOCHEMICAL STUDY OF THE EFFECT OF ALCOHOLIC EXTRACT OF *Catharanthus roseus* LEAVES IN SWISS ALBINO MICE

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## Abstract:

*The effect of the Alcoholic extract of the leaves of Catharanthus roseus was investigated. A dose of 10 mg alcoholic extract was dissolved in 50% alcohol and administered to Swiss albino female mice from day 7 to 9 post-coitum. The 10 mg alcoholic dose proved to be 100% effective in causing pregnancy interruption. The alkaline phosphatase and acid phosphatase enzyme activity of the uterus of the mice significantly decreases after treatment with the alcoholic extract of the leaves of Catharanthus roseus.*

***Key Words:*** *Catharanthus roseus, leaves, Alcohol extract, pregnancy interruption, antifertility, acid phosphatase, alkaline phosphatase, uterus*

## INTRODUCTION

The study of medicinal plants has garnered significant interest due to their potential therapeutic benefits and mechanisms of action. Among these, *Catharanthus roseus*, commonly known as the Madagascar periwinkle, or periwinkle or' sadabahar', a member of the family Apocyanaceae, is renowned for its diverse range of bioactive compounds, including alkaloids such as vincristine and vinblastine, which have been extensively studied for their anticancer properties. The decoction of the flowers of periwinkle with a few drops of alcohol is used as an eye wash in infants. Similarly, vinculin, an alkaloid isolated from *Catharanthus roseus* (Chopra *et.al.*, 1959), is used to cure diabetes. The leaf extract of this plant is used for indigestion and dyspepsia and is beneficial to the kidneys. Vinca leukoblastine and Leurocristine, the alkaloids of Catharanthus, are used against Hodgkin's disease and childhood leukaemia and breast cancer, respectively (Johnson *et. al.*, 1963; El Sayyed and Condell, 1981). Considerable antifertility activity of *Catharanthus* leaf has been reported in male rats and mice (Murugand *et.al.*, 1989; Murugand and Akbarsha, 1991; Chauhan and Mathur, 1992; Stanley and Akbarsha, 1992).

The administration of herbal extracts during critical gestation periods can significantly influence reproductive outcomes and fetal development. The period between days 7 to 9 post-coitum is particularly crucial, as this timeframe coincides with the early stages of embryonic implantation and placentation in mice (Sinha et al., 2019). Therefore, understanding the effects of the alcoholic extract of *Catharanthus roseus* leaves during this specific window is essential for assessing its safety and efficacy in pregnancy.

Previous studies have indicated that extracts from *Catharanthus roseus* can exhibit various pharmacological effects, including antioxidant, anti-inflammatory, and uterine toning properties. Prakash and Mathur (1976) screened the antifertility effects of pods of this plant, but they did not find any antifertility efficacy at least in the mouse. However, the specific biochemical interactions of these extracts within the uterine environment during early gestation remain inadequately explored. This study aims to investigate the biochemical effects of the alcoholic extract of *Catharanthus roseus* leaves on the uterus of pregnant Swiss albino mice when administered between days 7 and 9 post-coitum. By evaluating parameters such as uterine weight, histopathological changes, and biochemical markers associated with reproductive health, this research seeks to provide insights into the potential impact of *Catharanthus roseus* on pregnancy outcomes and uterine physiology.

## MATERIAL AND METHODS

**Plant extract and animals used**: The experimental plant Catharanthus roseus leaves were collected from agricultural farms near Jaipur, Rajasthan. They were then authenticated in the Herbarium, Department of Botany, University of Rajasthan, Jaipur, under specimen voucher No. RUBL-20841.The leaves were shade dried, powdered, and extracted with alcohol (90%) in a Soxhlet apparatus, to obtain a semi-solid, viscous, dark green mass, i.e., the extract.

