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Antifertility Efficacy of Alcoholic Extract of Mentha Arvensis L Leaves in Swiss Albino Mice

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Abstract

The effect of the Alcoholic extract of the leaves of *Mentha arvensis* 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, and on the other hand, the cholesterol content of the uterus increased significantly.

Keywords: Mentha Arvensis, Leaves, Alcohol Extract, Pregnancy Interruption, Antifertility, Glycogen Cholesterol, Uterus

Introduction

The global population surge has intensified the demand for effective, safe, and reversible contraceptive methods. While synthetic contraceptives are prevalent, their associated side effects and long-term health implications have prompted a shift towards exploring plant-based alternatives. Marvellous powers have been attributed to plants for the alleviation of different maladies in the indigenous medicine system of India. Millions of people in the third world use herbal medicines because they believe in them and regard them as their system of medicine. (Chaudhary, R.R.,1992).

Mentha arvensis or 'mint,' commonly known as wild mint, is a member of the family Labiatae, is an erect, hairy herb,10-60 cm high with 2.5-5 cm long leaves, which are shortly petiolate. Field mint is locally used as a stimulant and carminative and is extensively utilized in traditional medicine for its diverse therapeutic properties, including antimicrobial, anti-inflammatory, and antioxidant activities. The herb yields a volatile oil known as Japanese mint oil, which is used as a substitute for peppermint oil. (Chopra *et al.*,1956). Further, an infusion of leaves of Mentha affords a remedy for rheumatism and indigestion. (Acharya and Srivastava,2008). The leaves of Mentha arvensis are known for their abortifacient properties in folkloric medicine. Recent studies have highlighted its potential antifertility effects. For instance, Sharma and Jacob (2002) demonstrated that the methanolic extract of *M. arvensis* leaves, when administered orally to male albino mice, led to a significant reduction in fertility parameters such as sperm count, motility, and viability, without affecting overall health indicators. Based on these findings, the present study aims to evaluate the antifertility efficacy of the alcoholic extract of *Mentha arvensis* as a natural, reversible female contraceptive agent.

MATERIAL AND METHODS

Plant extract and animals used: Leaves of the experimental plant," Mint" (Mentha arvensis), 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 (12 h 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 Mentha arvensis leaves 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 interceptor effect of alcoholic extract of Mentha arvensis 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 highly significant increase in the cholesterol content of the uterus of experimental animals was observed on day 12pc. (Table 3).

Table 1: Effect of administration of alcoholic extract of the leaves of *Mentha arvensis* 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	32.7 ± 2.6	35.7 ± 2.6*	156 ± 22.6***

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 *Mentha arvensis* 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	53	0	0

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

Group		Dose Mg/day/rat	Glycogen Mean±SEM	Cholesterol Mean+SEM
Post-coital	Control	-	8.3± 0.6	11.08 ± 0.7
	Experimental	10	6.3 ± 0.6*	16.62 ± 0.9**

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

DISCUSSION

Several plant extracts have been reported to act as effective antifertility agents (Badami *et al.*, 2003; Vasudev and Sharma,2006). For a long time, leaves of *Mentha arvensis* have been a folklore remedy, used to terminate pregnancy. In the present investigation, it was observed that the alcoholic extract of leaves of *Mentha arvensis* caused a decline in the percentage of implantation as compared to corpora lutea.

For a long time, leaves of "Mint", *Mentha arvensis*, have been a folk lore remedy used to terminate pregnancy. *Mentha arvensis* has been reported to possess antifertility activity in various laboratory animals. However, the leaves of *Mentha arvensis* have not been subjected to extensive study for their antifertility effect, excepting for the initial reports by Kapoor *et al.*, (1974), Bodhankar *et al.*, (1974) and Garg *et al.*, (1978), Alcoholic extract of *Mentha arvensis* leaves is 60% effective in inhibiting ovulation among rabbits, when given orally at a dose of 100 mg/kg body weight once daily for three days (Kapoor, Garg and Mathur, 1974). Bodhankar *et al.*, (1974) reported that the alcoholic extract of *Mentha arvensis* showed 80 per cent and 100 per cent postcoital antifertility activity in rats at a dose of 100 mg and 500 mg/kg body weight. Similar, results were observed by Kanjanopathi et al., (1981), who reported that the uterotonic fraction of *Mentha arvensis* inhibits pregnancy in rats at a dose of 5 and10 mg/kg body weight. My results are like those reported by many other researchers (Kanjanopathi *et al*, 1981, Gupta and Mathur, 2009).

Body weight: In the present investigation administration of the alcoholic and extract leaves of *Mentha arvensis* 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 *Mentha arvensis* 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 implantation 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 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 phos-phorylase 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.

CHOLESTEROL:

The importance of cholesterol as a precursor molecule in the synthesis of steroid hormones is well known (Dorfman and Forchielli, 1963; Eik-Nes and Hall, 1962).

In the present investigation, the increase in the cholesterol content of the uterus after the administration of postcoitally effective antifertility doses of petroleum ether, alcoholic extract, and Chromatographic fractions (petroleum ether and benzene, 1:1 **V/V)** of the petroleum ether extract of leaves of *Mentha arvensis* may well be due to its estrogenic nature

The uterine muscle being involuntary, store large amount of cholesterol which maybe structural as well as precursoral. The exact role of cholesterol in the mammalian uterus is not clearly understood. Lal (1976) mentioned that estrogens influence the conversion of uterine cholesterol to other compound or may stimulate its synthesis from its acetate precursor leading to an increase or decrease in the rabbit uterus. In the rabbit estrogen or androgen increases the uterine cholesterol deposition from day 4 to 6 *pc* or from 7 to 9th day of gestation. (Lal, 1976). In the present investigation also, similar results were obtained specifically with the leaves of *Mentha arvensis*. It could be presumed that estrogens first increase the synthesis of cholesterol from its acetate precursor and secondly block the conversion of cholesterol to the esterified compounds required for the maintenance of pregnancy, thus possibly contributing in the interference of pregnancy.

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