EFFECTS OF MICROWAVE OVEN EXPOSED DIET ON SPERMATOGENESIS IN TESTICULAR TISSUE OF MICE AND COMPARATIVE EFFECTS OF MENTHA PIPERITA AND MELATONIN

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ABSTRACT

Objective: To observe the effects of microwave oven exposed diet on spermatogenesis in the testis of mice and comparative effects of Mentha piperita and melatonin.
Study Design: Laboratory based randomized controlled trial.
Place and Duration of Study: Anatomy Department, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad, from Apr 2015 to May 2015.
Material and Method: Study comprised of 32 adult male mice (BALBc strain) weighing 25-30 gms. Selection criteria based on non-probability (purposive) simple random sampling. Mice were divided into four equal groups of 8 mice each. Group 1, taken as control, was given standard diet 5-10gm/animal/day daily for four weeks. Group 2 was given 5-10 gm/animal/day of microwave oven exposed mice pellets for four weeks. Group 3 received Mentha piperita leaf extract (1g/kg b.wt./day) along with microwave oven exposed mice pellets (5-10 gm/animal/day) for 4 weeks and group 4 received oral dosage of melatonin 12mg/kg/day along with microwave oven exposed mice pellets (5-10gm/animal/day) for 4 weeks. After four weeks animals were dissected. The shape, color and any abnormal finding of the testis were observed. Testis were processed, embedded and stained for histological study. Spermatogenesis was assessed by the Johnsons scoring. SPSS 21 was used for statistical analysis. Chi square test was applied for intergroup comparison.
Results: Spermatogenesis was suppressed and Johnsons score was decreased from normal spermatogenesis (10) to (6-8) in the experimental group 2 and was more improved in the Mentha piperita treated group as compare to the melatonin.
Conclusion: Microwave oven exposed mice pellets suppressed spermatogenesis and Mentha piperita had better ameliorative effects than melatonin on the testis of mice.

Keywords: Microwave radiations, Spermatogenesis, Testis.

INTRODUCTION

Microwave is an advanced invention which helps in decreasing the cooking time. Just like north and south poles of a magnet, food and water molecules also have negative and positive ends. An extremely rapid alternating current with a frequency of a billion cycles per second is produced by the magnetron present in the microwave. So molecular friction is produced in the food stuff by this rapid bombardment that produces heat and causes distortion of the food molecules. While microwaving the food, amino acids are transformed to biologically inactive and even toxic forms[1]. In one study conducted on microwaved carrots and broccoli it was shown that the molecular organizations of nutrients was distorted and its cells became polarized because of creation of free radicals which interrupts the biological process. It is also observed that micro-nutrients of the food decrease as a result of molecular friction. As nutrition is important for spermatogenesis so it is mostly affected[2]. Hence, the present study was conducted to see whether the variation in the nutritional contents after exposure to microwave radiations effects the process of spermatogenesis.

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Plant extracts have been used for a long time to treat many health disorders. Natural products have achieved a significant consideration in many fields[3]. Mint (peppermint) is an aromatic herb of family Labiatae and genus Menthais an important culinary plant with many medicinal uses. Leaf extract of Menthapiperita provides good protection in radiational induced changes in gastrointestinal mucosa[4], amends serum acid and alkaline phosphatases levels and prevents chromosomal damage in bone marrow[5]. In addition to this, it is also a good free radical scavenger because of high phenolic contents[6]. In the present study protective effects of M. piperita leaf extract against microwave radiation-induced changes on the spermatogenesis were evaluated.

Melatonin (N-acetyl-5-methoxy-tryptamine, an important hormone produced by the pineal gland is an effective antioxidant, even more than vitamin E[7]. After its formation by the pineal gland it enters into the blood. Since it is both lipid and water soluble it easily crosses blood brain barrier[8] and the placental barrier[9]. Melatonin is degraded to 6-hydroxymelatonin in the liver[10], conjugated with sulfuric or glucuronic acid and eliminated in the urine as 6-sulfatoxy-melatonin[11]. Studies have shown that melatonin is very active scavenger of toxic hydroxyl radicals[12]. It is also known that melatonin is more potent antioxidant than vitamin D, glutathione and mannitol[13] however an increased concentration of melatonin is needed for all these effects. The rationale of this study was to observe the protective effects of melatonin and Mentha piperita leaf extract on spermatogenesis in mice exposed to microwaved food.

