DOI: https://doi.org/10.53350/pjmhs2023175369

ORIGINAL ARTICLE

Effects of Dried Leaves Extract of Spearmint (Mentha Spicata Labiateae) on Histology of Ovaries of Adult Female Wistar Albino Rats

UZMA ALI[1], AMBER SALMAN[2], HUMAIRA GULNAZ[3], MOMNA RIAZ[4], FAIZA HANIF[5], SHIBRAH RAFAQAT[6]
[1]*Assistant Professor, Faisalabad Medical University Faisalabad*
[2]*Associate Professor UMDC Faisalabad*
[3]*Associate Professor, Faisalabad Medical University, Faisalabad*
[4]*Assistant Professor, UMDC Faisalabad*
[5]*Senior Demonstrator, Deptt. Of Anatomy, KEMU, Lahore*
[6]*M.Phil PGR, Department of Anatomy, KEMU, Lahore*
Corresponding author: Uzma Ali, Email: druzmaaliazeemi@gmail.com

ABSRTACT

Spearmin is a herb which has aromatic and medicinal properties. It is also used as flavoring agent in toothpaste, chewing gums, confectionaries and pharmaceutical preparation. The objective of this study was to evaluate the toxic effects of dried leaves extract of spearmint on gross and histological parameters of ovaries of adult female Wistar albino rats.

Materials and Method: 60 female adult Wistar albino rats were taken in this study. After acclimatization rats divided into group A (Control), group B (Low dose experimental) and group C (High dose experimental) containing 20 rats each. Group A received distilled water whereas group B and C received dried leaves extract of spearmint in doses of 0.3 & 0.6 g / kg / day respectively, through gastric intubation for 30 days. Blood sampling and body weight of all the three groups were taken at the start & end of experiment. Ovaries of rats were dissected, observed for gross parameters and then prepared for histological examination.

Result: All quantitative parameters had skewed distribution. The body weight of both experimental groups was decreased significantly (p – value < 0.001). Paired ovarian weight was increased significantly (p – value < 0.001). The ovaries of both experimental groups showed atrophied antral, Graffian follicles and corpora lutea significantly (p – value < 0.001). The decreased levels of serum progesterone were also observed.

Conclusion: Use of spearmint in adult female rats showed significant harmful effects on morphology and histology of ovaries, hence it is recommended that further research work should be carried out to evaluate its other toxic effects on different organs of body

Keywords: Spearmin, Graffian follicles, Ovary, Leaf extract

INTRODUCTION

Herbs are plants that are produced specifically for their medicinal or fragrant qualities (1). The use and knowledge of herbs has been passed down for thousands of years (2). The Lamiaceae, or mint family, is a crucial one in the world of medicinal plants (3). Typically, fragrant plants belong to the family of herbs and shrubs (4). People of this species tend to be bisexual and verticillastrate. There are around 236 genera in the larger lamiaceae, with 6,900 to 7,200 species total. Saliva (900), Scutellaria (360), Stachys (300), Plectranthus (300), Teucrium (250), Vitex (250), Thymus (220), Nepta (200), and Clerodendrum (200) are the most abundant genera, each having hundreds of species (150) (5).

Spearmint stimulates the process of antioxidation of lipids, which causes peroxidation in tissues and the generation of oxygen species that are reactive (ROS) (6). ROS trigger cell death, which activates mitochondrial excitability, DNA breaking, and the Caspases P450 (CYP 3A4) machinery in the hepatocytes. This hepatic kinase causes corticosteroid hormone breakdown to rise. The quantities of these substances in the blood drop when they are digested in the liver.

There are 19 species of Mentha in the lamiaceae family, and these species may be found in many different habitats across the world (7). Moreover, there are 13 hybrids created by crossing Mentha Longifolia and Mentha Rotundifolia (7). Herbs like basil, mint, rosemary, sage, savoury, marjoram, oregano, thyme, lavender, and perilla are all examples of plants that have a fragrant scent throughout their whole plant (8). The purpose of this research was to examine the gross and histological changes to the ovaries of adult female Wistar albino rats caused by an extract of dried spearmint leaves.

