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Research Article

Effects of Microwave Oven Exposed, Mentha Piperita and Mel Diet on Spermatogenesis in Testicular Tissue of Mice

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ABSTRACT

Objective: The ultimate objective of in hand research paper is aimed on the observation that how spermatogenesis in mice testis and comparative effects of melatonin and Mentha pipe Rita are exposed by diet that is processed through microwave oven.

Study Design: The current research design is Laboratory based randomized controlled trial.

Place and Duration of Study: The research was a joint venture held at the venue of Army Medical College (AMC), Rawalpindi (Anatomy Department) and National Institute of Health (NIH), Islamabad. It was completed in the time span of April - May, 2015.

Material and Method: A total of thirty-two male adult mice (BALBc Strain) were short listed for the course work of the research paper. The weight rage of the mice was between twenty-five grams to thirty grams. Simple random sampling which was purposive in nature, purely non-probable in the selection of the research sample was adopted. Four randomly selected groups were made out of total sample of thirty-two mice. Every group was allocated eight mice. Control was given to Group-I as they were provided with standard mice diet for a period of consecutive four weeks at the rate of ten gram per mice every day. Microwave exposed diet (pellets) for the period of four weeks was given to every mice at the rate of five to ten grams per mice every day. Leaves extract of Mentha pipe Rita at the rate of (1 gram / kilogram b.wt. every day) and additionally microwave exposed diet of pellets was given to group-III mice at the rate of five to ten grams per animal every day was fed for a period of four weeks. Similarly group four was fed an oral dose of melatonin at the rate of twelve milligram per kilograms per day along with an exposed microwave pellets for the period of four weeks at the rate of five to ten grams per mice every day. Dissection procedure of mice was carried out at the end of fourth week for the identification of any abnormality in testis, color and shape. Testis were stained, embedded and processed for histological analysis. Evaluation of Spermatogenesis was completed by the scoring method of Johnsons. Statistical analysis was completed through SPSS – 21 and simultaneously Chi-Square test was utilized for the inter-group comparison.

Results: Johnsons score was lowered and level of Spermatogenesis was curbedin comparison to normal spermatogenesis (10) to (6-8) in Group-II and it was also better improvised for Menthapipe Ritagroup treatment in comparison to melatonin.

Conclusion: Mice pallets processed into microwave oven and exposed to electromagnetic radiation had curbed spermatogenesis in addition to that Menthapipe Ritawas improvedin ameliorative effects in comparison to melatonin when experimented on the mice testis.

Keywords: Testis, Spermatogenesis, EMW, Bombardment and Microwave radiations.

INTRODUCTION

Cooking time and effort is decreased through the advance developments in the shape of Microwave oven. It has few advantages and disadvantages. In the microwave an electronic process with the help of magnetron produces high frequency as per second billion cycles through alternating current[1]. Resultantly, the molecular friction in the food happens because of the striking of these waves, distortion is produced in the food because of this heat.

Amino acids in the food are transformed into inactive toxics of biological forms. A research study concludes that the food such as broccoli and carrots due to polarization process became radicalized and according to nutritionist its molecular construction is distorted and biological process is also disturbed within the food[2].

Micro nutrients are also damaged or decreased as a result of molecular friction created because of the bombardment of electromagnetic waves in the microwave oven. Because of its effectiveness the presence of nutrients is mandatory for the spermatogenesis[3]. For the same investigation of variation or loss of nutrients value after being exposed to oven was the objective of the research that how the process of spermatogenesis is affected.

Health disorders have been treated with the help of leaves and green extracts. Many fields rely onthe natural products for the treatment of many ailments. Mint and Menthais are aromatic herbs coming from the family of herbs called Labiate. These are used in variety of disorders in plenty[4]. Leaves extracts potentially aid in the treatment of gastrointestinal mucosa, alkaline phosphatases, serum acid and chromosomal damage of bone marrow.Additionally, it can also assistradical free scavenger as it contains high phenolic contents. M. pipe Ritaleaves are used in this research for the observation of microwave oven changes as happened and affected the spermatogenesis[5].Melatonin is one of the important hormone (N-acetyl-5-methoxy-tryptamine), which is being produced in the penile gland is considered antioxidant effective than Vitamin-E. It enters in blood after being produced. It is soluble in blood and water and can reach brain and placental.Melatonin is taintedup to six -hydroxy melatonin in liver, conjugation is made with glucuronic or sulfuric acid and ends in urine as six-sulfa-toxic-melatonin[6]. Researches have reflected melatonin as an active scavenger relating to toxic-hydroxyl radicals. It is even potent than Vitamin-D, mannitol and glutathione. Increased concentration is required for the desired effects. Purpose behind the research was observation of the protective effects of Menthapipe Rita and melatoninextracts of leaves after exposure to microwave on spermatogenesis of mice[7].

