

ORIGINAL ARTICLE

Effects of Microwave Oven Exposed Diet on Animal Weight and Testicular Tissue and Relative Role of *Mentha Piperita* and Melatonin

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ABSTRACT

Background: 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 weight and testis of mice along with protective effects of *Mentha Piperita* and Melatonin

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. After experimental period, wt of each mice was again recorded and then mice were sacrificed. Data analyzed by SPSS 21.0v.

Results: There was no statistically significant difference of weight gain of animals but there was significant reduction in weight of testis in group G2 but in G3 and G4 wt of the testis was close to control.

Conclusion: It was concluded that microwave oven exposed diet had no significant effect on overall weight gain of the animal but it significantly reduced weight of the testis in group G2. However, *Mentha Piperita* and Melatonin both had ameliorative effects on the wt of the testicular tissue.

Keywords: Mice, Testis, Weight, *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 form¹. In addition to it, nutrient cells get polarized because of creation of free radicals that disturbs the natural ongoing processes in the body².

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 outside³. Microwave radiations produce oxidative damage by increasing the production of enzymes like catalase, glutathione peroxidase and dismutase⁴.

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 group⁵. It was also shown that electro-magnetic radiations produce oxidative stress and disturbs the antioxidant status of testis⁶. 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 problems⁷. The usage of natural products obtained from plants is becoming popular as chemo preventive agents and valuable in certain cell/tissue based structures like *Mentha piperita*, also known as peppermint. Leaf extract of *Mentha piperita* protects intestinal mucosa from radiational effects. It also decreases serum acid phosphatase and increases alkaline phosphatase enzyme⁷. 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 rosmarinic acid, rutin, phenolic acids, rosmarinic acid, caffeic acid, lithospermic acid and many others⁸. Dorman explained that aqueous extracts of different species of *Mentha* possess antioxidant properties especially *Mentha Piperita* and this property was because of phenolic compounds⁹. Some other components like acetonitrile, ethyl acetate and water soluble extracts of *M. piperita* leaves were also seen to have free radical scavenging activity¹⁰. It was also studied that oil

and aqueous extract of *Mentha piperita* provides remarkable radioprotection¹¹. 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 it¹²⁻¹⁴. Role of melatonin on the male reproductive system and testosterone production from leydig cells have been studied on animals. As melatonin binding sites have been identified in the reproductive system of various species so it has a direct interaction with the steroidogenic cells of the reproductive organs¹⁵. Therefore in the light of above description, we carried current project to observe the protective role of *M. Piperita* leaf extract on the overall weight of animal and of testicular tissue was observed when animal was fed with microwave oven exposed mice pellets.

The objective of the study was to find the effects of microwave oven exposed diet on weight and testis of mice along with 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 °C for 36 hrs. Later vaporized to prepare its precipitated form¹⁶. Dose was given to each mouse by oral gavage. Daily dose of *Mentha piperita* was 1gm/Kg/day¹⁷. *Mentha* extract and melatonin was given to each mouse by oral gavage (Figure-1).

Fig-1: Photograph showing the steps of preparation of *Mentha piperita* extract.



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/day¹⁸.

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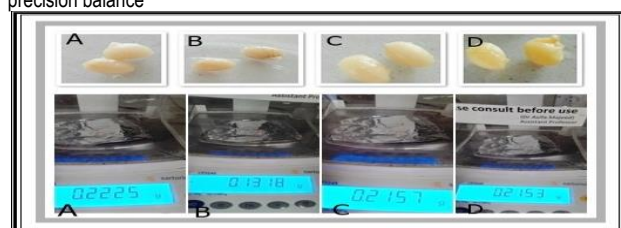
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Animal preparation: 32 mature male balb/c mice of about 6-8 weeks old, weighing b/w 25 ± 5 gm were taken for this study and divided into 4 groups: Group-1 (control): Mice were fed with standard diet (5-10 gm/day) for each mice for 4 weeks. Group-2: Animals were fed with microwave oven exposed pellets (5-10 gm/day) for same duration²⁰. 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 weeks¹⁸. Group-4: Mice were given microwave oven exposed mice pellets (5-10gm /day) plus oral melatonin 12mg /kg/day for 4 weeks¹⁹.

Weight Recording: The body weight of all the animals was recorded at end of study just before the sacrifice of animals

Sacrifice and Dissection: The mice were given anesthesia in a jar with cotton wool soaked with chloroform. The abdominal cavity was opened by a midline incision in the abdominal wall for the exposure of the reproductive tissues. Testicular tissue was then identified and separated by cutting deep fascia. Color, shape and texture of the tissues were noted. The weight of testis of each animal was taken by an electronic analytical and precision balance (Figure-2).