MATERIAL AND METHODS

A randomized controlled trial was conducted at the Anatomy Department, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad, from April to May 2015. Thirty two adult male mice (BALB/c strain) weighing 25-30grams selected by simple random sampling were used for the study. They were kept in the NIH animal house. Animals were divided into four groups. Animals in group 1 served as control and were given standard mice pellets for 4 weeks. The experimental group 2 was given microwave oven exposed mice pellets 5-10 gm/day/animal for 4 weeks[14]. The experimental group 3 received 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[15] and the experimental group 4 received oral dosage of melatonin 12 m[16]/ kg /day[3] along with microwave oven exposed mice pellets (5-10 gm/ animal /day) for 4 weeks. At thes end of one month, the animals were sacrificed and dissected. Both right and left testis of all animals were removed. General features like color, shape etc were noticed. Testis were placed in 10 percent formalin. For infiltration and embedding paraffin wax with melting point 58^{o}C was used. The blocks were allowed to solidify in the cold temperature. Five micrometer thick cross sections were obtained by using rotary microtome. The staining of sections was done with hematoxylin and eosin (H&E) for histologic parameter.

Spermatogenesis

For each animal, mid testis cross section for both right and left testis were observed under the light microscope at 40X magnification and 10 rounded tubules were evaluated and mean was calculated. Each tubule was given a score ranging from 10 to 1. The tubules having complete inactivity scored as 1 and those with maximum activity (at least five or more spermatozoa in the lumen) scored 10[17]. This method includes scores from 1-10 as follows:

- Complete spermatogenesis with many spermatozoa; regular organization of the germinal epithelium.
- Many spermatozoa present but germinal epithelium disorganized.
- Only a few spermatozoa.
- No spermatozoa but many spermatids.
- No spermatozoa but few (<5) spermatids.

- No spermatozoa or spermatids but many spermatocytes.
- No spermatozoa or spermatids but few (<5) spermatocytes.
- Spermatogonia are the only germ cell present.
- No germ cells but sertoli cells are present.
- No cells in tubule section.

IBM SPSS version 21 was used for data analysis. Spermatogenesis was presented by frequency and percentages. Chi square test was applied for intergroup comparison. A *p*-value <0.05 was considered as statistical significant.

RESULTS

On gross examination, testis of all the groups were normal in shape (oval) and color (light pink). They were soft in consistency with smooth shiny surfaces. Blood vessels on the surface were normal and healthy.

In the control group 1, Johnsons score was 10 with complete spermatogenesis. But in the experimental group 2, 50% of cases had Johnsons score 8 (only a few spermatozoa) and 25% of cases had Johnsons score 7 (no spermatozoa but many spermatids) and 25% of cases had Johnsons score 6 (no spermatozoa but few <5 spermatids) and these results were significant in comparison with control group 1 (table-I & II).

In the experimental group 3, there were 37.5% cases with complete spermatogenesis (score=10) while 50% cases showed Johnsons score 9 and rest 12.5% cases showed Johnsons score 8. These results were statistically significant in comparison with group 2 (*p*-value=0.012).

Table-I: Johnsons scoring and percentage for spermatogenesis among the groups.

Johnsons scoring for spermatogenesis	G1	G2	G3	G4
Complete spermatogenesis with many spermatozoa, germinal epithelium organized in regular thickness (score=10)	8 (100%)	0 (0%)	3 (37.5%)	1 (12.5%)
Many spermatozoa present but germinal epithelium disorganized (Johnsons score =9)	0 (0%)	0 (0%)	4 (50%)	3 (37.5%)
Only a few spermatozoa (Johnsons score = 8)	0 (0%)	4 (50%)	1 (12.5%)	4 (50%)
No spermatozoa but many spermatids (Johnsons score = 7)	0 (0%)	2 (25%)	0 (0%)	0 (0%)
No spermatozoa but few (less than 5 spermatids) (Johnsons score = 6)	0 (0%)	2 (25%)	0 (0%)	0 (0%)
No spermatozoa or spermatids but many spermatocytes (Johnsons score = 5)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
No spermatozoa or spermatids but few (<5) spermatocytes (Johnsons score = 4)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Spermatogonia are the only germ cell (Johnsons score = 3)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
No germ cells but Sertoli cells are present (Johnsons score = 2)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
No cells in the tubule cross section (Johnsons score = 1)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table-II: Inter group comparison of *p*-values of spermatogenesis.