Colony-bred adult healthy male of proven fertility (8-12 weeks old) and parous female Swiss albino mice (5-10 weeks old) weighing 25 + grams were used in the present investigation. The mice were housed in standard cages and maintained under standard conditions (12h light/dark cycle, room temperature) and provided standard laboratory chow (Ashirwad Food Industries, Chandigarh, India) and water were provided ad libitum. The extract was dissolved in 50% alcohol and administered intramuscularly. The study was approved by the Institutional Ethical Committee of the Department of Zoology, University of Rajasthan, Jaipur. The Indian National Science Academy (2000), New Delhi, guidelines were followed for the maintenance of experimental animals.

**Experimental design;**

**Female antifertility test:**

**CONTROL:** Parous female mice were administered 0.1 ml of 50% alcohol as a vehicle only and were treated as controls. A minimum of five animals were used in each experiment.

**EXPERIMENTAL**: 10 mg alcoholic extract dissolved in 0.1 ml of 50% alcohol was administered during post coital stages to adult, healthy parous female mice for 3 consecutive days from day 7-9post-coitum (pc). These females were then cohabited with males of proven fertility. Mating was confirmed by the presence of a vaginal plug or spermatozoa in the vaginal smear. The day of mating was taken as day 0.

**Autopsy schedule:** The animals were weighed, and an autopsy was performed on day 12 post-coitum (pc). The reproductive tract was quickly exposed and cleared of adherent tissue.

**Body and Organ Weight:** The animals' initial and final body weights were recorded. The uterine horns were dissected, cleared of adherent tissues and blood, and weighed to the nearest milligram.

**Fertility Test:** Number of Corpora lutea (CL) and implantation sites (IS), Resorbed implantation sites (RIS), living foetus (LF)and dead foetus (DF), if any, were counted and recorded.

**Tissue Biochemistry:** Uterine horns were frozen at -20 °c for biochemical estimations. The uterus was assayed for acid phosphatase, and alkaline phosphatases were determined by the method of Fiske and Subbarrow as given by Hawk, Oser and Summerson (1965). The ph. of the buffer was maintained at 5.0. Colorimetric readings were taken at 640 $\mu$. _

**Statistical Analysis:** Data are expressed as mean + SEM. Student's t-test was used for statistical comparisons.

## RESULTS

**Body and organ weights**: The 10 mg dose of the alcoholic extract of leaves of *Catharanthus roseus* did not significantly change the mean body weights but caused a statistically significant decline in the wet uterine weights of the experimental rats compared to the control mice (Table 1).

**Fertility Test:** A total pregnancy interceptory effect of alcoholic extract of *Catharanthus roseus* was observed at a dose of 10 mg/day/mice as compared to the control animals. (Table 2).
**Tissue Biochemistry:** In the present study, acid phosphatase and alkaline phosphatase activity of the uterus of the mice significantly decrease after treatment with the alcoholic extract of leaves of *Catharanthus roseus*.

**DISCUSSION**
The present study investigated the antifertility potential of the alcoholic extract of *Catharanthus roseus* leaves and demonstrated a significant impact on early pregnancy in Swiss albino female mice. Administration of a 10 mg dose of the extract during the critical window of embryonic implantation (days 7–9 post-coitum) resulted in 100% pregnancy interruption, indicating a potent abortifacient effect of the plant extract.
**Body weight:** In the present investigation, administration of the alcoholic and extract leaves of *Catharanthus roseus* does not significantly alter the body weights when administered post-coitally to female mice. In gross terms, this possibly indicates that the extracts do not have any apparent toxic or adverse effect on the general physiology of the test animal.
**Organ Weight:** In the present investigation, administration of the alcoholic and extract leaves of *Catharanthus roseus* shows a dose-dependent decline in the weight of the uterus on day 12 *post-coitum.* The control uterus is heavy on day 12 *pc* when the fetal sites are well-marked and well-developed fetuses are present in the uterus. As a result of abortions occurring in treated females, the uterine weight decreases considerably, and its appearance is like that of a normal uterus. Gopala Krishnan *et al.* (1970) reported a decrease in uterine weight of rats treated with Carica papaya fruit during the post-stages of pregnancy. Similarly, Sharma (1989) reported a dose-related decrease in the uterine weight of rats treated with the alcoholic extract of Nigella sativa and Carica *papaya* seeds from day 1 to 3 *post-coitum.* In contrast, Sizzirmani (1962) reported that estrogens exert a stimulatory effect on the female genital tract.

