

EFFECTS OF MENTHA PIPERITA AND MELATONIN ON THE SEMINIFEROUS TUBULAR DIAMETER AND HEIGHT OF THE GERMINAL EPITHELIUM IN THE TESTIS OF MICE FED CONTINUOUSLY WITH MICROWAVE OVEN EXPOSED DIET.

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ABSTRACT

Background: Microwave exposed food intake among human is on rise. **Objective:** To determine the comparative effects Mentha piperita and melatonin on the seminiferous tubular diameter and height of the germinal epithelium in the testis of mice fed continuously with microwave oven exposed diet. **Methodology: Study Design:** Randomized control trial. **Place and Duration of Study:** Department of Anatomy, Army Medical College Rawalpindi, in cooperation with National Institute of Health (NIH), Islamabad, from 1st April to 31st May 2016. Thirty two adult male mice (BALBc strain) weighing 25-30gms were divided into four equal groups having 8 mice each. Group G1 (control group), was given standard diet 5-10 gm/animal/day for the four weeks. Group G2 received 5-10 gm/animal/day of microwave oven exposed mice pellets daily for the four weeks¹. Group G3 was given leaf extract of Mentha piperita (1g/kg b.wt./day)² along with microwave oven exposed mice pellets (5-10 gm /animal /day) for 4 weeks and the Group 4 was given melatonin 12 mg /kg/day orally³ along with microwave oven exposed mice pellets (5-10 gm /animal /day) for 4 weeks. After the experimental period was over, the animals were sacrificed. General features of the testis were noticed. Testis were processed embedded and stained for histological study. SPSS 21 was used for data analysis. ANOVA test was applied for intergroup comparison of quantitative variables. **Results:** In the experimental group G2, seminiferous tubular diameter and height of the germinal epithelium was decreased with significant p value of 0.05 and in the group G3 and G4, both parameters were improved close to the control group and in comparison of p values of G2 v G3 and G2 v G4. **Conclusion:** Microwave oven exposed diet decreases the seminiferous tubular diameter and height of the germinal epithelium and Mentha piperita has more potent ameliorative effects than melatonin.

Keywords: Microwave radiations, Histology, Testis, Germinal Epithelium.

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INTRODUCTION

With increasing development, our need for electronic gadgets has increased. One of the invention is microwave oven.^{1,2} A microwave oven is a kitchen appliance in which microwave radiations are absorbed by the materials and then are transformed into heat energy. There is rapid heat transfer and less time is needed to get higher temperatures.^{3,4} Microwaved food has molecules and energies that are not present in food cooked in the conventional way.² Unnatural molecules production is inevitable. Isomeric changes occur in amino acids and they are changed into toxic forms. It becomes difficult for the body to metabolize the unidentified by-products produced in the microwaved food.⁴ In addition to it, production of male and female hormone which play important role in the body is also changed by taking microwaved food for long time.

A big proportion of the world population presently

relies on traditional medicines in the field of health and most of this treatment involves usage of plant extracts, often in aqueous solutions.⁵ Water soluble extracts of *M. piperita* leaves were seen to have free radical scavenging activity.⁶

Melatonin is a naturally occurring substance produced from pineal gland.⁸ Studies have shown that melatonin is very effective scavenger of toxic hydroxyl radicals and very effective antioxidant.⁹ It enhances the antioxidant enzyme activity like of superoxide dismutase, glutathione peroxidase and catalase in conditions of increased oxidative stress and regulate mRNA levels in the cells for these enzymes.¹⁰ This study was conducted to determine the effect of Mentha piperita melatonin on the seminiferous tubular diameter and height of germinal epithelium in the testis of mice fed continuously with microwave oven exposed diet.

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METHODOLOGY

The randomized control trial was accomplished in the Anatomy Department, Army Medical College Rawalpindi, in association with National Institute of Health (NIH), Islamabad, from 1st April to 31st May 2016. Thirty two adult male mice (BALB/c strain) weighing 25-30grams were randomly selected. They were housed in NIH under controlled condition of temperature and light and divided into four groups. Group G1 served as control and was given standard laboratory mice pellets for 4 weeks. The second Group G2 was given microwave oven exposed mice pellets 5-10 gm/day/animal for 4 weeks. The third group G3 was given *Mentha piperita* leaf extract (1 g/kg b.wt./day) along with microwave oven exposed mice pellets (5-10 gm /animal /day) for 4 weeks and the experimental group G4 received oral dosage of melatonin 12 mg /kg/day along with microwave oven exposed mice pellets (5-10 gm /animal/day) for 4 weeks.

