EFFECTS OF SPEARMINT (PEPPERMINT) ON MORPHOLOGICAL, HISTOLOGICAL AND BIOCHEMICAL ASPECTS OF OVARIES

Uzma Ali,[1] Amber Salman,[2] Momna Riaz,[3] Shireen Hamid,[4] Faiza Hanif,[5] Saqib Mansoor[6]

ABSTRACT

Background and Objective: Spearmint (Peppermint), known for its aromatic and medicinal properties, is used in various products globally, including food, confectionaries, chewing gum, and toothpaste. While it has been known for its potential therapeutic benefits, this study aimed to investigate the effects of spearmint on the morphology, histology, and biochemistry of ovaries in Wistar albino rats.

Methods: This Experimental animal study involved 30 adult female albino mice (200–250 g), divided into three equal groups. Group A (Control) received 15 ml/kg distilled water, Group B (Low Dose) received 0.3 g/kg body weight ethanol extract of peppermint leaves, and Group C (High Dose) received 0.6 g/kg body weight. Treatments were administered via gastric infusion for 30 days. Ovaries were dissected, weighed, and histological slides were prepared using H&E staining. Parameters, including capsule thickness, follicle diameter, and corpus luteum, were analyzed with ImageJ® software. Data were processed using SPSS 20.0, with significance level as $p < 0.05$.

Results: Final ovarian weights (Group A: 0.19±0.02 g, Group B: 0.36±0.09 g, Group C: 0.49±0.05 g; $p<0.001$) and Relative Tissue Weight Index (RTWI; Group A: 0.08, Group B: 0.17, Group C: 0.27; $p<0.001$) increased significantly in experimental groups. Histological analysis revealed decrease in follicle diameters with higher doses of spearmint in both secondary and graafian follicle (secondary follicle; Group A 29.10±4.11 µm, Group B 22.31±5.51 µm, Group C 15.20±2.94 µm and Graafian follicle; Group A 53.75±8.01 µm, Group B 35.87±4.97 µm, group C 23.64±4.96 µm). Decreased corpus luteum size, along side significant reductions in serum progesterone levels post-experiment (Group A: 55.29±5.94 ng/ml, Group B: 23.01±8.89 ng/ml, Group C: 4.12±0.70 ng/ml; $p<0.001$) were also observed.

Conclusion: This study reveals detrimental effects of excessive spearmint leaf consumption on rat ovarian histology, body weight, and progesterone levels. Caution, particularly during pregnancy, is advised due to potential risks to hormonal and ovarian function. Further research is essential to understand the mechanisms and assess the implications for human health.

Key words: spearmint, ovarian morphology, progesterone level

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Spearmint (Peppermint), has been cultivated for its distinctive medicinal and fragrant qualities, therefore commonly used in confectionaries, food, chewing gum and tooth pastes.[1] It has the ability to induce cytochrome P450, is a potent regulator of fatty acid peroxidation.[2] This enzyme is mainly produced by liver, and works by producing reactive oxygen species (ROS), especially free radicals. Peppermint has also a remarkable inhibitory effect on mutagens found in cooked meat, especially heterocyclic amines. This anti-mutation quality makes it valuable in beverages and teas.[3]

The peppermint by inducing P450 enzyme is also involved in the metabolism of hormones, thus can

1. *Department of Anatomy, Faisalabad Medical University, Faisalabad(Previously in Sheikh Zayed Postgraduate Institute of Medicine Lahore)*
2,3. *Department of Anatomy, University Medical and Dental College, Faisalabad*
4. *Department of Pathology, Aziz Fatima Medical College, Faisalabad*
5. *Department of Anatomy, King Edward Medical University, Lahore*
6. *Departmet of Anatomy, Niazi Medical And Dental College, Sargodha*

Correspondence:
Dr. Amber Salman, *Ph.D Anatomy, Associate Professor, Department of Anatomy ,University Medical and Dental College, Faisalabad.*
dr.ambersalman2@gmail.com

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used to treat the hormonal imbalances, especially in the treatment of polycystic ovary syndrome, where its anti-androgenic properties can improve the symptoms.[4] It could be due to reduction in high testosterone, FSH and LH levels after drinking mint tea as compared to placebo.[5] Similarly hirsutism and facial hairs associated with PCO's due to increases androgenic effects of male hormones were also reduced after consuming peppermint herbal tea.[6] A histopathological study on effects of peppermint on uterine tissue observed apoptotic cells in the surface and glandular epithelium, with diffuse infiltration of leucocyte in both the endometrial and myometrial layers.[7] There are studies that reported its toxic effects on kidney, liver, placenta and fetuses.[8-10] While its effects on biochemical parameters are well documented, previous studies have not examined the gross and histological aspects of ovarian function. To address this gap, this study was conducted to investigate the effects of spearmint on morphological, histological and biochemical aspects of ovaries in Wistar albino rats.