MATERIALS AND METHODS

The method of research was an experiment. This experimental research was carried out at the Shaikh Zayed, Postgraduate Medical Institute in Lahore, Pakistan, in conjunction with the Department of Anatomy there. The duration of the study was twelve months. Wistar albino rats are the study population. Mature, disease-free female albino rats (age range, 3-6 months) weighing between 200 and 250 grammes are eligible for inclusion. Rats with an excess of 250 g in weight, rats younger than 3 months of age, and rats with any visible signs of illness was not considered. For this investigation, 60 female Wistar albino rats measuring 200-250 grammes were chosen and acquired from the Veterinary Research Institute in Lahore.

The rats were stretched out on the dissection tray in supine position. Abdomen was opened through midline incision running from pubic region to lower end of sternum. Then ovaries were identified and removed, weighed, observed for gross anomalies and fixed in 10% Natural Buffered Formaldehyde solution for histological evaluations. 5µm sections were cut with the help of rotary microtome and stained with Haematoxylin and Eosine (H&E) for detailed histological examinations. Markedly distorted follicles, like damaged during tissue preparation, were also excluded from study. Diameter of ovarian follicle was calculated by taking horizontal and vertical measurement at right angle to each other and taking their average. Four follicles were used from each slide and the mean was taken. The reference and test teams were compared. Data entered and analyzed by using SPSS 20.0. P value< 0.05 considered significant

RESULTS

The capsule is a thin layer of connective tissue underline the germinal epithelium which was simple cuboidal in nature. It was normal and intact in all the three control A, experimental groups B and C. The secondary follicles of the control group A were occupying a deeper segment of the cortical stroma. The oocytes were spherical in shape, surrounded by polyhedral shaped granulosa cells with rounded to oval shaped centrally placed nuclei. These cells were lying on a thin basement membrane separating them from the theca cells. Irregular fluid filled spaces were present around them. The cells of theca interna were variable, composed of spindle shaped to polygonal cells with oval central nuclei having blood vessels between them.

The secondary follicles of experimental groups B and C also comprised of a rounded oocyte surrounded by granulosa and theca cells. They occupied in a deeper portion of the ovarian cortex. The number and size of the follicles decreased in both

experimental groups B and C as compared to the group A. Small blood vessels were seen in between theca cells. Theca interna was blended with theca externa in all the groups.

Theca interna showed a mixture of cells varying between polygonal and flattened cells with rounded to elliptical nuclei respectively in both experimental groups B and C. The examination of secondary follicles revealed that there was decreased growth of follicles. The size of secondary follicle was decreased in 10 animals of group B and 18 animals of group C (Table 1.1)

Table 1: Status and comparison of secondary follicle for animals in control and experimental groups after exposure to dried leaves extract of spearmint.

	Group							
	A		B		C		Total	
	N	%	N	%	n	%	N	%
Normal	20	100.0	10	50.0	2	10.0	32	53.3
Atrophied	0	0.0	10	50.0	18	90.0	28	46.7
Total	20	100.0	20	100.0	20	100.0	60	100.0

Key: A Control group, B Low dose group, C High dose group, N Number of animals

The group wise comparison showed that the difference between group B and C was significant when compared to group A. This difference was insignificant with p-value 0.068 when comparison was done between group B and C (table 1.2)

Table 2: Group wise comparison of corpora lutea status among control and experimental groups after exposure to the dried leaves extract of spearmint.

(I) Group	(J) Group	Chi-square	Df	P-value
A	B	14.41	1	<0.001**
	C	29.19	1	<0.001**
B	C	3.33	1	0.068

The ovarian medulla of control group A was vascular showed blood vessels of different size embedded in stroma. There was no congestion found in 20 animals of control group A. The blood vessels of 16 animals of group B and 20 animals of group C were found congested. The difference of two experimental groups was highly significant from control group with p-value <0.001 (Table 24)

Table 3: Status and comparison of congestion in BV for animals in control and experimental groups after exposure to dried leaves extract of spearmint.