Fig-2: Depicting weighing of testicular tissue by an electronic analytical and precision balance



Statistical analysis: Data analyzed by SPSS 21.0v. Chi square test applied for intergroup comparison 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. There was no resistance in cutting the tissue. Blood vessels on the surface were normal and quite healthy.

Weight of the tissue (testis): Mean values of initial and final weight of animals for each group represented in table-1 and net weight gain and weight of testicular tissue were shown in table-1. On intergroup comparison, there was no statistically significant difference of weight gain of animal.

Table-1: Description Of Weight Gain Among Different Groups as Mean \pm SD

Parameter	G1 (n=8)	G2 (n=8)	G3 (n=8)	G4 (n=8)
Initial weight of animal (gm)	27.3 \pm 1.487	26.9 \pm 1.445	28.1 \pm 1.268	27.3 \pm 1.066
Final weight of the animal (gm)	36.2 \pm 1.298	34.9 \pm 1.083	36.5 \pm 1.309	36.1 \pm 1.251
Animal Weight gain (gm)	8.96 \pm 1.714	8.02 \pm 0.822	8.40 \pm 1.256	8.96 \pm 0.504
Weight of the testis (gm)	0.226 \pm 0.007	0.211 \pm 0.006	0.228 \pm 0.004	0.226 \pm 0.008

On intergroup comparison, when G2 was compared with G1, there was statistically significant difference in weight of the testis with p value 0.001. In comparison of G3 and G4 with G1, there was statistically non-significant difference. But when G2 was compared with G3 and G4, there was statistically significant difference with p value 0.00 and 0.001 respectively

DISCUSSION

In the present study, all animals remained active and healthy. There was statistically non-significant difference in the mean initial body weight in all the groups because the animals selected were of similar age and sizes. Similarly in the final body weight and weight gain of animal, again there was no statistically significant difference. In this experiment, non-significant change in the body weight following microwave oven exposed food agrees with the results of who exposed experimental group to microwaves²⁰. However, this does not agree with the outcomes of who observed that there is change in body weight when rats are

exposed to electromagnetic radiations for the long time²¹. Similarly Gonzalez-Riola²² indicated that there is no significant difference in the initial and final body weights of mice after exposure to electromagnetic fields. In one of the article entitled, "Do You Microwave Your Food?" Mercola et.al²³ explained that nutritional value of food decreased and it also decreased the appetite which might also lead to decrease in total body weight after long-term exposure. The difference in opinions may be because of different modes of exposure to radiations and different duration of exposure of radiations.

In this study when mean weight of the testis in the control G2 microwave food exposed group was compared with G1, there was statistically significant reduction in the testicular weight and p-value was 0.001. Weight of the testis of the experimental groups G3 and G4 were close to the control. This finding was in the agreement with the similar finding in the study of Raghuvanshi et.al²⁴ who did the evaluation of the changes in sperm morphology, sperm count and gonadotropic ratio of swiss albino male mice fed continuously with microwave exposed food. Paul et.al²⁵ described that the decrease in the weight of the testis was due to absence of spermatids and spermatozoa from the testis and accessory organs. The weight of testis is known to be a good index of FSH secretion. The reduction of FSH causes a significant decrease in the weight of testis and accessory organs in the male.

In the group G3, weight of the testis was close to normal. These results were in agreement with study of Samarth²⁰ in which animals treated with radiation showed reduction in the weight of the testis during all days of observation. Whereas, in leaf extract of *M. piperita*-pretreated irradiated group there was a significant increase in the weight of the testis as the histology of testis revealed near normal histoarchitecture and lumen full of sperms.

In another study by Hussein et.al²⁶ did the morphological evaluation of the radio protective effects of melatonin in the radiations induced damage in the testis of rats in which radiations induced marked weight reduction with depletion of Sertoli cell and spermatogenic cells but in melatonin pretreated group, both Sertoli and spermatogenic cells had relatively normal architecture and cytological features.

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

CONCLUSION

It was concluded that microwave oven exposed diet had no significant effect on overall weight gain of the animal but it significantly reduced weight of the testis in group G2. However, *Mentha Piperita* and Melatonin both had ameliorative effects on the wt of the testicular tissue.

Conflict of interest: None

Funding: None

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

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