METHODS

This experimental animal study was conducted in Department of Anatomy, from January 2023 to December 2023 at Sheikh Zayed Postgraduate Institute of Medicine, Lahore after ethical approval (Letter # F-38/NHRC/AQMIN/FRB/205)

Thirty adult female albino mice weighing 200-250 gm were selected for the study and were divided into three groups (n=10). Group A (Control) received 15ml/kg of distilled water, Group B (Low dose) received Ethanol extract peppermint leaves at 20 g/l (0.3 g/kg body weight) while Group C (High Dose) received Ethanol extract peppermint leaves at 40 g/L (0.6g/kg body weight) for 30 days by gastric infusion. Wister rats were weighed at the start and end of the experiment. At the end, these rats were dissected, ovaries were removed, weighed, and then histological slides were prepared and stained with Hematoxylin & Eosin (H & E). Ovaries were evaluated for histomorphometric parameters, including capsule thickness, corpus luteum, and follicle diameter and analyzed for intra- and myometrial thickness, shrinkage, and cellular damage. Image analysis software ImagJ® was used to microscale these sections.

Data were entered and analyzed using SPSS 20.0. The quantitative data, such as body weight, fetal weight, placental weight, and placental weight are presented as mean ± S.D. Comparisons between groups for these parameters were made using one-way ANOVA and Chi-square test, with significance set at $p < 0.05$.

RESULTS

The mean initial body weights (g) of adult Albino rats in groups A, B, and C showed no significant differences (p-value: 0.140). However, the final body weights were significantly increased in all groups by the end of the experiment. The paired ovarian weight at the end of the study was significantly higher in groups B and C compared to the control group (A). A similar trend was observed in the Relative Tissue Weight Index (RTWI), which showed a significant increase from group A to group B and a further significant increase from group B to group C. (Table1).

The ovaries of group A had normal pink colour, while in group B, only 20% had normal pink ovaries and 80% had dark purple ovaries. Group C consisted of all animals with dark red and black spotted ovaries. The differences between all three groups were highly significant (Table 2). While the uterine morphology and shape of the ovaries appeared to be normal with a compact ovoid structure and irregular peripheral margins, with no significant differences in group wise comparisons.

Table 1: *Initial and Final Body Weight, Paired Ovarian Weight, and Relative Tissue Weight Index (RTWI) for Groups A, B, and C, with p-values Indicating Comparisons Among the Three Groups.*

Parameters	Group A	Group B	Group C	P value
Initial Body Weight (gm)	246.0± 22.8	238.4± 6.1	251.4±3 1.2	0.14
Final body Weight (gm)	248.7± 4.4	205.6 ±8.4	180.9± 10.7	<0.001
Paired Ovarian weight	0.19± 0.02	0.36± 0.09	0.49± 0.05	<0.001
Relative Tissue Weight Index (RTWI)	0.08	0.17	0.27	<0.001

The histological examination of control group A and experimental groups B and C revealed normal cervical muscle and tissue. Within the ovarian stroma, the cortex contained microvascular growth at different stages, while the tissue contained blood vessels, smooth muscle and connective tissue. (Figure 1)

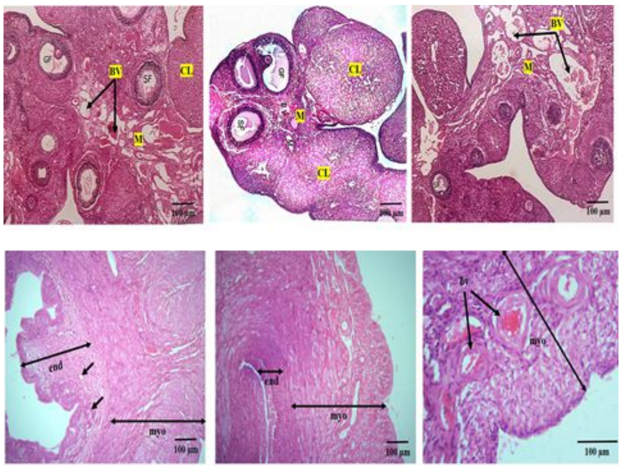

Figure 1: *Histological images of the ovaries (first row), uteri (second row) collected from groups A, B, and C are presented. Ovaries from Group A oocytes have well-developed corpus luteum (CL), Graphian follicles (GF) and secondary follicles (SF) in the cortex region, while blood vessels are present in the medullary region but histological changes like vascular congestion, reduction in size and vacuolation in CL, GF and SF are seen in both experimental groups (B and C) (H&E, 100X).*

The histological sections uteri of group A had evenly distributed uterine endothelial glands (black arrow) with well intact uterine mucosa. The inner circular and outer longitudinal smooth muscles in myometrial layers (myo) with normal blood vessels (bv). The gropu B and C showed decreased endometrial and myometrial thickening with moderately to severely slightly dilated blood vessels in Group B and C respectively.

