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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 7-9 post-coitum. The 10 mg alcoholic dose proved to be 100% effective in causing pregnancy interruption. The glycogen content of the uterus of the treated animal declined significantly; on the other hand, the cholesterol content of the uterus increased significantly.**

**Keywords: Catharanthus roseus, leaves, Alcohol extract, pregnancy interruption, antifertility, glycogen cholesterol, uterus**

## Introduction

The use of plant-based compounds in treating and preventing various diseases has gained increasing attention in recent years due to their broad pharmacological potential and lower side-effect profiles compared to synthetic drugs.The use of herbal antifertility agents has been on record from time immemorial. "Rig Veda", the oldest repository of human knowledge mentions medicinal use of plants.Casey (1960)also provides a glossary of plants which have been shown to possess abortifacient properties in folklore medicine.

A perusal of literature reveals that numerous plants with possible antifertilityactivityare available (Kirtikar and Basu, 1935, Nadkarni, 1954; Chopra, *et.al.*, \956; 1968; Sharma, *et.al.* 1976; Jacob, *et.al.*, 1988; Kamboj, 1987). Karnkov and Mats-(1981) have also described various species of plants belonging to the genera *Humulus, Gleditschia, Lupinus, Medicago, Pisum,Gossypium, Lithospermum, Lycopus, Ambrosia* and *Solidago* to possess promising contraceptive properties.Among these medicinal plants, *Catharanthus roseus* (L.) Periwinkle belongs to the Apocynaceae family and is widely recognised for its diverse medicinal properties. Traditionally used in Ayurveda and other folk medicine systems, *C. roseus* is known for its rich phytochemical profile, including alkaloids, flavonoids, tannins, and saponins, which contribute to its antioxidant, antidiabetic, anti-inflammatory, antimicrobial, and anticancer activities.The leaves of C. roseus are particularly abundant in therapeutic alkaloids such as vincristine and vinblastine, both of which are clinically used in chemotherapy. While much of the existing research has focused on the plant's anticancer effects, there is still limited data on its general pharmacological and toxicological effects when administered systemically, especially in animal models. Alcoholic extraction, using solvents such as ethanol or methanol, is known to efficiently isolate these bioactive constituents, making such extracts particularly suitable for pharmacological evaluation. The decoction of the flowers of periwinkle with few drops of alcohol is used as an eye wash in infants. Similarly, vinculin, an alkaloidisolatedfromCatharanthus rose us (Chopra et.al., 1959) is used for curing diabetes. The leafextract ofthis plant is used for indigestion, dyspepsia and is beneficial to the kidney. Vincaleukoblastine and Leurocristine, the alkaloids ofCatharanthus are used against Hodgkin's disease and childhood leukemia and breast cancer respectively, (Johnson et. al., 1963; ElSayyedand Condell 1981).

Considerable antifertility activity of *Catharanthus* leaf has been reported in male rat and mice (Murugand*et.al.*, 1989; Murugand and Akbarsha, 1991; Chauhan and Mathur, 1992, Stanley and Akbarsha, 1992). However, little attention has been paid on the antifertility efficacy of *Catharanthus roseus* in the mammalianfemale. Prakash and Mathur (1976) screened the antifertility effects of pods of this plant but they did not find any antifertility efficacy atleast in the mouse.

Swiss albino mice are widely utilised in experimental pharmacology and toxicology due to their genetic uniformity, ease of handling, and responsiveness to various bioactive compounds. Evaluating the effects of alcoholic extracts of *C. roseus* leaves in these models provides a foundation for understanding the systemic responses, including physiological, behavioural, and biochemical changes.

This study was conducted to study the effects of an alcoholic extract of *Catharanthus roseus* leaves in Swiss albino mice, thereby contributing to the scientific validation of its medicinal use and providing a basis for further pharmacological and toxicological investigations.

## MATERIAL AND METHODS

**Plant extract and animals used**: The experimental plant Catharanthus roseus leaves were collected from agricultural farms near Jaipur, Rajasthan. They were thenauthenticated 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 Swissalbino 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 initial and final body weights of the animals 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 glycogen (Montgomery, 1957) and cholesterol (King, 1959).

**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:** The glycogen content of the uterus of experimental mice showed a statistically significant decline in comparison to the control animals. On the other hand, a significant decrease in the cholesterol content of the uterus of experimental animals was observed on day 12pc. (Table 3).

## 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 theextracts 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-stagesof pregnancy. Similarly, Sharma (1989) reported a dose-relateddecrease 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 in general exert a stimulatory effect on the female genital tract.

**Uterine Biochemistry**

**Glycogen**

Glycogen as carbohydrate is the principal source of energy stored in the uterine tissue and its content is influenced by hormonal secretion. According to Cecil *et al.,* (1967) metabolism of uterine carbohydrate in the female is controlled and regulated by the ovarian hormone.

In general, the exogenous estrogens increase the glycogen content of the mammalian uterus (Wallas, 1952; Boettiger, 1946). Bo *et al.,* (1967) have reported that estrogens increase the glycogen concentration in the smooth muscles of the uterus of the ovariectomized rat. They indicated that estrogens stimulated glycogenolysis by increasing the glycogen synthetase activity and suppressing glycogenolysis by inhibiting the phosphorylase activity. Chandhoke and Gupta (1978) have found that *Datura lactone* increases the glycogen content in the uterus of the ovariectomized rat. Similarly, Bitman *et al.,* (1965,1967) have shown that estrogens induced a marked increase in glycogen synthesis in the uterus of the ovariectomized rat.

The effectiveness of the treatment may be linked to the biochemical alterations observed in the uterine environment. A marked reduction in uterine glycogen content suggests impaired endometrial receptivity, as glycogen is a crucial energy substrate required for successful implantation and early embryonic development. The depletion of glycogen might have disrupted the metabolic support necessary for maintaining early pregnancy.

Conversely, the significant increase in uterine cholesterol levels post-treatment may reflect disrupted steroidogenesis or membrane integrity. Cholesterol is a precursor for steroid hormones such as progesterone and estrogens, which are vital for the maintenance of pregnancy. An accumulation of cholesterol could indicate a feedback inhibition in steroid biosynthesis or altered lipid metabolism, both of which could contribute to the failure of pregnancy maintenance.

The results align with existing literature that highlights the pharmacological properties of *Catharanthus roseus*, a plant known for its bioactive alkaloids such as vincristine and vinblastine. While these compounds are primarily noted for their antineoplastic activity, the findings of this study suggest they may also exert significant influence on reproductive physiology.

It is important to note that while the extract showed complete effectiveness at the tested dose, the underlying mechanisms of action require further elucidation. Histological studies of the uterus, hormonal assays, and investigation into specific molecular pathways would be necessary to understand the precise mode of action. Additionally, evaluating the dose-response relationship and potential systemic toxicity is crucial before considering any therapeutic application.

In conclusion, the alcoholic extract of *Catharanthus roseus* leaves demonstrated strong abortifacient properties, likely mediated through significant alterations in uterine biochemistry. These findings open avenues for further research into plant-based contraceptives or abortifacients, although caution must be exercised given the potential implications for reproductive health and systemic safety.

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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 ofmicein 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 on the Biochemical Parameters of the uterus of female mice (Number of mice in each group= 5)**

| Group | | Dose | Glycogen | Cholesterol |
|---|---|---|---|---|

| | | Mg/day/rat | Mean±SEM | Mean+SEM |
|---|---|---|---|---|
| Post-coital | Cntrol | - | 8.3± 0.6 | 11.08 ± 0.7 |
| | Experimental | 10 | 6.2± 0.5* | 8.1± 0.8** |

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

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