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Histomorphological study of the effect of mint on the uterus and ovary of adult Wistar rats

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ABSTRACT

Mentha piperita is a medicinal plant used for treating human diseases for thousands of years. However, the traditional medicinal usage of herbs by humans is imperfect and unscientific by modern standards. It is the result of countless trial-and-error tests that people have conducted, and so traditional usage points the way to natural therapeutic usage. "Natural" does not necessarily mean "safe." This study aimed to evaluate the effects of ethanolic extract of *Mentha piperita* leaves on histo-morphological changes in the ovary and uterus of adult female Wistar rats. Hence this study was undertaken to evaluate its impact on the gonadal organ in female Wistar albino rats in 18 female albino rats of age 6-8 weeks which were divided into 3 groups. Group I was treated as control, Group II and III received orally 200mg/kg and 400mg/kg of ethanolic extract of *Mentha piperita* respectively for 60 days. Then the rats were sacrificed and the ovaries and uterus were processed for haematoxylin and eosin staining to study the effect on the estrous phase, ovulation, and implantation. The ovarian germinal epithelium was found to be flattened at places and disrupted. The stroma shows more fibrosis with severe degeneration. Granulosa cells are also disrupted. Follicles show features of degeneration. Degenerating oocytes are also seen in places. The endometrium lining epithelium is disrupted, vascularity was increased and the myometrium has become edematous. It is shown in previous studies that *Mentha arvensis*, *Mentha spicate* (spearmint), and *Mentha piperita* have got adverse effects on the reproductive system but in our study on female fertility, we found that *Mentha piperita* showed increased folliculogenesis and many degenerated follicles at different stages of development.

1. Introduction

Mentha piperita is a medicinal plant used for treating human diseases for thousands of years (Aryal et al., 2019; Singh et al., 2015). As we later stress, "natural" does not necessarily mean "safe." Some herbal products are extremely effective but so dangerous that they should only be used in the hands of skilled medical professionals. Others, however, are sufficiently safe that they can be used by laypeople to help prevent or alleviate minor health problems. Sometimes herbal drugs are preferable, but as we stress throughout this work, qualified medical personnel should always be consulted.

Mentha piperita is currently one of the most economically important aromatic and medicinal crops. It is commonly known as Peppermint, Brandy mint, Candy mint, Lamb mint, Balm mint, Vilayati pudina, or Paparaminta and belongs to the family Lamiaceae (Rita and Animesh, 2011). It is a popular medicinal plant in several traditional systems of medicine. In Ayurveda, this is an important ingredient of several compound formulations used in the management of gastrointestinal and skin disorders. Moreover, it has been used to treat a variety of digestive complaints such as colic in infants, flatulence, diarrhoea, indigestion, nausea and vomiting, morning sickness, and anorexia, and as a spasmolytic to reduce gas and cramping. The oil is also used for toothache, rheumatism, and muscular pains and to relieve menstrual cramps. *M. piperita* is currently used to treat irritable bowel syndrome, Crohn's disease, ulcerative colitis, gallbladder and biliary tract disorders, and liver complaints (Tyler,

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1992; Fleming, 1998). Medicinal plants have remedial effects but are not always safe and may be capable of causing toxicity (Spiteri, 2011).

2. Material and method

This histomorphology study was undertaken to study the effects of *Mentha piperita* on fertility in Wistar albino rats and to find out the morphological and histological differences between the control group of rats and the rats treated with different doses of *Mentha piperita*. All the experiments were carried out in the daytime from 09:00 hours to 16:00 hours. IAEC-approved experimental protocol and care of animals were taken as per CPCSEA guidelines, Dept. of Animal Welfare, Government of India.

Twenty Wistar albino female rats, 6-8 weeks old, weighing 120-150 grams were procured from Ingenex ltd, Bhubaneshwar. They were kept in the Central Animal House, Department of Pharmacology, MKCG Medical College, Berhampur. They were housed in clean polypropylene cages (6 rats/ cage) which were maintained under controlled room temperature with a relative humidity of 45-55% under 12 hour light and dark cycle.