At the end of experimental period, the animals were sacrificed. Testicular tissue was then identified and separated by cutting deep fascia. Colour, shape and texture of tissue was noted. Collected tissues were washed in saline and then fixed in formalin solution. Afterward they were dehydrated by passing through alcohol and processed in paraffin wax. Tissues were then cut in 5 microns thick sections with help of microtome. Stained with Haematoxylin and Eosin and then it was permanently mounted. These slides were examined for following histologic parameters. The data was entered and analyzed by using SPSS version 21. ANOVA test was applied to compare groups. P-value of <0.05 was taken as significant.

Seminiferous tubules diameter:

The diameter of seminiferous tubules with profiles that were round or nearly round were estimated for each animal with help of ocular micrometer and a mean \pm SD of the diameter was determined by taking the average of two diameters, D1 and D2 (perpendicular to one another). For each animal randomly two sections were observed one for the right and one for the left and then mean tubular diameter was calculated for each right and left testis and then finally average mean was taken for each animal.

Height of the germinal epithelium

In each tubular cross-section, the height of the epithelium was measured four times at right

angles in each tubule. The height of the seminiferous epithelium was estimated in three tubules per section and mean was calculated and measured from the basal membrane towards the lumen of the tubule. For each animal randomly two sections (one from Rt testis + one from Lt testis) were analysed by this method.

RESULTS

Seminiferous tubular (ST) diameter (μm):

Mean and standard deviation of ST diameter in control group G1 was $246.25 \pm 5.14\mu\text{m}$. Mean and the standard deviation of ST diameter in the experimental group G2 was $217.43 \pm 3.83\mu\text{m}$. Similarly in the experimental groups G3 and G4 were $242.52 \pm 5.16\mu\text{m}$ and $241.37 \pm 2.18\mu\text{m}$ respectively. (Figure I)

Figure I: Photomicrograph at 40X magnification, H&E staining showing: Comparison of seminiferous tubular diameter of the control group G1 (A—control), G2 (B—group exposed to microwave oven mice pellets) with decreased ST diameter, G3 (C—microwave oven exposed mice pellets + *Mentha piperita*) with ST diameter close to control and G4 (D—microwave oven exposed mice pellets + melatonin) showing ST diameter near the control group.

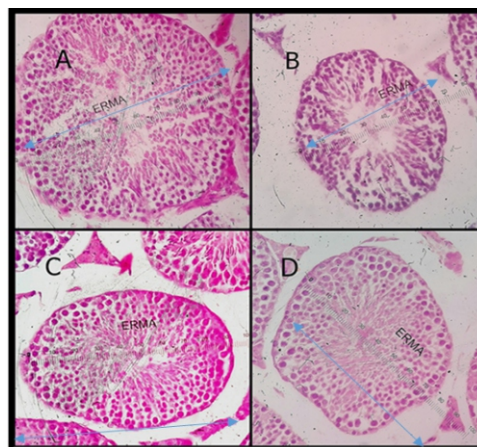
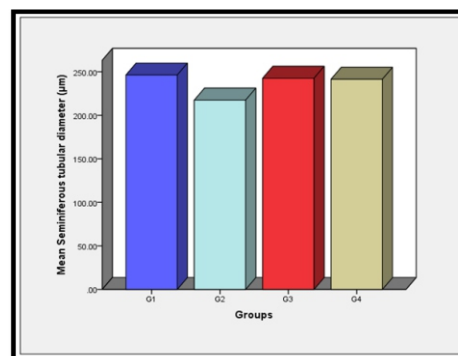


Figure II: Bar chart showing comparison of mean values of seminiferous tubular diameter among the control group G1 and experimental groups G2, G3 and G4