**Uterine Biochemistry**
**ACID PHOSPHATASE**
The physiological role of acid phosphatase (ACPase)is also not clearly understood, particularly in the reproductive process. According to Novikoff (1961), ACPase is located mainly in the lysosome of the uterine tissue. Manning *et al.* (1971) suggested involvement of ACPase in the heterolytic and autolytic processes, which in turn are involved in the formation of new membrane. Connell *et al.* (1967) suggested a possible role of the enzyme related to regressive phenomena within the cell rather than to growth and differentiation. Malone (1966) suggested its relation to the degeneration of secretory product and the digestion of tissue debris during the late secretory phase. The uterine phosphatases have been implicated in a variety of functions concerned with controlled degradation of the uterine components and the modification of the uterine epithelium (Boshier,1963). Joshi and Tejuja (1969) observed the ACPase activity in menstruating women. They observed that ACPase activity in normal endometrium of the uterus remains constant during the cycle through the mid-secretory phase. During the late secretory phase, there was a slight but significant increase in enzyme activity.
In the present study, ACPase activity of the uterus of the mice significantly decreases after treatment with the alcoholic extract of the leaves of Catharanthus *roseus.* These results are similar to those reported by Kabir *et al.* (1984). They reported that the benzene extract of *Hibiscus rosa-sinensis* flowers at four different dose levels, when administered from day 1 to 4 *pc* significantly reduces the ACPase activity of the uterus. A chlorohydrin, an antifertility substance, significantly reduces ACPase activity of the uterus of the house rat (Agarwal, 1977). Dixit *et al.* (1978) similarly reported a decline in ACPase activity of the uterus of the gerbil after daily administration of quinacrine hydrochloride.
On the other hand, Prakash (1979 b) showed that 50% ethanolic or benzene extracts of *Hibiscus rosa-sinensis* flowers increase ACPase activity of the rat uterus. Prakash and Mathur (1979) reported that 50% ethanolic and benzene extracts of *Embelia ribes* seeds increase the ACPase activity of the uterus of intact and ovariectomized rats. Dugan *et al.* (1968) and Karkun and Mehrotra (1973) also found an increase in the uterine ACPase after estrogen treatment in the rat and mouse. Similarly, Pakrashi and Ganguly (1982) reported that a single oral dose of aristolochic acid on day 1 or day 6 of pregnancy in mice elevates the ACPase content of the uterus.
While it would be premature to assign any functional significance to the uterine ACPase in the antifertility effect of the plant materials, the observed changes in the phosphatase content of the experimental mice indicate the definite role of uterine ACPase in the antifertility process.

**ALKALINE PHOSPHATASE**
Alkaline phosphatase (ALPase) is associated with the transport of substances across the cell membrane (Goldfischer *et al.,* 1964). ALPase is also responsible for tissue growth, differentiation and secretory activity (Malone, 1966). Sharma (1974) and Raizaday (1974) studied the ALPase activity in the uterus of the mouse, rat and gerbil, respectively. They reported that ALPase activity increases during the estrous and metaestrous stages and explained that this increase is probably due to the enhanced secretion of estrogen during the estrous, and estrogen plus progesterone during the metaestrous phase. Atkinson and Engle (1947), Arzac and Blanchet (1948), McKay *et.al* (1956), Joshi and Tejuja (1969) have examined the ALPase activity in menstruating women. They have concluded that the enzyme is present in high concentrations in the endometrium.