They were provided with a standard laboratory diet, water ad libitum and kept for one week to acclimatize to laboratory conditions before starting the experiment. The following drugs and chemicals have been used in the experiment:

1. Test drug used was the extract of *Mentha piperita* procured from Sapiens bioanalytical lab.
2. Vehicle used was Tween 80 procured from Himedia.

2.1. Experimental design

The rats were randomly divided into different groups containing 6 animals in each group as shown in Table 1.

2.2. Methodology of female fertility test

2.2.1. Effect on estrous cycle

The test drug and vehicle were given daily for 30 days. Vaginal smears were collected daily using a cotton swab technique between 9 a.m. to 10 a.m. The smear was placed on the slide and examined under a light microscope. Rats exhibiting a four-stage and four-day estrous cycle of proestrous-estrous-metestrous-diestrous were selected for this study. The duration of each phase of the estrous cycle was noted throughout the treatment period (30 days).

2.2.2. Effect on ovulation

In this test, vaginal smears from each rat were examined daily for 15 days and the rats exhibiting 3 regular cycles were used. Drugs and vehicles were started in the estrous phase and administered daily for 30 days. On the 31st day, 24 hours after the last treatment, the rats from each group were sacrificed by cervical dislocation. The ovary and uterus were dissected out, weighed quickly, and processed for histological examination and were fixed in 10% formal saline. The tissues were dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Serial sections of 5µ thickness were obtained using a rotatory microtome. The de-paraffinized sections were stained with hematoxylin and eosin. The sections were explored for any abnormalities under the following headings:

- Ovary: Germinal epithelium, cellular organization, connective tissue stroma, follicular atresia, Graafian follicle, any signs of nuclear degeneration, vascularity
- Uterus: Endometrial architecture, epithelial cell status, uterine glands, uterine lumen, uterine diameter, uterine musculature, connective tissue stroma, vascularity, and uterine glands.

2.2.3. Effect on implantation

Female rats in a pro-estrous phase were kept with male rats in a ratio of 2:1. The female rats were examined the following morning for the presence of thick clumps of spermatozoa in the vaginal smear and were considered as day 1 of pregnancy. Drug treatment was done from day 1 to day 10 of pregnancy. All the animals were sacrificed by cervical dislocation on day 10 and laparotomy was performed to determine the number of implantation sites and resorption sites in both uteri horns (Singh, 1990).

Table 1: Experimental design for drug dosage during estrous phase, ovulation, and implantation

Group (n=6)	Drug and dose	Route of administration	No. of days		
			Estrous	Ovulation	Implantation
I	Vehicle (Tween 80)	orally	21	30	10
II	Ethanolic extract of *Mentha piperita* (MPE)200 mg/kg body weight	orally	21	30	10
III	Ethanolic extract of *Mentha piperita* (MPE)400 mg/kg body weight	orally	21	30	10

Statistical Analysis: Data obtained were analyzed by one-way ANOVA followed by Dunnett's test. Histopathological scores (non-parametric data) were subjected to Krushkal Wallis one-way ANOVA followed by Dunn's test using the statistical software Graph Pad Prism Version. $P<0.05$ was considered as minimal level of significance.

3. Results

3.1. Ovary

The control group I in Fig. 1 shows the histology of the normal ovary of Albino rats in its diestrous phase with follicles seen at different stages of development. In group II in Fig. 2, the germinal epithelium shows flattening at places. The developing follicles (primary and secondary) are relatively decreased in number but the size of the oocyte has increased with reference to the control. The zona pellucida is swollen. Granulosa cells are found dispersed at places due to edema. The theca of the secondary follicle is thinned out at places and is disrupted. The amount of corpus luteum has increased relative to that of control but vacuolations are seen which are suggestive of signs of degeneration and necrosis. Stroma is edematous with dispersed interstitial cells and an increased number of blood vessels can be observed as compared to that of the control. Follicles are seen as a homogenous mass with lymphatic infiltration and mild necrosis. In group III in Fig. 3, the germinal epithelium is totally flattened and disrupted with the stroma showing more fibrosis with severe degeneration. Granulosa cells are disrupted. Follicles with features of degeneration with degenerating oocytes are also seen in places. All observations of groups II and III are described in Table 2.

