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ORIGINAL ARTICLE

Consequences of Microwave oven Exposed Diet on The Basal Lamina of Seminiferous Tubules of Mice and Relative Role of Mentha Piperita and Melatonin

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ABSTRACT

Usage of electronic gadgets like microwave oven is increasing day by day that heats the food by exposing it to electromagnetic radiations which has many hazardous effects on human health including fertility.
Aim: To find the effects of microwave oven exposed diet on basal lamina of seminiferous tubules of mice alongwith protective effects of *Mentha piperita* and melatonin on the same tissue.
Study Design: Randomized control trial.
Methodology: Adult male mice (n=32) were divided into four groups. Control group (G1) received standard pellets prepared for mice. Second group (G2) was given mice pellets exposed to microwave oven. Third group (G3) received *Mentha Piperita* leaf extract along with mice pellets exposed to microwave oven and the fourth group (G4) received oral melatonin along with pellets exposed to microwave oven. Later their testicular tissue was removed for histological examination while basal lamina disruption was assessed by scoring. Data analyzed by SPSS 22.0v.
Results: In group G2, there was slight disruption in the basal lamina in 75% of the cases while in experimental group G3, there was slight disruption of basal lamina only in 12.5% of the cases. However, in group G4, only 25% specimen had slight disruption of basal lamina
Conclusion: It was concluded that microwave oven exposed diet produced severe disruption of basal lamina in group G2 that decreased in *Mentha piperita* and melatonin treated groups. However, *Mentha piperita treated group* produced better results than melatonin treated group.
Keywords: Mice, Testis, Basal Lamina, *Mentha piperita* and Melatonin

INTRODUCTION

Microwave oven is a new technological invention found in almost every home and has reduced cooking time. It contains magnetron that produces very high frequency current that produces molecular friction in the food particles. This molecular friction produces heat and distorts the food particles. In the same way, amino acids are distorted in to toxic and inactive form[1]**.** In addition to it, nutrient cells get polarized because of creation of free radicals that disturbs the natural ongoing processes in the body[2]**.**

While comparing heating of food by conventional means with the microwave oven heating, in conventional means there is heating of the exterior and later on heat transfers inside the material by conduction, but in the microwave oven heating, the electromagnetic energy is converted into heat energy inside the material then it moves outside[3]**.** Microwave radiations produce oxidative damage by increasing the production of enzymes like catalase, glutathione peroxidase and dismutase[4]**.**

In one study, effects of radiations with 2.45GHz frequency emitting from wireless devices on the oxidative status of blood were noticed and it was seen that lipid peroxidation levels in blood plasma and erythrocytes were considerably greater in the experimental group[5]**.** It was also shown that electro-magnetic radiations produce oxidative stress and disturbs the antioxidant status of testis[6]**.** Usage of plant extracts is becoming very common while treating many health problems. Several medicinal plants comprise active pharmacological ingredients that are used for many therapeutic problems[7]**.**

Mint (peppermint), genus Mentha is an aromatic plant with many pharmaceutical, culinary and medicinal uses. It contains more than 40 different biochemical compounds like acacetin, diosmin, methyl rosmarinate, rutin, phenolic acids, rosmarinic acid, caffeic acid, lithospermic acid and many others[8]**.** Dorman explained that aqueous extracts of different species of Mentha possess antioxidant properties especially *Mentha Piperita* and this property was because of phenolic compounds[9]**.** Some other components like acetonitrile, ethyl acetate and water soluble extracts of *M. piperita* leaves were also seen to have free radical scavenging activity[10]**.** It was also studied that oil and aqueous extract of *Mentha piperita* provides remarkable radioprotection[11]**.**

Pineal gland produces melatonin in human body. It controls our sleep wake cycle. As previously documented, other tissues in body like ovary, bone marrow and testis also produce it[12-14]**.** It has free radical scavenging as well as antioxidant activities even more effective than glutathione and vitamin D[15,16]. So in this study, aqueous

extract of *Mentha piperita* and melatonin was used to see their protective effect in the testicular tissue of mice when they were fed with microwave oven exposed diet.

The objective of the study was to find the effects of microwave oven exposed diet on basal lamina of seminiferous tubules of mice alongwith protective effects of *Mentha piperita* and melatonin on the same tissue.