Congestion in B. V	Group							
	A		B		C		Total	
	N	%	N	%	N	%	N	%
Present	0	0.0	16	80.0	20	100.0	36	60.0
Absent	20	100.0	4	20.0	0	0.0	24	40.0
Total	20	100.0	20	100.0	20	100.0	60	100.0

Chi-sq= 46.67 p-value < 0.001**

The difference between both experimental groups B and C was also significant with p-value 0.014 (Table 1.4)

Table 4: Group wise comparison of congestion in BV in between control and experimental groups after exposure to dried leaves extract of spearmint.

(I) Group	(J) Group	Chi-square	Df	P-value
A	B	26.67	1	<0.001**
	C	40.00	1	<0.001**
B	C	5.99	1	0.014

DISCUSSION

Spearmint stimulates the process of antioxidation of lipids, which causes peroxidation in tissues and the generation of oxygen species that are reactive (ROS). Their harmful effects on liver, kidney, uterus and testis have been proved (9). Due to its excessive usage the present study was designed to evaluate the effects of dried leaves extract of spearmint on ovaries of adult female albino rats. In the present study the mean body weight of the rats at the end of experiment in control group A was 248.7gm while the mean weight of the rats in group B and C was 205.6gm and 180.9gm respectively. The contrast of the treatment and reference categories respectively revealed a substantial drop in muscle mass in both treatment arms, with a p-value of 0.001.

These findings are consistent with the findings of a previous research in which Zulfiqar A et al evaluated the very same dosage of spearmint on pregnant mice and found a substantial loss of body weight (10). This is also similar with the results of Guan et al, who looked at the impact of soya (a Lamiaceae member of the family with the same component as spearmint) on the growth of young rats and discovered a substantial decrease in body weight (11). Similarly, Bano et colleagues studied the effects of Dill (a Lamiaceae member) on obese adult rats and found a considerable loss of weight (12). This finding is consistent with another research in which Lamizumi K et al administered adult rats Carvone (active element of spearmint) including essential oil and found a substantial decrease in body weight (13). Guney M et al, on the other hand, looked into the impact of spearmint on rats for 30 days at a dosage of 20gm/L (estimated 2.2gm/Kg body weight) and didn't find any substantial loss in body weight (14). Nozhat et al also reported that at low dose of spearmint cause no effect on the weight of adult male rats (15).

These findings are consistent with those of Thaukar et al, who discovered that the impacts of Carum Carvi and Carum Longa (both representatives of the Lamiacae genus) on the physiological and sexual parameters of female rats, as well as a decrease in the reduction of auxiliary and Graafian follicles (16). Nevertheless, similar investigation contrasts the current research, in which Brasil et al looked into the impacts of Soya (member of the Lamiaceae family) on the endocrine cycle of rats and found no influence on the dimension of Graafian and secondary follicles (11). Likewise, Monsefi et al. investigated the impacts of Dill on oocytes and reproduction in adult female rats and discovered no impacts on secondary and Graafian follicle size (17).

In the present study serum progesterone levels were measured at the start and end of experiment. The mean of serum progesterone levels at the start of experiment for group A, B and C was 55.54 ng/ml, 54.46 ng/ml and 53.99 ng/ml respectively. There was no significant difference in the serum progesterone levels among three groups A, B and C with p-value 0.735. The mean of serum progesterone levels at the end of experiment were 55.29 ng/ml, 23.01 ng/ml and 4.12 ng/ml for group A, B and C respectively. It showed that at the end of experiment serum progesterone was markedly reduced in both experimental groups, more in group C than group B as compared to control group A. The difference was significant with p-value <0.001. Similar findings were observed in a study in which effects of phenols (constituent of spearmint) were observed on the ovaries of common carp and reduction in the levels of serum progesterone was found (18).

CONCULUSION

The research found that dehydrated leaf extracts of spearmint had a detrimental influence on ovarian cytology. While these benefits were shown at large doses (equivalent to 4 - 8 cups of spearmint tea daily), it is recommended that spearmint be avoided, particularly during conception, since it lowers blood progesterone levels.

Further Recommendations: Further study on spearmint extract's impact on cycle and fertility is suggested. A thorough examination of the hypothalamo-pituitary-ovarian axis at various periods of the menstrual cycle is also necessary. As a result, it may be taken securely throughout the reproductive period and also during pregnancy.