MATERIAL AND METHODS

The current research design is Laboratory based randomized controlled trial. The research was a joint venture held at the venue of Army Medical College (AMC), Rawalpindi (Anatomy Department) and National Institute of Health (NIH), Islamabad. It was completed in the time span of April - May, 2015. A total of thirty-two male adult mice (BALBc Strain) were short listed for the course work of the research paper. The weight rage of the mice was between twenty-five grams to thirty grams. Simple random sampling which was purposive in nature, purely non-probable in the selection of the research sample was adopted. Four randomly selected groups were made out of total sample of thirty-two mice[8]. Every group was allocated eight mice. Control was given to Group-I as they were provided with standard mice diet for a period of consecutive four weeks at the rate of ten gram per mice every day.Microwave exposed diet (pellets) for the period of four weeks was given to every mice at the rate of five to ten grams per mice every day. Leaves extract of Mentha pipe Rita at the rate of (1 gram / kilogram b.wt. every day) and additionally microwave exposed diet of pellets was given to group-III mice at the rate of five to ten grams per animal every day was fed for a period of four weeks[9]. Similarly group four was fed an oral dose of melatonin at the rate of twelve milligram per kilograms per day along with an

exposed microwave pellets for the period of four weeks at the rate of five to ten grams per mice every day. Dissection procedure of mice was carried out at the end of fourth week for the identification of any abnormality in testis, color and shape. Testis were stained, embedded and processed for histological analysis. Evaluation of Spermatogenesis was completed by the scoring method of Johnsons. Statistical analysis was completed through SPSS – 21 and simultaneously Chi-Square test was utilized for the inter-group comparison[10].

In the formalin in quantity of ten percent testis were positioned. For the purpose of embedding and infiltration paraffin was used as wax at 58ºC for melting purpose.In cold temperament blocks were solidified. Thick cross-sections of 5 mm were acquired with the help of rotary microtome. To note the histological parameters staining was done in eosin and hematoxylin[11].

Spermatogenesis

A sample of testis from mid portion from left and right testis was taken and evaluated under 40-X magnification (microscope)&ten rounded tubules were analyzed for the calculation of mean for every animal. Scoring was allocated to every tubule from ten to one[12]. The tubules having complete inactivity scored as one and other with maximum activity (at least 5 or more spermatozoa in lumen) scored ten.In this method score 1 – 10 is included as under:

- Spermatozoa in many Complete spermatogenesis; systematic organization of germinal epithelium.
- Presence of maximum spermatozoa but with disorganized germinal epithelium.
- Very few spermatozoa.
- Many spermatids with zero spermatozoa.
- (<5) spermatids with zero spermatozoa.
- Many spermatocytes with zero spermatozoa.
- (<5) spermatocytes with zero spermatozoa.
- Spermatogoniaas only available germ cell.
- Sertolicells with zero spermatozoa.
- Zero cells in tubule section.

IBM SPSS V-21 was instrument of data analysis. Shiny surfaces reflected Spermatogenesis. Healthy and normal blood vessels were observed on the surface.

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

Johnsons scoring for spermatogenesis	G-1	G-2	G-3	G-4
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

Through Johnsons score was ten in the controlled group-I with full spermatogenesis. In second group, fifty percent of the cases reflected a score of eight (few spermatozoa) and twenty-five percent of the cases had score of seven 7 (many spermatids with zero spermatozoa) and twenty five percent remaining cases had score of six(<5 spermatids with zero spermatozoa). These findings were significant in the comparative study of percentages and frequency. Chi-square test was employed for the comparison between groups.Statistical significant p-value <0.05 was calculated in the research and data analysis.

RESULTS

Normal testis was observed at the end of research in every group and animal. Color of the testis was after test light pink and shape of the testis was oval. In control group one with characteristics of smooth and soft as reflected in Table-I & II). On the other hand, in third group, 37.5 percent of the cases had complete spermatogenesis with a score of ten, while 50 percent of the cases reflecteda score of nine and remaining 12.5 percent of the cases indicated the score of eight. In comparison to second group the results were significant in statistical evaluation with a p-value that equals to 0.012.