MATERIALS AND METHODS

Mentha Extract: National Agricultural Research Institute provided leaves of *Mentha piperita,* 100 grams leaves were washed with distill water, dried, pulverized and extracted with 1.5 L of double-distilled water by refluxing at 80℃ for 36 hrs. Later vaporized to prepare its precipitated form[17]**.** Dose was given to each mouse by oral gavage. Daily dose of *Mentha piperita* was 1gm/Kg/day[18]**.**

Melatonin: Melatonin was bought from the suppliers of General Nutrition Corporation. Melatonin dose per serving was 10mg. Melatonin was dissolved in ethanol and was given by oral gavage. Dose of melatonin was 12mg/kg/day[19]**.**

Animal preparation: 32 mature male balb/c mice of about 6-8 weeks old, weighing b/w 25 ± 5 gm were taken for this study and divided into 4 groups: Group-1 (control)**:** Mice were fed with standard diet (5-10 gm/day) for each mice for 4 weeks. Group-2: Animals were fed with microwave oven exposed pellets (5-10 gm/day) for same duration[20]**.** Group-3: Animals were fed with mice pellets exposed to microwave oven (5-10gm /day) along with leaf extract of *Mentha piperita* (1g/kg body wt /day) for 4 weeks[18]. Group-4: Mice were given microwave oven exposed mice pellets (5-10gm /day) plus oral melatonin 12mg /kg/day for 4 weeks[19]**.** After 4 weeks, mice were dissected to remove their testicular tissue and then fixed in 10% formalin solution, afterward their sections were infiltrated and embedded in paraffin wax. 5µm thick cross sections were obtained by using rotatory microtome. Then tissues were stained with Periodic acid Schiff stain. This processing and staining methods were performed in histology unit CREAM II lab of Army Medical College.

For basal lamina disruption, mid-testis cross-sections of both right and left testis were taken and randomly 10 rounded seminiferous tubules were selected and given a score from 0 to 3 on basis of the following criterion[21]:

0 = Normal seminiferous histology, no disruption of basal lamina.

1 = Slight effect, <50 % of the tubule cross sections show disruption of basal lamina.

2 = Moderate effect, >50% tubule cross-sections show disruption of basal lamina.

3 = Severe effect, >70% of tubule cross-sections show disruption of basal lamina**.**

Statistical analysis:Data analyzed by SPSS 21.0v. ANOVA was used for intergroup comparison followed by Post Hoc Tukey Test having P-value <0.05 as statistically significant.

RESULTS

Overall during the experimental period, all 32 animals remained healthy and alive. On naked eye examination, shape (oval) and color (pink) of testis was normal, with soft, smooth and shiny surface. No unusual finding was noticed.

Disruption of basal lamina:: To see disruption of basal lamina, we did the scoring which included Normal, Slight, Moderate and Severe disruption (table-1).There was no evidence of disruption of basal lamina of seminiferous tubules in Control Group G1 (Photomicrograph, Table-1). In the Experimental Group G2, there was slight disruption in the basal lamina in 75% of the cases.

Table-1: Disruption scale of the basal lamina

Disruption scale	Group G1	Group G2	Group G3	Group G4
Normal, no disruption count, % within the group	8 (100%)	2 (25%)	7 (87.5%)	6 (75%)
Slight disruption count, % within the group	0 (0 %)	6 (75%)	1 (12.5%)	2 (25%)
Moderate disruption count, % within the group	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Severe disruption Count, % within the group	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)

Table-2: Comparison Between *Mentha piperita* and Melatonin Groups

Parameters	P-value comparison	
	Groups (G2 Vs G3)	Groups (G2 Vs G4)
Basal lamina disruption	0.020*	0.070

* Significant

On comparison with Group G1, disruption of basal lamina was found to be statistically significant (p = 0.003). In experimental Group G3, there was slight disruption of basal lamina only in 12.5% of the cases which was statistically significant with p-value of 0.02 when compared with Group G2 (Table-2) but insignificant when compared with groups G1 and G4. Whereas in Experimental Group G4, 25% of the specimen showed slight disruption of basal lamina that was statistically non-significant with p-value of 0.07 when compared with experimental group G2 (Table-2).