REFERENCES

1. Salmerón-Manzano E, Garrido-Cardenas JA. Worldwide Research Trends on Medicinal Plants. 2020;17(10).
2. Šantić Ž, Pravdić N, Bevanda M, Galić K. The historical use of medicinal plants in traditional and scientific medicine. Psychiatr Danub. 2017;29 Suppl 4(Suppl 4):787-92.

3. Uritu CM, Mihai CT, Stanciu GD, Dodi G. Medicinal Plants of the Family Lamiaceae in Pain Therapy: A Review. 2018;2018:7801543.
4. Mamadalieva NZ, Akramov DK, Ovidi E, Tiezzi A, Nahar L, Azimova SS, et al. Aromatic Medicinal Plants of the Lamiaceae Family from Uzbekistan: Ethnopharmacology, Essential Oils Composition, and Biological Activities. Medicines (Basel, Switzerland). 2017;4(1).
5. Naghibi F, Mosaddegh M, Motamed SM, Ghorbani A. Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. Iranian Journal of Pharmaceutical Research. 2022;4(2):63-79.
6. Wu Z, Tan B, Liu Y, Dunn J, Martorell Guerola P, Tortajada M, et al. Chemical Composition and Antioxidant Properties of Essential Oils from Peppermint, Native Spearmint and Scotch Spearmint. 2019;24(15).
7. Ramos da Silva LR, Ferreira OO, Cruz JN. Lamiaceae Essential Oils, Phytochemical Profile, Antioxidant, and Biological Activities. 2021;2021:6748052.
8. Kagawa N, Iguchi H, Henzan M, Hanaoka M. Drying the leaves of Perilla frutescens increases their content of anticancer nutraceuticals. Food science & nutrition. 2019;7(4):1494-501.
9. Atanassova M, Atanassov L, Valere Tsouh Fokou P.
10. El-Nekeety AA, Hassan ME, Hassan RR, Elshafey OI, Hamza ZK, Abdel-Aziem SH, et al. Nanoencapsulation of basil essential oil alleviates the oxidative stress, genotoxicity and DNA damage in rats exposed to biosynthesized iron nanoparticles. Heliyon. 2021;7(7):e07537.
11. Guan L, Huang Y, Chen ZY. Developmental and reproductive toxicity of soybean isoflavones to immature SD rats. Biomedical and environmental sciences : BES. 2008;21(3):197-204.
12. Bano F, Ahmed A, Ahmed M, Parveen T. Anethum graveolens seeds aqueous extract stimulates whole brain 5-hydroxytryptamine metabolism and reduces feeding behavior and body weight in obese rats. Pak J Pharm Sci. 2015;28(1):221-5.
13. Boukhris M, Bouaziz M, Feki I, Jemai H, El Feki A, Sayadi S. Hypoglycemic and antioxidant effects of leaf essential oil of Pelargonium graveolens L'Hér. in alloxan induced diabetic rats. Lipids in Health and Disease. 2012;11(1):81.
14. Wyllie AH. The genetic regulation of apoptosis. Current opinion in genetics & development. 1995;5(1):97-104.
15. Nozhat F, Alaee S, Behzadi K, Azadi Chegini N. Evaluation of possible toxic effects of spearmint (Mentha spicata) on the reproductive system, fertility and number of offspring in adult male rats. Avicenna journal of phytomedicine. 2014;4(6):420-9.
16. Thakur S, Bawara B, Dubey A, Nandini D, Chauhan NS, Saraf DK. Effect of Carum carvi and Curcuma longa on hormonal and reproductive parameter of female rats. International Journal of Phytomedicine. 2009;1:31-8.
17. Monsefi M, Ghasemi A, Alaee S, Aliabadi E. Effects of Anethum graveolens L. (dill) on Oocyte and Fertility of Adult Female Rats. Journal of reproduction & infertility. 2015;16(1):10-7.
18. Das S, Majumder S, Mukherjee D. Effect of Phenol on Ovarian Secretion of 17β-Estradiol in Common Carp Cyprinus carpio. Archives of environmental contamination and toxicology. 2013;65.