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# Changes in the Biochemical Milieu of the Uterus of Pregnant Rats Treated with Alcoholic Extract of the Leaves of *Mentha arvensis* L.

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**ABSTRACT:** A uterotonic fraction of *Mentha arvensis* (UM-fraction) was tested for antifertility effect in rats. Subcutaneous administration of the UM-fraction to rats pregnant from day 1 to day 10 caused a significant interruption in pregnancy. The effect was pronounced during the post-implantation period. At the effective dose, the UM-fraction did not exhibit significant estrogenic or antigonadotropic activities. However, it enhanced the estrogenic effect of estradiol when administered concurrently.

**KEYWORDS:** *Mentha arvensis*, antifertility, pregnant, rats, biochemical, leaves, extract, dose

## I. INTRODUCTION

***Mentha arvensis***, the **corn mint**, **field mint**, or **wild mint**, is a species of flowering plant in the mint family Lamiaceae. It has a circumboreal distribution, being native to the temperate regions of Europe and western and central Asia, east to the Himalaya and eastern Siberia, and North America. *Mentha canadensis*, the related species, is also included in *Mentha arvensis* by some authors as two varieties, *M. arvensis* var. *glabrata* Fernald (North American plants such as American Wild Mint) and *M. arvensis* var. *piperascens* Malinv. ex L. H. Bailey (eastern Asian plants such as Japanese mint).It grows in moist places, especially along streams. Wild mint is a herbaceous perennial plant generally growing to 10–60 cm (4–24 in) and rarely up to 100 cm (40 in) tall. It has a creeping rootstock from which grow erect or semi-sprawling squarish stems.The leaves are in opposite pairs, simple, 2–6.5 cm (¾–2+½ in) long and 1–2 cm (½–¾ in) broad, hairy, and with a coarsely serrated margin.The flowers are pale purple (occasionally white or pink), in whorls on the stem at the bases of the leaves. Each flower is 3 to 4 mm (⅛ to 5⁄32 in) long and has a five-lobed hairy calyx, a four-lobed corolla with the uppermost lobe larger than the others and four stamens. The fruit is a two-chambered carpel. The leaves have been made into tea to treat colds or aid digestion. They can also be eaten raw.

Chemical substances that can be extracted from wild mint include menthol, menthone, isomenthone, neomenthol, limonene, methyl acetate, piperitone, beta-caryophyllene, alpha-pinene, beta-pinene, tannins and flavonoids.[15][16] Mint extracts and menthol-related chemicals are used in food, drinks, cough medicines, creams and cigarettes.[16] Menthol is widely used in dental care, as a mouthwash potentially inhibiting streptococci and lactobacilli bacteria. Two main diseases that can significantly damage Japanese mint (*M. arvensis* var. *piperascens)* and its yield are the rust fungus and the mildew attacks. Mildew attacks usually only occur on the west coast of United States where the weather can be foggy and humid, a condition that attracts mildew. Rust fungus is a disease that is common for most of the Mentha plants such as peppermint and spearmint. These diseases are flagged due to the almost to none probability of controlling once it starts in a mint farm. They are typically cut immediately when discovered to help reduce the probability of contaminating the rest of the plant leaves.

## II. DISCUSSION

The dried seeds of *Mentha arvensis* were finely pulverized into powder and then macerated in 80% methanol for 48 hours. The solution was filtered, and methanol was evaporated using rotary evaporator under reduced pressure, and the concentrated extract was dried using lyophilizer to completely remove the solvent residue. The average yield of dried *Mentha arvensis* seed extract from the dried seed was 15.95%. The extract was kept in a tightly sealed container at -20°C until use. Throughout the course of this investigation, healthy nulliparous female Swiss albino rats (weighing 200 to 220 g and age of 12-14 weeks) were used. The rats were obtained from the laboratory animal breeding facility of the EPHI.

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The animals were housed in suspended stainless steel cages in an environmentally controlled room (22-23°C) and relative humidity of . A cycle of 12 hr of light and 12 hr of dark was maintained at all times. They were acclimatized for 5 days. During the period of adaptation, all the animals were provided with free access to standard pellet laboratory diet and water *ad libitum*. The handling of animals and all experimental procedures were carried out according to internationally accepted guidelines .

For mating purpose, the female Swiss albino rats should be added into the male cage in 1 to 2 ratios. After an overnight mating, a female rat was inspected for the presence of a copulatory plug the following morning and vaginal smears were taken for microscopic determination of the presence of sperm. The presence of spermatozoa in the vaginal smear was considered as day 0 of gestation . On the basis of oral reports of high consumption of the leaves and seeds for diet mixed with cultural foods, the chosen dose of extract was highest (5000 mg/kg body weight). On the first day of the test, one female Swiss albino rat fasted for 3 hours was given 5000 mg/kg of the crude extract orally using oral gavages. Then, the rat was kept under strict observation for physical or behavioral changes for 24 hr, with special attention during the first 4 hours. Because mortality was not observed in the first rat, other two female rats fasted for 3-4 hours were sequentially given a single dose of 5000 mg/kg of the seed extract and then observed in the same manner. The observation was continued for a total of 14 days for any sign of toxicity and mortality.

