

A Histomorphological Study of the Effect of Mint on the Testes of Albino Rats

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Abstract: Since times immemorial mint has been used as food additives such as flavoring agent. Despite its wide use there are some reports about its adverse impact on different systems including reproductive system. Hence this study was undertaken to evaluate its impact on gonadal organ in male wistar albino rats. 18 male albino rats of age 6-8 weeks were divided in 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 testes were processed for Haematoxylin and Eosin staining. With 200 and 400 mg/kg the gonadal index was reduced and on histology the general architecture of seminiferous tubules was grossly damaged. Vacuolation and degenerative changes in testicular tissue was observed. This study suggests that mint has reproductive toxicity in male on long term continuous use, however further study is needed to establish these findings.

Keywords: MPE=Mentha piperita Extract, Mint

I. Introduction

Use of food additives such as spices, flavoring agents and coloring agents is a common practice in human beings¹. Either food additives act as preservatives, enhancer of palatability, digestion, carminatives and appetizers; but their effect on body system is not widely reported scientifically. Despite their taste stimulation and appetite enhancement, many of them have adverse impacts.² The magic of Indian plants in reducing fertility of mammalian species is well established. Herbal drug therapy is a common practice adopted in traditional and alternative medicine and has been used in the treatment of many diseases from ancient times.³ A number of Indian plants have been experimentally tested using modern techniques for their anti-fertility activity.⁴ Approximately 318 different plants are used worldwide of which 227 plants are of Indian origin. So far 74 plants have been screened for their anti-fertility potential and 48 of them have been found to be effective.⁵

The MINT (*Mentha arvensis* and *Mentha pulegium*)⁶ have proven its anti-fertility effect in male as well as female albino rats in many studies. Out of them *Mentha piperita* is an important medicinal herb belongs to family Lamiaceae, and commonly known as peppermint, which is potentially used as a flavoring agent from chewing gum, cosmetics and pharmaceutical products.⁷

II. Material And Method

The study seeks to find the morphological and histological differences between control group of Wistar Albino Rats and the Rats treated with different doses of *Mentha piperita*. Different literatures and methods done by different workers have been pursued.

1. Animal: Male Wistar Albino rats of 6-8 weeks old weighing 120-150 grams were procured from Ingenex Ltd Bhubaneswar. They were kept in the Central Animal House, Department of Pharmacology, MKCG Medical College, and Berhampur. They were housed in clean polypropylene cages (6 rats/ cage) and maintained under controlled room temperature with relative humidity of 45-55% under 12 hours light and dark cycle. They were provided with standard laboratory diet, water ad libitum and kept for one week to acclimatize with laboratory conditions before starting the experiment.

All the experiments were carried out in the day time from 09:00 hrs to 16:00 hrs. IAEC approved the experimental protocol and care of animals was taken as per CPCSEA guidelines, Dept of Animal Welfare, Government of India.

2. Drugs And Chemicals:

| SL | NAME | PURPOSE |
|----|-----------------------------------|-----------|
| 1. | Extract of <i>Mentha piperita</i> | Test drug |
| 2. | Tween 80 | Vehicle |

Methodology of Male fertility test

18 healthy adult male wistar albino rats of 6-8 weeks were selected for the study and divided into 3 groups having 6 animals in each. The vehicle/ mint extract was orally administered daily to each rat every morning for 60 days. On 61st day the animals were weighed and sacrificed by cervical dislocation. Immediately blood was collected from each rat by cardiac puncture and centrifuged.

Histomorphological Examination:

The testes was removed, cleaned of accessory tissues and weighed. The testicular dimensions were noted using precision callipers. The gonadosomatic index for testis was calculated by dividing the testis weight by body weight of each animal¹.The testes were processed for histological examination and fixed in 10% formal saline. The tissue was 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 deparaffinised sections were stained with Haematoxylin and Eosin. Photomicrographs of the sections were explored for the abnormalities under microscope with motic software.