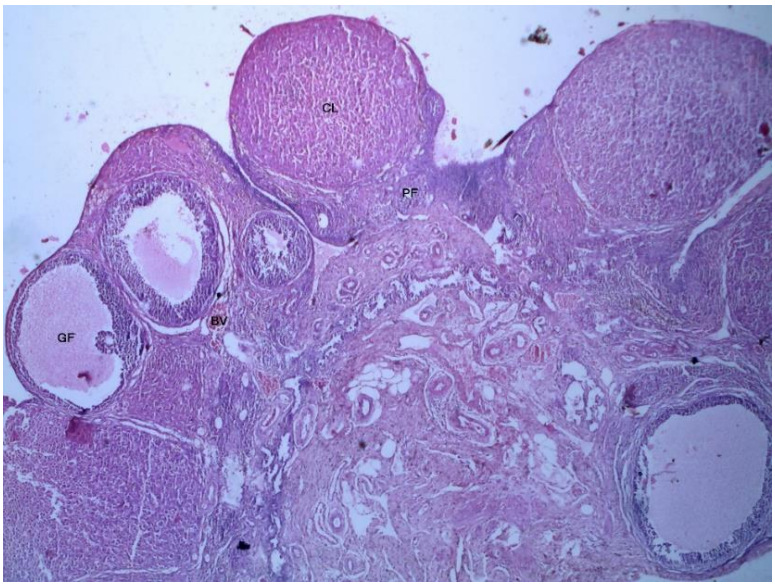

Fig. 1: Rat ovary of group I (control) at 100X magnification showing primary follicle (PF), secondary follicle (SF), Graafian follicle (GF), corpus luteum (CL), and blood vessels (BV)

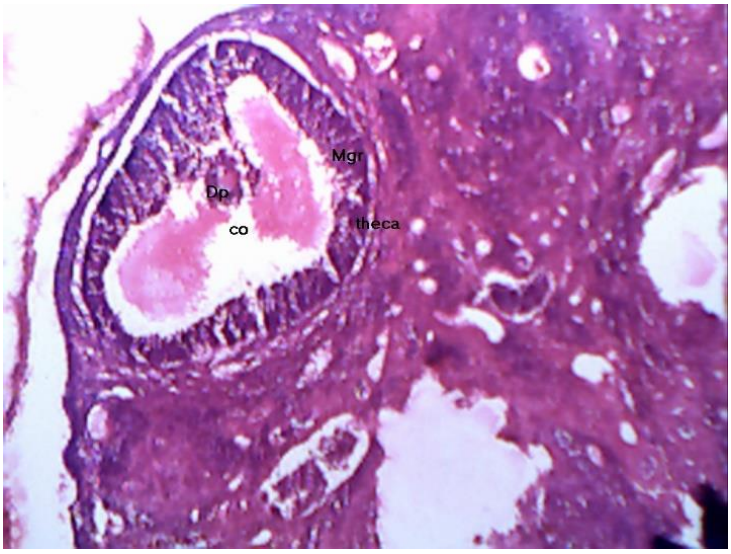

Fig. 2: Rat ovary of group II (200mg/kg MPE) at 100X magnification showing discus proligenous (Dp), cumulus oophoricus (CO), membrana granulosa cells (Mgr), and theca (T)

3.2. Uterus

The control group I in Fig. 4 shows the normal histology of the albino rat. In group II in Fig. 5, the lining epithelium has changed to large cuboidal cells. The lamina propria is edematous with decreased number of uterine glands and increased lymphatic infiltrations. The myometrium is intact. In group III

in Fig. 6, the lining epithelium is completely disrupted, vascularity is increased and myometrium has become edematous. All observations in group II and III are described in Table 3. Moreover, the implantation sites were also observed to be decreased in number in the drug treated group (Table 4).