Fig-1: Frequency of disruption of the basal lamina in groups (G1,G2, G3 and G4)

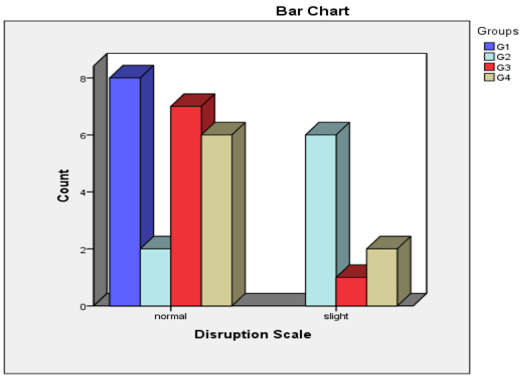
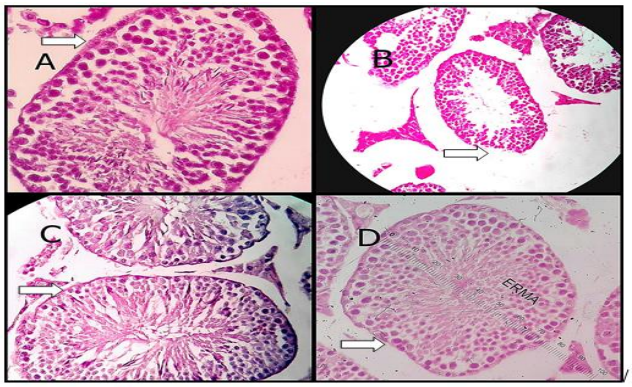


Fig-2: PAS stain showing disruption of basal lamina in the experimental group G2 and its comparison with other groups.



DISCUSSION

In today's world, usage of microwave oven is increased to the extent that very few homes and cafes are seen without them. It was believed that no harmful effects are produced by microwave ovens[21] however, literature review revealed that microwave oven destroy important food nutrients[20]**.** Hence, we noticed the effects of microwave oven exposed diet on very sensitive tissue of body and also saw the protective role of antioxidants and free radical scavengers like *Mentha piperita* and melatonin.

In the experimental group-G2, 75% of the cases had severe basal disruption. Comparison of p value of basal lamina disruption of group G2 with the control group was 0.005. This study was in agreement with the results of Khaki[23] who worked with light microscopy and TEM to see the effects of electromagnetic radiations on the boundary wall of seminiferous tubules. According to his study, boundary wall of seminiferous tubule has four layers. According to his conclusion, basal lamina disruption occurs because of biological stress and free radicals which cause the myoid cell to become inert with heterochromatic nuclei, loss of mitochondria, few actin filament and loss of continuity between adjacent myoid cells. Lacy and Rotblat[24] explained that myoid cells are modified smooth muscle cells that sustain a firm pressure to facilitate sperm discharge.

In this study, basal lamina disruption in the experimental group-G3 was close to the normal and there were only 12.5% cases with slight disruption having statistically significant difference in the basal lamina disruption of group G3 and G2 (p-value=0.02). As already explained *Mentha piperita* contains phenols, flavonoids, and flavonols (antioxidants) thus making it free radical scavenger. Highman and Hanks explained that radiations depletes alkaline phosphatase which is normally present in the basal lamina, maintains its integrity and permeability so *Mentha piperita* plays a beneficial role by restoring alkaline phosphatase[25]**.**

Similarly in this study in the experimental group G4 (microwave exposed mice pellets + melatonin) there was slight basal lamina disruption in 25% of the cases and in comparison with the experimental group G2, disruption had decreased but significance was 0.07. These results are in contrast with the results of Take where there was severe disruption after exposure to radiations and in melatonin treated group disruption was markedly reduced[26]**.**

Limitations: Our limitations included small sample size, time with financial constrains and limited resources.

CONCLUSION

It was concluded that microwave oven exposed diet produced severe disruption of basal lamina in group G2 that decreased in *Mentha piperita* and melatonin treated groups. However, *Mentha piperita treated group* produced better results than melatonin treated group.

Author's contribution: KN & MSA: Conceptualized the study, analyzed the data, and formulated the initial draft. MY & HA: Contributed to the histomorphological evaluation. FM & MA: Contributed to the analysis of data and proofread the draft. TL: Contributed to the proofreading the manuscript for intellectual content.

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Conflict of interest: Nil

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