In Fourth group, 12.5 percent of the cases reflected score as ten, 37.5 percent of the cases scorednineand remaining 50 percent of the casesreflected a score of eight. In comparison to second group the results were significant in statistical evaluation with a p-value that equals to 0.09 as reflected in figure-1 and figure-2.

DISCUSSION

As we assessed Spermatogenesis by Johnsons scoring and it resembles in its outcomes as of Raghuvanshi. The scoring range was from ten to one. Arrested spermatogenesis was observed by him after exposed to microwave oven[13]. According to Salama studied electromagnetic radiation effects on testis function and observed a significant fall in the concentration of sperms. According to Kesariobserved additional histone kinase 1 activity in the rat semen. This fall directly relatesthe fall in G-2/M phase activity that leads to the decrease of spermatogenesis[14]. According to Goyal& Dixit reproductive system of males requires regular presence of androgens for their structural repair and integrity of functionfor differentiation control of primordial germ cells in spermatids[15]. Zare and Jelodarclarified that fall in serum testosterone possibly because of theradiation effects on ley-dig cells, hypothalamus or pituitary[16].

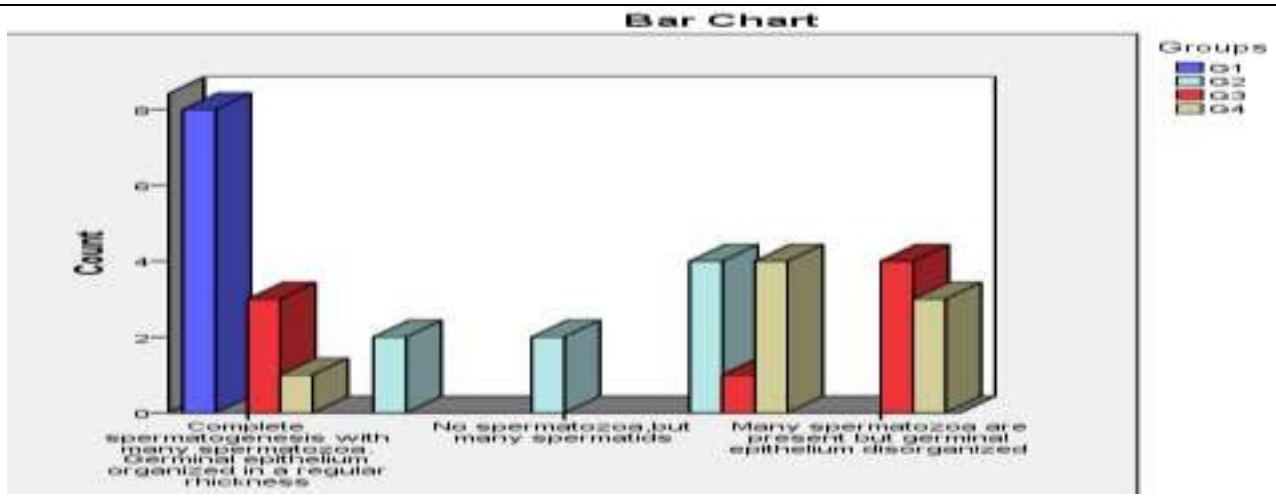

Figure-1: Clustered bar chart showing frequency of spermatogenesis (Johnsons scoring) of the control group 1 and experimental groups 2, 3 & 4.

In third group (microwave processed mice pellets and Mentha pipe Rita), Johnsons score improved by score of 8–10 with 37.5 percent as significantly noticed fall in the male testis tissue sperm count of Wister rats after being processed by electromagnetic radiations[17]. Similar findings werebeingstated by Aitken, Roman and Johnson but differing from the findings of Ozgunerand companions. According to Ozgunermicrowaves radiations are not associated in affecting spermatogenesis[18]. It was also concluded by Johnson and Roman &Aitken, radiations decrease Vitamin-E important for suppressing the lipid peroxidation that is why because of the deficiency of Vitamin-Ean oxidative stress is produced and it changes both testosterone and spermatogenesis production[19]. According to Kesari, electromagnetic waves(EMW) exposure creates significant fall in mean cases with an addition of complete spermatogenesis[20], fifty percent have a score of 9 and 12.5 percent have a score of 8. According to Samarthexplored the protective purpose of Menthapipe Rita on the damage of testicular due to radiation. He also suggests that radiation exposure enhances acid phosphatase[21]. This acid is available in the spermatozoa acrosome and lysosome of spermatocytes, spermatids andSertoli cells. Alkaline phosphatase is also depleted through radiation which issignificant in material transportation from Sertoli to germinal cells;additionally[22], in proliferation and differentiation of germinal epithelium. Menthapipe Ritalowers lipid peroxidation and reinstates alkaline phosphatase; which is responsible for the improvement ofmaterial transportation from Sertoli to germinal cells and lowers the level ofacid phosphatase[22].