4. Discussion

Fertility regulation comprising both contraception and management of infertility is an important component of reproductive health. Many medicinal plants have contraceptive properties. The definite effect of some Indian plants in controlling the fertility of mammalian species is well established and a number of them have been experimentally tested using modern techniques for their anti-fertility effect. Maurya et al. (2004) have given a review to provide an account of the studies carried out on traditional plants which are used for fertility regulation (Maurya et al., 2004). *Mentha piperita* is an herbal plant used in many diseases, however, it is shown in some studies that *Mentha arvensis*, spearmint (*Mentha spicata*), and *Mentha piperita* have got adverse effects on the reproductive system (Nasri, 2013).

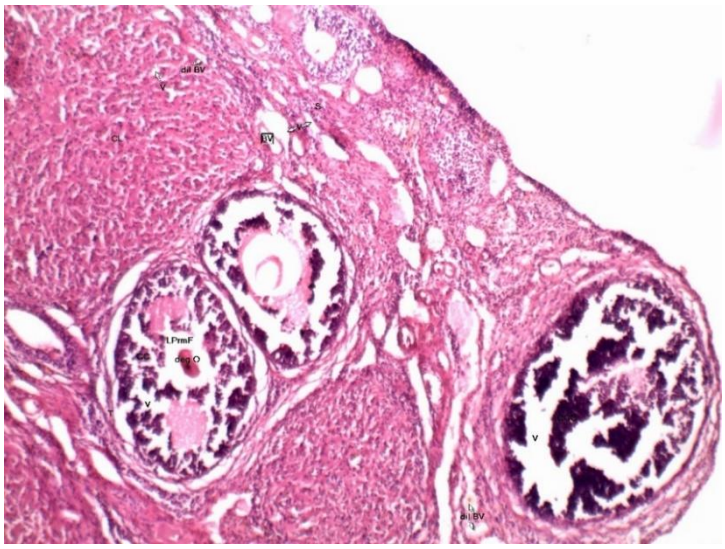

Fig. 3: Rat ovary of group III (400mg/kg MPE) at 100X magnification showing degenerating follicle (deg F), degenerating oocyte (deg O), granulosa cells (GC), blood vessels (BV), corpus luteum (CL), theca (T), and Graafian follicle (GF)

Table 2: Histomorphology changes in Wistar rat ovary in different groups

Study tissue		Parameters	Control	MPE 200mg/kg	MPE 400mg/kg
Surface epithelium			Cuboidal epithelium	Flattened cells in some places	Disruption of lining epithelium with flattened cells
Follicular characteristics	Primordial and Growing follicles		Primary follicles	Normal	Normal
			Growing follicles	Increased+	Increased++
	Graafian follicle	Number	Normal	Increased+	Increased++
		Size of oocyte	Normal	Increased+	Increased++
		Swelling of zona pellucida	Normal	Increased+	Increased++
		Granulosa cell disruption	Normal	Increased+	Increased++
		Theca thinning and disruption	Normal	Increased+	Increased++
	Corpus luteum	Vacuolation	--	Increased+	Increased++
		Swelling of luteal cells	--	Increased+	Increased++
		Sign of degeneration and necrosis	--	Increased+	Increased++
STROMA	Edema		--	Increased++	Increased+++
	Vacuolar degeneration		--	Increased++	Increased+++
	Vascularity		Normal	Increased+	Increased++
	Polymorphonuclear cells		--	Increased+	Increased++
	Sign of necrosis and fibromuscular degeneration		--	--	Increased+

+: slightly increased; ++: moderately increased; +++: highly increased

Table 3: Histomorphology changes in Wistar rat uterus in different groups

Study tissue	Parameters		Control	MPE 200mg/kg	MPE 400mg/kg
Endometrium	Lining epithelium		Simple columnar	Large cuboidal (reduced height)	Large cuboidal
	Lamina propria	Blood vessels	Normal	Increased+	Increased+++
		Uterine glands	Normal	-	--
		Polymorphonuclear cells	Few	Increased+	Increased+++
		Oedema	absent		
Myometrium			Normal	Normal	Normal
Perimetrium			Normal	Normal	Normal