The simple cubic connective tissue layer capsule filled with germinal epithelium remained intact and undamaged in all three groups (A, B, C).

Primary follicles were found in both control group A and experimental groups B and C, located in the peripheral cortex below the capsule. They are spherical oocytes surrounded by a thin layer of follicular epithelial cells in the oocyte stroma.

The secondary follicles of control group A reached deep into the cortical stroma. They consisted of spherical oocytes, polymeric granulosa cells with a central nucleus, and fluid-filled zones separated from the theca cells by a narrow bottom Theca interna cells varied in size surrounding blood vessels. Secondary follicles had spherical oocytes surrounded by granulosa and theca cells located deep in the ovarian cortex in all the groups but in both the experimental groups number and size of follicles were small as compared to group A. This difference in diameter was not only significant between group A and experimental groups B and C (p value <0.001) but also between groups B and C.

The graafian follicles occupied the superficial cortex with primarily oocytes surrounded by granulosa cells in all the groups, but the size of the Graafian follicles was reduced in group B and C. This reduction in size of graafian follicle was highly significant, with p-values <0.001 (Table 3).

In group A, there were more corpora lutea in different parts of the cortex, especially in the superficial area. These corpora lutea contained multilayered granulosa luteal cells, large oval nuclei, and eosinophilic vacuolar cells. Theca-luteal cells were also rectangular

Table 2: *Status and comparison of color of ovary for animals in control and experimental groups after exposure to dried leaves extract of spearmint.*

Color of Ovary	**% of normal and abnormal Coloured ovaries in Groups**							
	A		**B**		**C**		**Total**	
	N	%	N	%	N	%	N	%
Normal	20	100	4	20	0	0.0	24	40
Abnormal	0	0.0	16	80	20	100	36	60
Total	20	100	20	100	20	100	60	100

but smaller, with dark round nuclei. The corpora lutea was scattered with numerous blood vessel. In experimental groups B and C, corpora lutea were present in superficial and deep corpora regions. It consisted of granulosa luteal and theca luteal cells: small granulosa luteal cells and dense chromatin in theca luteal cells. Although no blood exudates were observed, the size of the corpora lutea was reduced in both experimental groups B and C.

Initial serum progesterone levels were similar among the three groups: Group A (54.54±6.28 ng/ml), Group B (54.046±5.49 ng/ml), and Group C (53.99 ± 5.43 ng/ml) while at the end of experiment these levels for group A, B and C were 55.29±6.20, 23.01±8.89 and 4.12±0.70 ng/ml respectively. Significant differences in progesterone levels were observed in all three groups, with group C showing the lowest levels of progesterone and significantly different from groups A and B (Table 3).

DISCUSSION

Spearmint, scientifically known as Mentha Spicata Labiateae, is a herbal plant renowned for its medicinal and aromatic qualities[1]. It is indigenous to North America and Egypt, and widely employed as a flavoring agent in various applications, including medicines, toothpaste, and candies.[11,12] This versatile herb is utilized to alleviate conditions such as cold, headaches, vomiting, chest problems, and allergic reactions.[13,14] Moreover, spearmint has demonstrated effectiveness in the treatment of autoimmune arthritis. Additionally, its essential oil is harnessed for fumigation and as an insecticide, and it exhibits antibacterial, antiviral, and anti-mutagenic properties.[15-18]

Spearmint promotes lipid peroxidation, resulting in tissue oxidative stress by generating reactive oxygen species (ROS).[2] These ROS trigger apoptosis, leading to mitochondrial depolarization, DNA fragmentation, and the activation of the liver enzyme Caspases P450 (CYP 3A4), which, in turn, accelerates the breakdown of steroid hormones metabolized in the liver, reducing their concentrations in the blood.[2,7] The harmful effects of spearmint on the liver, kidneys, uterus, fetuses and testes have been established.[7-10,19] This study focuses on assessing the impact of dried spearmint leaf extract on the ovaries of adult female albino rats due to concerns about its excessive use.

Both experimental groups showed a significant decrease in body weight compared to the control group. These results coincide with the many previous studies where in same dose of peppermint and the related herbs significantly reduced body weight.[20,21] Peppermint can reduce body weight by inhibiting growth hormone and increasing serotonin levels, which can activate satiety centers. In addition, its role in enhancing lipid peroxidation may contribute to weight loss.