Group 1 Vs Group 2	Group 1 Vs Group 3	Group 1 Vs Group 4	Group 2 Vs Group 3	Group 2 Vs Group 4	Group 3 Vs Group 4
0.001*	0.026*	0.002*	0.012*	0.092	0.23

In the experimental group 4, only in 12.5% cases Johnsons score was 10, and 37.5% of cases had Johnsons score 9 and rest 50% of cases showed Johnsons score of 8. These results were statistically non-significant when compared with group 2 (*p*-value=0.09) (fig-1, fig-2).

DISCUSSION

Spermatogenesis was assessed by Johnsons scoring which has scores from 10-1. Our results are in agreement with the results of Raghuvanshi[14]. He noticed arrested spermatogenesis after giving microwave oven exposed mice pellets. Salama et al[15] studied the effects of electromagnetic radiation on the testicular function and noticed significant drop in the sperm concentrations. Kesari et al[16] also noticed significant decrease in the sperm count in testicular tissue of male Wister rats after exposure to electromagnetic radiations. Same results were observed by Johnson[17] and Aitken & Roman[18] but these results were in contrast with the results of Ozguner et al[19] in which microwaves had no significant effect on spermatogenesis. Johnson et al[17] and Aitken & Roman[18] concluded that radiations lead to decrease in vitamin E which plays important role in suppressing lipid peroxidation so deficiency of this vitamin produces oxidative stress and alters both spermatogenesis and testosterone production. Kesari et al[20] showed that EMW radiation exposure causes significant decrease in mean activity of histone kinase 1 in semen of rats. This decline in the level of histone kinase, indicates a decrease in G2/M phase activity leading to decrease spermatogenesis.

Dixit and Goyal[21] proposed that male reproductive system requires continuous presence of androgens for structural maintenance and functional integrity for control of differentiation of primordial germ cells into spermatids. Jelodar and Zare[22] explained that decrease in serum testosterone may be due to the effects of radiation on leydig cells, the pituitary or the hypothalamus.

In the group 3 (microwave oven exposed mice pellets + Menthapiperita), Johnsons score was improved with the score of 8–10 with 37.5% cases with complete spermatogenesis, 50% having score 9 and 12.5% with score 8. Samarth et al[23] studied protective role of Mentha piperita on radiational induced testicular damage and he suggested that exposure to radiations increases the acid phosphatase. Acid phosphatase is present in the acrosome of spermatozoa and in the lysosome of Sertoli cells, spermatocytes and spermatids. These radiations also deplete alkaline phosphatase which plays an important role in transport of material from sertoli cells to germinal cells and in the differentiation and proliferation of the germinal epithelium. Mentha piperita decreases the lipid peroxidation so restores alkaline phosphatase which improves the

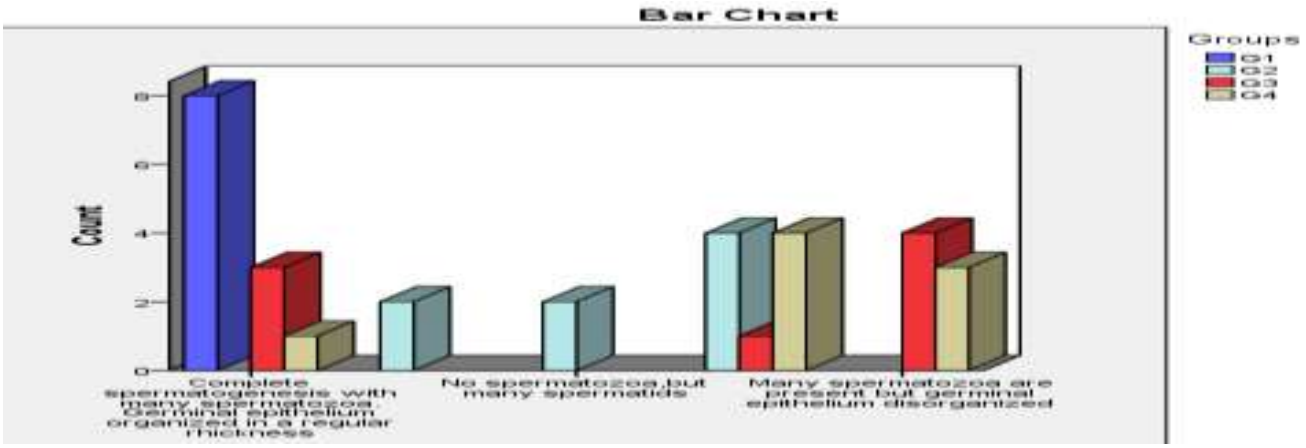

Figure-1: Clustered bar chart showing frequency of spermatogenesis (Johnsons scoring) of the control group 1 and experimental groups 2, 3 & 4.

transport of material from Sertoli cells to germinal cells and decrease acid phosphatase.