+: slightly increased; +++: highly increased

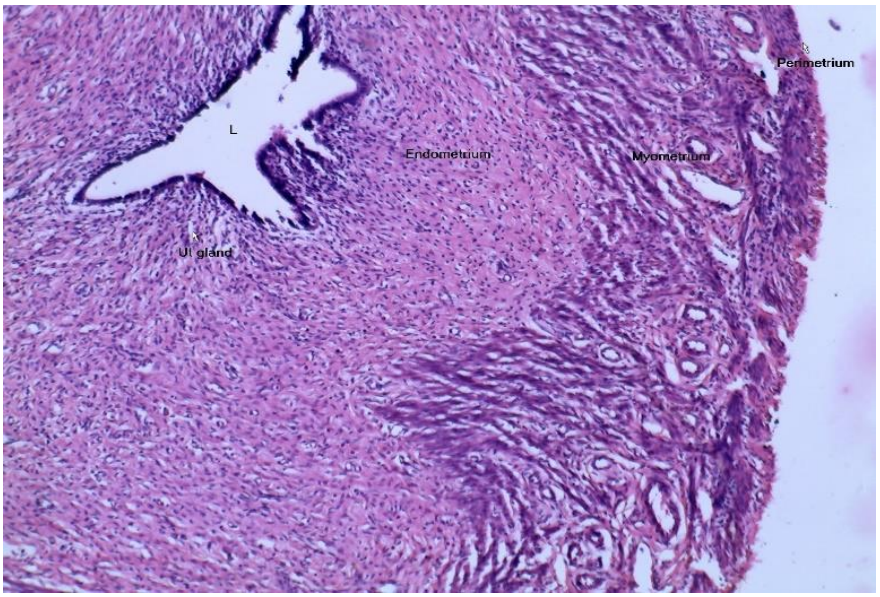

Fig. 4: Rat uterus of group I (control) at 100X magnification showing uterine gland (Uterine G) and uterine lumen (UC)

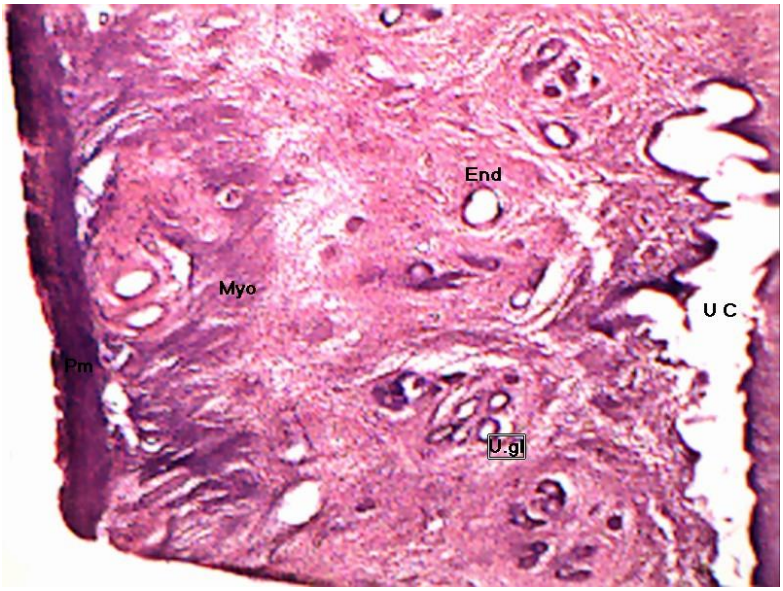

Fig. 5: Rat uterus of group II (200mg/kg MPE) at 100X magnification showing uterine lumen (UC), uterine gland (UG), endometrium (End), myometrium (Myo), perimetrium (Pm)