Similarly, Ovarian weight increased in experimental groups, with significantly more increase at a

Table 3: *Comparison of mean diameter of secondary & secondary & graafian follicile and pre-post serum progesterone levels among control in experimental groups. (One way ANOVA)*

Parameter	Group A	Group B	Group C	p-value	
Diameter of Secondary Follicle (µm)	29.10 ± 4.11	22.31 ± 5.51	15.20 ± 2.94	Group A vs Group B	<0.001
				Group A vs Group C	<0.001
				Group B vs Group C	<0.016
Diameter of Graafian Follicle (µm)	53.75 ± 8.01	35.87 ± 4.97	23.64 ± 4.96	Group A vs Group B	<0.001
				Group A vs Group C	<0.001
				Group B vs Group C	<0.001
Pre-Experiment Serum Progesterone (ng/mL)	55.54 ± 6.28	54.46 ± 5.93	53.99 ± 6.89	Group A vs Group B	<0.001
				Group A vs Group C	<0.001
				Group B vs Group C	<0.001
Post-Experiment Serum Progesterone (ng/mL)	55.29 ± 5.94	23.01 ± 8.89	4.12 ± 0.70	Group A vs Group B	<0.001
				Group A vs Group C	<0.001
				Group B vs Group C	<0.001

higher dose. Few studies used member of lamiacae family having same constituent like spearmint reported the same effects on adult ovaries of rats.[22,23]

Increased uterine weight in response to peppermint may be due to possibly increased levels of pyruvate transaminase due to effects of peppermint on the liver resulting in uterine contractions, and causing blood vessel dilation.

Relative Tissue Weight Index was also increased significantly ($p<0.001$) in both experimental groups. These findings are consistant with the previous study where significant increased RTWI was noticed. It could be due to enzyme induction which caused necrosis in ovaries.[19]

Ovarian color in group C showed a marked dark red color due to vascular congestion, likely dose-dependent but there was no change in shape observed in both experimental and control group. This finding concide with the previous studies.[22,24]

The study assessed histological parameters, including capsule, primordial and secondary follicles, graafian follicles, and corpus luteum. Ovaries in all groups exhibited a typical ovulatory cycle from primordial to Graafian follicles, with normal differentiation of theca and granulosa, and the formation of antrum, corpus luteum and cumulus oophorus. These findings are consistent with many previous studies.[7,22,24]

In the present research, the diameter of the secondary and graafian (Fig 1) follicles significantly ($p< 0.001$) reduced in the size in both experimental groups as compared to control. These results concide with previous studies where other members of Lamiacae family caused reduction in the size of both the secondary and Graafian follicles, but, there are studies that found no effects on the size of these follicles.[22,24,25] The reduction in size of secondary Graafian glands may be due to the phytoestrogen content of mint, which is converted to estrogen after metabolism in which this endogenous estrogen negatively affects hypothalamic GnRH release , reduces FSH from the anterior pituitary and inhibits follicular development by potentially interfering with ovarian estrogen receptors.[22]

Atrophied corpora lutea were significantly ($p<0.001$) observed in majority of the experimental groups, as reported previously possibly due to the reduction in Graafian follicle size.[22,25] The medulla of experimental groups exhibited dilated blood vessels with significant congestion, possibly linked to elevated pyruvate transaminase levels and the effects of spearmint's phytoestrogens.[25] Serum progesterone levels significantly ($p<0.001$)decreased in both experimental groups. Similar findings were reported in a study where phenols, found in mint, laid the same effects on ovaries and also reduced the serum progesterone levels.[7] This may be due to phenols inhibiting steroidogenesis by affecting p450-β hydroxylase enzyme and GnRh levels.[7]

The results of this study has shown effect on ovaries both morphologically, biochemically and histologically but its major limitation being animal study, the results of this study needs careful consideration before generalizing on human.

CONCLUSION

This study has have shown adverse effects of dried mint leaf consumption on ovarian histology, whole body, and weight of oocyte pairs. Although these effects have been noted in high doses equivalent to 4-8 cups of peppermint tea per day, precautions should be taken to reduce the possible levels of progesterone in the blood, in particular during pregnancy.

Ethical Approval: This study was approved by Ethical Review Committee of TSheikh Zayed Postgraduate Institute of Medicine, Lahore, vide No. ERB No.: F-38/NHRC/AQMIN/FRB/205)

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AUTHOR'S CONTRIBUTION

Conceptualization and study design	UA, AS, MR
Data Acquisition	UA, AS, MR, SH
Data Analysis/ interpretation	UA, AS, SH, SM
Manuscript drafting	UA, SH, SM
Manuscript review	UA, AS, SH, FH

All authors read and approved the final draft.

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