Parameters of evaluation:

- Height of germinal epithelium
- Tubular diameter
- Number of Leydig cells
- Interstitial area

(STATISTICAL ANALYSIS :)

Data obtained were analyzed by one way ANOVA followed by Dunnet’s‘t’ test. Histopathological scores (non parametric data) were subjected to Krushkal Wallis one way ANOVA followed by Dunn’s test using a statistical software Graph Pad Prism Version. P<0.05 was considered as minimal level of significance.

III. Observation And Results

TABLE -1: Effect of ethanolic extract of Mentha piperita on body and organ weight on male wistar albino rats

| Treatment group | Initial body weight(g) | Final body weight(g) | Weight gain(gm) | Testis weight(gm) | Gonado somatic index (GSI) |
|-----------------|------------------------|----------------------|-----------------|-------------------|----------------------------|
| I(Tween 80) | 172.5±5.73 | 215.8±6.37 | 45.00±1.82 | 1.617±0.13 | 0.746±0.054 |
| II(200mg/kg) | 179.2±4.90 | 205.8±5.54 | 26.67±3.33** | 1.197±0.044* | 0.582±0.018* |
| III(400mg/kg) | 165.8±8.98 | 179.2±12.0 | 13.33±4.014*** | 0.963±0.10*** | 0.541±0.053* |
| F | | | 24.82 | 10.93 | 5.762 |
| P | | | <0.001 | <0.0012 | 0.0139 |

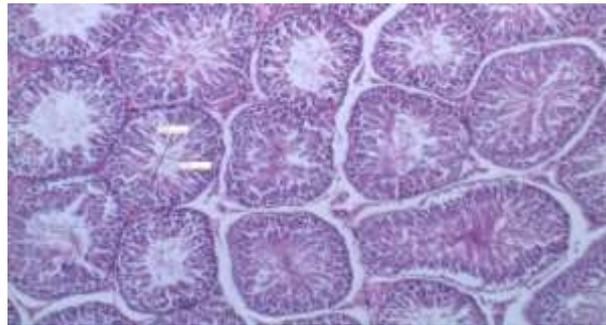
TABLE 2: Histological outcomes of testis with MPE treatment

| Group (n=6) | Testicular histological parameters | | |
|--------------|------------------------------------|---------------------------|--------------------------------------|
| | Tubular diameter | Ht of germinal epithelium | No of Leydig cells/interstitial area |
| I | 356.4±9.75 | 131.5±12.54 | 33.93±1.39 |
| II | 293.7±22.44* | 84±3.018 | 18.17±1.24 |
| III | 251±12.42 | 75.28±3.32 | 12±1.06 |
| F | 11.19 | 19.18 | 82.91 |
| P | <0.001 | <0.0001 | <0.0001 |

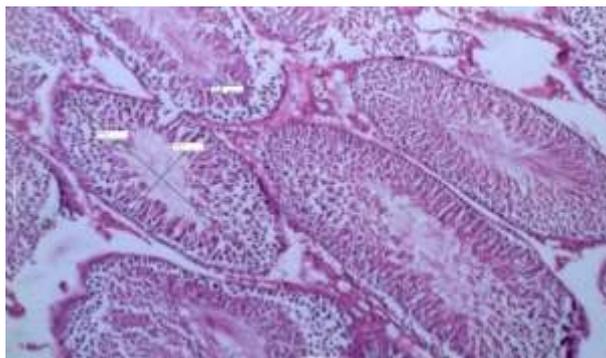
Table 1 shows the body weight changes and organ weight of rats treated with vehicle and ethanolic extract of Mentha piperita leaves for 60 days. It was observed that the net weight gain with MPE 200mg/kg treated rats was significantly less than that of control rats (p<0.01). But with MPE 400mg/kg dose the net weight gain after 60 days treatment was very less in comparison to control group which was a highly significant change. (p<0.001).Hence the gonadosomatic index of both group II and III rats was significantly less (p<0.05) when compared to control group.