In the experimental group 4 (microwave oven exposed mice pellets + melatonin), only 12.5% cases had complete spermatogenesis, 37.5% with score 9 and rest of 50% had score 8. If we compare *p*-value of group 4 with 2, it is 0.09. Johnsons score improved from 6–8 to 8–10. Similar results were shown by the experiment performed by Meena et al in 2013, who investigated the protective effects of melatonin against oxidative stress-mediated testicular impairment due to long-term exposure of MWs[24].

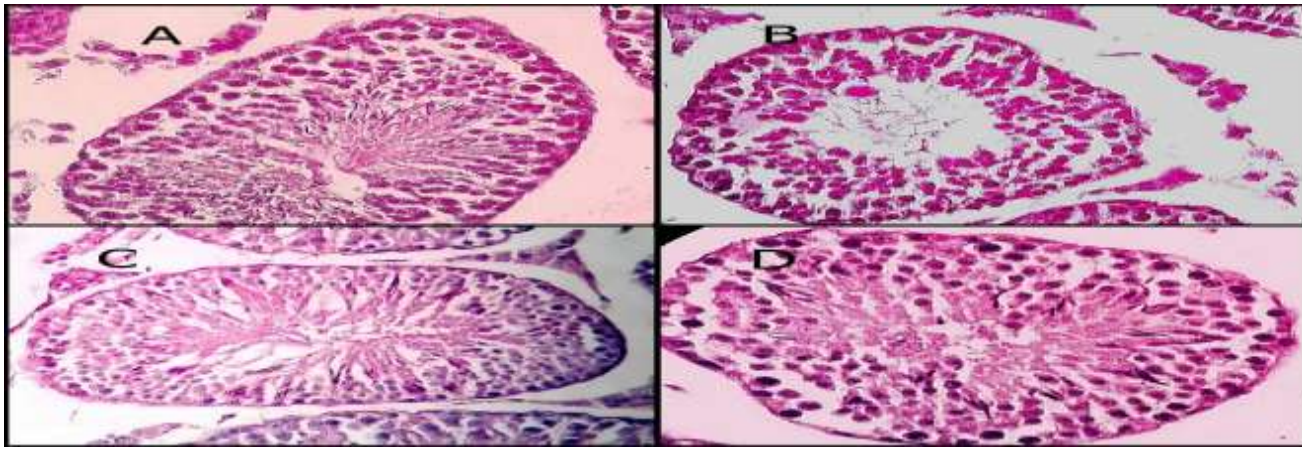

Figure-2: Photomicrograph at 40X magnification, H&E stain showing intergroup comparison of the Johnsons scoring for spermatogenesis. (Group-1 control) with Johnsons score 10, (Group-2group to microwave oven mice pellets) with Johnsons score 7, (Group-3 microwave oven exposed mice pellets + Menthapiperita) with Johnsons score 10 and (Group-4 microwave oven exposed mice pellets + melatonin) with Johnsons score 9. Arrow is indicating maturing spermatids.

They showed that melatonin prevents oxidative damage biochemically by significant increase ($p<0.001$) in the levels of testicular LDH-X, decreased ($p<0.001$) levels of MDA and ROS in testis ($p<0.01$). Aitken and Roman explained that melatonin is soluble in both lipid and aqueous environments so easily crosses the blood–testes barrier to protect the germinal epithelium[18]. It also decreases the lipid peroxidation and increases enzymatic and non-enzymaticanti-oxidants[25].

In the previous studies, comparison of role of Mentha piperita and melatonin on microwave oven induced changes on spermatogenesis has not been found and in this study when the role of Mentha piperita and melatonin on microwave oven induced changes on spermatogenesis was compared by its *p*-values, it was found that Mentha piperita was more potent than melatonin in improving the spermatogenesis.

Microwave ovens seem to be an absolute necessity in today's fast-paced world. They are currently present in most of the homes due to their ability to cook and reheat foods in a simple, rapid way. But because of the side effects of microwave radiations, we must avoid the use of microwave ovens especially for a baby, a child, pregnant lady, for one already suffering from disease, or are already bombarded with more radiation than the average person and we must also use antioxidants in our daily life.

CONCLUSION

Microwave oven exposed mice pellets suppressed spermatogenesis in the testis and Mentha piperita has more potent ameliorative effects on spermatogenesis as compared to melatonin.

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CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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