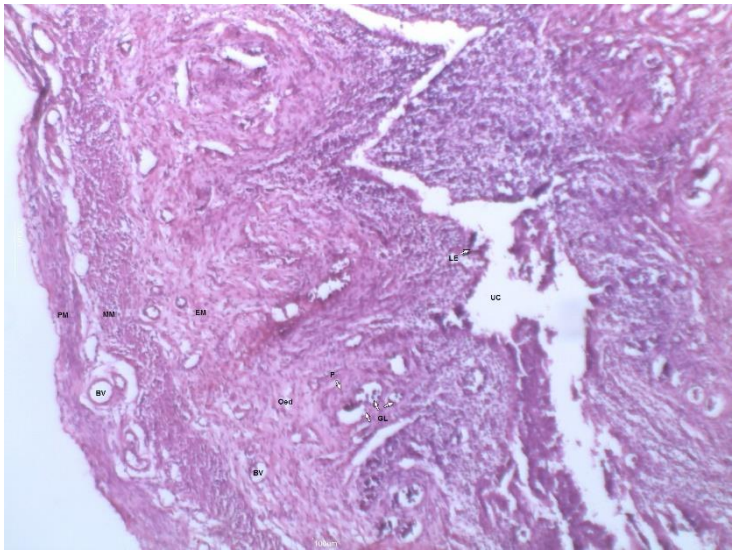

Fig. 6: Rat uterus of group II (400mg/kg MPE) at 100X magnification showing uterine lumen (UC), blood vessels (BV), epithelium (LE), uterine gland (UG), endometrium(End), myometrium (Myo), perimetrium (Pm), edematous endometrium (Oed)

Table 4: Effect of MPE on implantation

Group (n=6)	No. of implantation (mean±SE)	p
I (Tween 80)	9.667±0.7149	
II (MPE 200)	5.667±0.4944	<0.05
III (MPE 400)	4.667±0.3333	<0.01
k.w	12.13	
p	0.0002	

Rat is frequently used to study reproductive physiology as they have a short reproductive cycle than other large animals. Hence, Wistar albino rats were selected for the study (Krinke, 2000). In this study, the histopathological observations of the mint-treated group showed the ovarian tissue with an increased number of follicles in various stages of development indicating increased oogenesis. Thus it can be tried in patients presenting with complaints of infertility. Though the damaging effects in the growing follicles are minimal, the Graafian follicle showed a greater degree of damage with increasing doses. There are disrupted granulosa cells and many showed features of degeneration. The oocytes are also seen at different degrees of degeneration. There is a varying degree of fibrosis, vacuolar and fatty degeneration in the stroma. Large vacuoles abound in the field and the number of blood vessels is proportionately increased. Similar histological observations are described in the work of Ali et al. (2014) on albino rats administered with MSG which is in consonance with our observations (Ali et al., 2014). *M. piperita* can be used as a therapeutic agent to treat infertile patients whose infertility occurs due to poor oocyte quality and anovulation (Abasian et al., 2018). Previous studies have also documented that *M. piperita* showed an anti-androgenic effect by reducing increased androgen levels and preventing ovarian cell dysfunction in PCOS to improve fertility (Grant, 2010).

We found that the uterine lining epithelium changed to cuboidal in the treated group with increased vasculogenesis in the lamina propria, making it more edematous as compared to the control group. It has also been studied by previous authors that using extracts of Mentha induces menstruation and regulates menstrual cycles (Mokaberinejad et al., 2012).

5. Conclusion

Mentha piperita showed increased folliculogenesis but many degenerated follicles at different stages of development. However further investigations are necessary to shed more light on the effects of *Mentha piperita* on the female fertility system. Also, the beneficial effects of *Mentha* along with its safety, availability, and low cost; a future therapeutic role in women with amenorrhea and oligomenorrhea is expected.

It has been concluded that with its vast and diversified pharmacological potential *M. piperita* has a strong future in the world market. This plant is now well-acclimatized and cultivated in different parts of India and enjoys a strong export potential for the volatile oil extracted from it. Some herbal products are extremely effective but, in some cases, manifest toxic effects, and they should only be used in the hands of skilled medical professionals.

Compliance with ethical standards

Ethical consideration

IAEC-approved experimental protocol and care of animals were taken as per CPCSEA guidelines, Dept. of Animal Welfare, Government of India.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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