Table 2 reveals the effect of MPE on testicular histological parameters. The mean tubular diameter of seminiferous tubule of control group was observed to be 356.4±9.75µm whereas the tubular diameter was reduced with MPE treatment to 293.7±22.44 and 251±12.42µm with 200& 400 mg/kg respectively and this change with both the doses were significantly reduced from that of control group. (p< 0.05). Similarly the height of germinal epithelium cell were reduced to 84.0±3.018 &75.28± 3.32 with 200 & 400 mg/kg b.w MPE

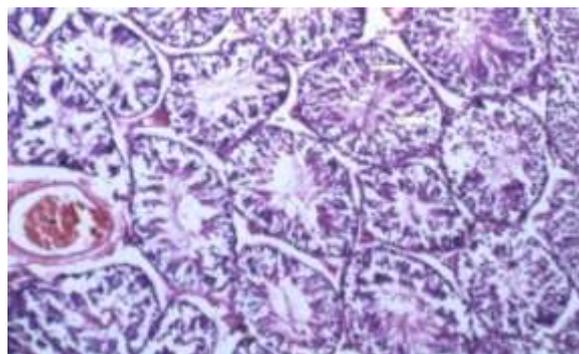
treatment from that of control group i.e 131.5 ± 12.54 showing a significant reduction in height of germinal epithelium. ($p < 0.05$).



Photomicrograph 1 of testis of control group of rats (100X Magnification) ST=Spermatid, Int=interstitium



Photomicrograph 2 of testis treated with 200mg/kg MPE (100X magnification) GE= Germinal epithelium, BM= Basement membrane V= Vacuole, Int=interstitium



Photomicrograph 3 of testis treated with 400mg/kg MPE (100X Magnification) Int= interstitium, BV= blood vessel, ST= Spermatid

IV. Discussion

Fertility regulation comprising of both contraception and management of infertility is an important component of reproductive health. Many medicinal plants have contraceptive properties. Definite effect of some Indian plants in controlling fertility of mammalian species is well established. A number of plants of Indian origin have experimentally been tested using modern techniques for their anti fertility effect. Maurya et al. (2004)⁵ have given a review to provide an account for the studies carried out on Indian traditional plants which are used for fertility regulation

In the present study the ethanolic extract of *Mentha piperita* at 200mg/kg and 400mg/kg was administered orally for sixty days in male rats to cover four spermatogenic cycles. The male rats showed a highly significant weight difference from that of control group as shown in table no 1. The weight of testes was reduced in comparison to control group to a significant extent (table 1). Hence the gonadosomatic index was also reduced significantly with MPE treatment as shown in table no 1 which corroborates with the study of

N.S.Nosseir et al¹.Edema and thickening of the lining epithelium was more marked in the drug treated groups as compared to that of control .Detachment of primary spermatocyte layer from lining epithelium was more marked in 400mg/kg MPE treated rats as compared to control and 200mg/kg of MPE treated rats. Sertoli cell degeneration was seen more in 400mg/kg drug treated rats as compared to that of control and 200mg/kg of MPE treated rats. The stroma showed increased interstitial edema, number of blood vessels and their callibers had increased, vacuolation was well marked and the amount of polymorphonuclear cells had also increased.

Table 2 reveals the effect of MPE on testicular histological dimensions. The mean tubular diameter of seminiferous tubule was decreased in both the drug treated groups as compared to that of control. Similarly the height of germinal epithelium was reduced in the drug treated groups as compared to that of control.

V. Conclusion

Mentha piperita a medicinal plant is used for treating human ailments for thousands of years. Traditional medicinal usage of herbs by men, however seemingly imperfect and "unscientific" as per modern standards, is the result of countless trial-and-error tests that people have conducted, and so traditional usage points the way to natural therapeutic usage. The usual notion that all natural medicaments are safe is often far from truth.

- In our study *Mentha piperita* showed damage to the the cytoarchitecture of the epithelium of seminiferous tubules and a definite anti-spermatogenic effect in albino rat.
- In our study *Mentha piperita* in the test doses show a definite anti-spermatogenic effect in albino rats. Hence it may be envisaged to be used as a potential male anti-fertility drug.
- However further investigations are necessary to shed more light on the effects of *Mentha piperita* on the male fertility system.

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