Group Four (microwave processed mice pellets and melatonin), 12.5 percent of the cases reflected complete spermatogenesis, 37.5 percent had a score of nine and remaining 50 percent showeda score of eight[23]. In comparison of p-value of group four with group two, it was calculated as 0.09. Improvement is observed in Johnsons score ranging from 6–8 and 8–10 respectively. Meena also found similar outcomes in 2013, during the investigation of protective effects of melatonin in comparison to testicular impairment oxidative stress-mediationbecause of long-term radiation exposure of microwave[24].

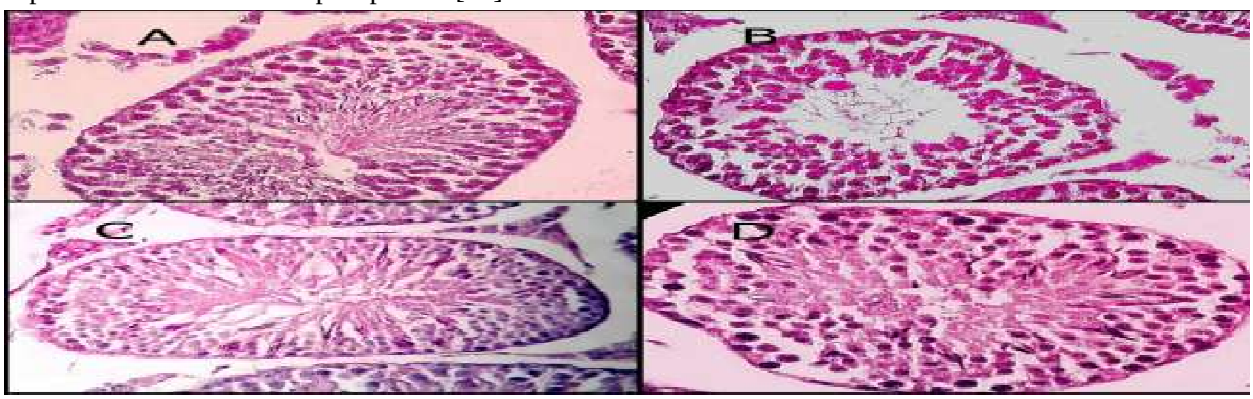

Figure-2: Photomicrograph at 40X magnification, H&E stain showing intergroup comparison of the Johnsons scoring for spermatogenesis. (Group-1 control) with Johnsons score 10, (Group-2group to microwave oven mice pellets) with Johnsons score 7, (Group-3 microwave oven exposed mice pellets + Menthapiperita)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

Melatonin and Menthapipe Ritain microwave variations on spermatogenesis werecontrasted in term of its P-values, results reflected that Menthapipe Rita was strongerin comparison to melatonin for the improvement of spermatogenesis[25].

Microwave has become a permanent an inevitable household that has made the life even easier but on the base of our findings its use must be discouraged especially for children, babies and ladies in the state of pregnancy.

It was observed that melatonin haltsbiological damage of oxidative with a significant increase in the level ($p<0.001$) of testicular LDH-X, lowered ($p<0.001$) MDA levels and testis ROS ($p<0.01$)[26]. It was further explained by Roman and Aitken that melatonin is soluble in both aqueous &lipid settingsand crosses easily the barriers of blood and testes for the protectionof germinal epithelium[27]. It is also responsible to lower the level of lipid peroxidation with added increase of enzymatic and non-enzymaticanti-oxidants[28].

Changes were observed in the previously available researches on the radiated melatonin and Menthapipe Rita on spermatogenesis but this study has failed in the observation of any association in the role of any disease. Routine use of anti-oxidantscounts and bombardment of EMW on usually consumed food after the microwave exposure[29].

CONCLUSION

Mice pallets processed into microwave oven and exposed to electromagnetic radiation had curbed spermatogenesis in addition to that Mentha pipe Rita was improved in ameliorative effects in comparison to melatonin when experimented on the mice testis.

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