The pregnant rats were randomly divided into five groups (3 experimental and 2 control groups) with each group consisting of five rats. Group I was treated with distilled water and served as the pair-fed control group. Groups II, III, and IV were treated with 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the crude *Mentha arvensis* seed extracts, respectively. Group V was unrestricted-fed *ad libitum* group.

The various doses of the *Mentha arvensis* extract were selected based on the result of acute toxicity study. The extract was weighed and mixed with distilled water and continuously shacked with a vortex shaker. Final volume was 2 ml/100 g with the vehicle (distilled water), and oral gavage was used for oral administration. The treatment period was from days 6 to 12 of gestation. The rationale for administration of the extract from day 6 through day 12 of gestation is because this period represents a period of active embryogenesis and organogenesis.

## III. RESULTS

On day 12 of gestation, at 12 : 00 hours, the pregnant rats were euthanized by inhalation of 4% diethyl ether. This was achieved by putting the animal in a tight desiccator jar having cotton rinsed with high dose of diethyl ether. The rats were kept in the desiccators until they lose their consciousness. In addition, cervical dislocations were done to lose their consciousness. Once the rats lose their consciousness, they were removed from the desiccators and placed in a supine position on an operating board. The limbs were stretched and fixed; the abdominal cavity was opened by abdominal midsagittal skin incision. The flap of the skin and the abdominal muscles on both sides were reflected laterally and held with pins. Then, the uterine horns were removed and placed in Hank's balanced salt solution. The pregnancy outcomes such as the number of implantation and resorption sites were counted. The uterine horns were then incised along the antimesometrial border to reveal the embryos. With the aid of fine forceps and a dissecting microscope, the membranes surrounding the embryos were removed to reveal the underlying visceral yolk sac. The yolk sac circulation and development were evaluated. The embryos were then explanted, and embryonic growth such as development of the circulatory, nervous, visual, auditory, olfactory, and skeletal systems, as well as craniofacial development, was assessed quantitatively on the basis of 16 recognizable developmental endpoints (morphological scores), In addition, the numbers of somites were also counted.

The data regarding pregnancy outcome were analyzed using one-way ANOVA. Five Swiss albino rats within each group were analyzed. The result showed that the number of implantation site did not appear to be different from all the other groups. There was high incidence of fetal resorptions at a dose of 1000 mg/kg compared to all the other groups.

Embryonic growth indices used were the number of somites and morphological score. The number of somites present was considered as one of the most important criteria for assessing embryonic growth. Compared to all other groups, the number of somites following treatment with 1000 mg/kg *Mentha arvensis* seed extract was significantly decreased. Embryos of 1000 mg/kg *Mentha arvensis* leaf extract-treated group show a significant reduction in the morphological score compared to all the other groups .

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The numbers of fetuses in M250 mg/kg, M500 mg/kg, and M1000 mg/kg *Mentha arvensis* -treated groups were 43, 43, and 41, respectively. There was a dose-dependent reduction in the number of fetuses and implantation sites among the groups but not statistically significant. With respect to fetal resorptions, there was a high incidence of fetal resorptions in M1000 mg/kg treated groups compared to all the other groups. The number of live fetuses was significantly decreased in M1000 mg/kg treated groups compared to both pair-fed control and *ad libitum* groups. Compared to all other groups, there was also a high incidence of fetal death in this group (M1000 mg/kg). But, the relationship was not statistically significant at the level of 0.05 (ANOVA)

## IV. CONCLUSIONS

In addition, frequencies of fetal resorptions per litter and the number of dead fetuses also increased statistically significantly. This study also revealed a dose-dependent decrease in the number of fetuses in each dam, but this was not statistically significant. This developmental delay in prenatal growth may be due the presence of high amount of alkaloid in *Mentha arvensis* seed which may cause developmental defects through the disruption of cholinergic neurotransmission [28]. However, in all fetuses of animals treated with all doses of *Mentha arvensis* no developmental anomalies and general external organ abnormalities were observed. Findings in the developmental toxicity test suggest that the methanolic seed extracts of *Mentha arvensis* are not safe to rat embryos and fetuses at the highest dose. Its toxic effects were evidenced by significant delay in embryonic and fetal development and increase in fetal resorptions and fetal death. Therefore, excessive intake of *Mentha arvensis* seed may be unsafe.

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