In the present study, the petroleum ether, alcoholic extract and chromatographic fraction (petroleum ether and benzene, 1:1 V/V) of the petroleum ether extract of *Mentha* and *Catharanthus* leaves significantly reduce the ALPase content of

the uterus of the post coitally treated animals.
These observations are similar to those of Prakash (1979a, b). Thus, ALPase content of the uterus of the rat decreases after administration of 50% ethanolic and benzene extracts of flowers of *Hibiscus rosa sinensis* to intact animals (Prakash 1979a) and of seeds of *Artobotrys odoratissimus* to intact and ovariectomized rat (Prakash,1979 b). Pakrashi and Ganguly (1982) found that aristolochic acid obtained from *Aristolochia indica* caused a decline in the ALPase content of the uterus of treated mice.
Seshadri *et al.* (1978) reported that embelin, isolated from *Embelia ribes, elevates* the ALPase level in the mouse uterus. Further, Prakash and Mathur (1979) also observed that 50% ethanolic and benzene extracts of *Embelia ribes* increase the level of ALPase in the intact and ovariectomized rat uteri.
Mehrotra and Kamboj (1979 a) have also observed in rats an increased ALPase activity during the estrous phase. Atkinson and Engle (1947) in monkey and Dempsey *etal.*, (1949), Dugan *etal.*, (12968) and Karkunand Mehrotra (1973) in the rat, have noticed that estrogenic treatment to ovariectomized animals elevates the ALPase content of the uterus.
The precise physiology of ALPase in the process of implantation still needs elaborate studies in laboratory animals. It will still be premature to consider the direct relationship between changes in the uterine ALPase activity and anti-implantation effect of estrogenic and antiestrogenic sub- stances. Prakash (1979 b) discussed that uterine alkaline phosphatase con- tent was due to the antiestrogenic nature of the plant extract of *Artobotrys odoratissimus.* He further suggested that the decrease in the ALPase level changes secretory activity of the uterus through its influence on the membrane permeability, which in turn disturbs the uterine milieu required for the maintenance of the implantation. Thus, it is clear that the plants with estrogenic or antiestrogenic properties cause a decline in the ALPase activity of the uterus of experimental animals. This drop in ALPase activity might be playing a role in preventing pregnancy. It is also known that the fundamental biochemical significance of the uterine phosphatases is their ability to catalyze the hydrolysis of phosphoric esters of sugars, amines and nucleotides (Atkinson and Engle, 1947), and this might be playing an important role in facilitating implantation of the blastocyst**.**

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**Table 1.Effect of administration of alcoholic extract of the leaves of *Catharanthus roseus* on the body weight and uterine weight of female mice. (Number of mice in each group: 5)**

| Group | | Dose Mg/day/mice | Initial body weight Mean ± SEM | Final body weight Mean ±SEM | Uterine weight Mean ± SEM |
|---|---|---|---|---|---|
| Post-coital | Control | -- | 33.5 ± 0.8 | 35.7 ± 1.03 | 372.2 ± 31.94 |
| | Experimental | 10 | 29.5 ± 3.2 | 30.5 ± 3.3* | 107 ± 13.07*** |

Significant difference at: *P<0.05 (Almost Significant) **P<0.01 (Significant) ***P<0.001 (Highly Significant)

**Table -2 .Effect of administration of alcoholic extract of the leaves of *Catharanthus roseus* on the on fertility of female mice (Number of mice in each group= 5)**

| Group | | Dose Mg/day/mice | Corpora lutea | Implantation sites | Percentage Implantation |
|---|---|---|---|---|---|
| Post-coital | Control | - | 61 | 52 | 85.24 |
| | Experimental | 10 | 56 | 0 | 0 |

**Table-3. Effect of administration of alcoholic extract of the leaves of *Catharanthus roseus* on the Biochemical Parameters of the uterus of female mice (Number of mice in each group: 5)**

| Group | | Dose Mg/day/rat | Acid phosphatase Mean ± SEM | Alkaline phosphatase Mean+SEM |
|---|---|---|---|---|
| Post-coital | Control | - | 5.5 ± 0.3 | 6.1 ± 0.5 |
| | Experimental | 10 | 3.7 ± 0.5*** | 3.4 ± 0.2*** |

Significant difference at: *P<0.05 (Almost Significant) **P<0.01 (Significant) ***P<0.001 (Highly Significant)

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