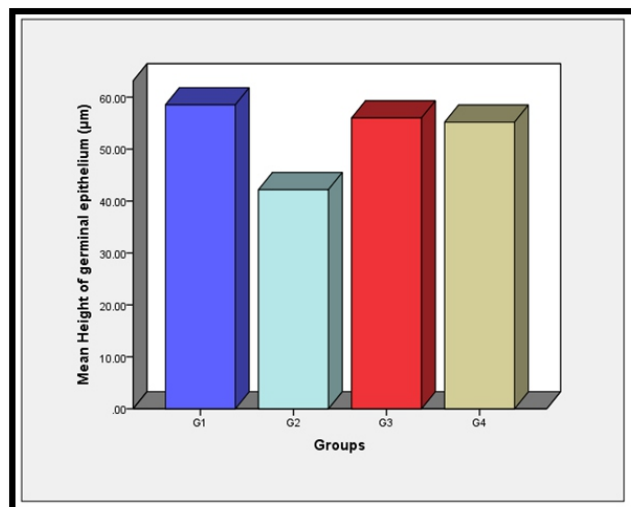


On intergroup comparison when group G2 was compared with group G1, there was a statistically significant difference in ST diameter with p value <0.05 but when experimental groups G3 and G4 were compared with G1, there was no statistically significant difference in ST diameter. ($P=>0.05$) Similarly when G2 was compared with G3 and G4, there was statistically significant difference ($P=0.00$). (Figure II) In comparison of ST diameter of group G3 and G4, there was statistically non-significant difference. ($P=>0.05$)

Height of the germinal epithelium (μm):

Mean height of the germinal epithelium in control group was $58.52 \pm 3.87\mu\text{m}$, in the experimental group G2 was $42.18 \pm 4.09\mu\text{m}$, in experimental groups G3 and G4 was $56 \pm 1.77\mu\text{m}$ and $55.1 \pm 0.96\mu\text{m}$ respectively. (Figure III)

Figure III: Bar chart showing comparison of mean values of height of the germinal epithelium among the control group G1 and experimental groups G2, G3 and G4



On intergroup comparison when group G2 was compared with group G1, there was a statistically significant difference in height of the germinal epithelium with p value <0.05 . But when experimental groups G3 and G4 were compared with G1, there was no statistically significant difference in the height of the germinal epithelium ($P=>0.05$) and ($P>0.05$). Similarly when G2 was compared with G3, p value was 0.004 and when G2 was compared with G4, p value was 0.046. In comparison of group G3 and G4, there was statistically non-significant difference in the height of the germinal epithelium. ($P=>0.05$).

DISCUSSION

In this study, seminiferous tubular diameter of the experimental group G2 was reduced as compared to control group G1 and this reduction was statistically significant (p value 0.00). These results are in agreement with the results of Raghuvanshi¹ who observed the histologic changes in the testis of mice continuously fed with food exposed to microwave radiations. Similar results that microwave radiation causes decrease in diameter of seminiferous tubules were shown by Dasdagetal.¹² These results are in contradiction with the study by Saygin¹³ who revealed the changes in histopathology of the testis of mice exposed to 2.45-GHz EMR which did not cause any significant change in diameter of the seminiferous tubules. Kata¹⁴ proposed that decreased seminiferous tubular diameter was probably due to the inhibition of spermatogenesis which resulted in decreased sperm count. Sharmaetal¹⁵ showed that there was decrease in seminiferous tubular diameter. In current study, seminiferous tubular diameter of group G3 (microwave exposed food + Mentha piperita) was close to group G1 but there was statistically significant difference between seminiferous tubular diameter of group G2 and G3 with p value of 0.00. These results were in agreement with results of Samarth and Samarth.¹⁶ Schrupf and Charley¹⁷ did a study on microwaved food and he explained that the cells in the nutrient got polarized due to microwaves and create free radicals. Gracy¹⁸ explained that free radicals produce detrimental effect in living systems.

Similarly in results of current study the seminiferous tubular diameter of the group G4 (microwave oven exposed food + melatonin) was close to the control group but there was statistically significant difference between the results of group G4 and G2 with the p value of 0.00. Melatonin is an important antioxidant and an efficient scavenger of OH, nitric oxide radical (NO) and peroxy radicals.^{19,20}

In current study, in the group G2 exposed to only microwave exposed diet has significantly decreased height of the germinal epithelium as compared to the control group. These results are in agreement with the results of and Samarth and Samarth.¹⁶ In current study height of the germinal epithelium in group G3 (microwave oven exposed mice pellets + Mentha piperita) was close to the control group. These results are in agreement with the results of Samarth.²¹

In the group G4, again the height of the germinal epithelium (microwave oven exposed mice pellets + melatonin) was close to the control group and there

was statistically significant difference in the height of germinal epithelium of the group G4 and G2. Melatonin is an important antioxidant and an active free radical scavenger.¹⁹ It augments the ability of cells to resist oxidative damage by inhibiting the pro-oxidant nitric oxide synthase.^{20,22} The protective role of melatonin in various types of oxidative stress-induced damage in testis is established.^{23,24,25}

CONCLUSION

Microwave exposed oven mice pellets decreases the seminiferous tubular diameter and height of the germinal epithelium in the testis and *Mentha piperita* has better ameliorative effects in these parameters than melatonin.

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Conflict of interest:

The authors have declared no conflict of interest.

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