Current Journal of Applied Science and Technology

34(4): 1-11, 2019; Article no.CJAST.18602
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Biophysical Effect of EMR with 5GHz on Male Reproductive System of *Mus musclus* Mice

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Authors' contributions

This work was carried out in Mosul University, College of science, Biophysics Department. Author QKHA designed the study, collected and prepared the samples, participated in the laboratory procedures and wrote the first draft of the manuscript and participated in the literature searches. Author AHI supervised the design and analysis of the study. Author YAAJ analyzed the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2019/v34i430138
Editor(s):
(1) Ya-mei Gao, Heilongjiang Bayi Agariculture University, P.R.China.
Reviewers:
(1) Musa Yakubu Tula, Federal Polytechnic Mubi, Nigeria.
(2) Abdullahi Muhammad, Nigeria.
Complete Peer review History: http://www.sdiarticle3.com/review-history/18602

Original Research Article

Received 19 March 2015
Accepted 16 October 2015
Published 06 April 2019

ABSTRACT

Aim: To study the effect of microwave radiation with power density 100,150 and 200 microwatt / cm^2 (μW/cm^2) of exposure on male reproductive system of *Mus musclus* Swiss albino mice.
Study Design: Twenty mice were used in the experiment, they were divided randomly and assigned into 4 groups of 5 animals each. White mice were used in this study *Mus musculus* Blub/c which range between 2-3 months of age and 23 -30 gm of weight . Healthy mice were obtained from the college of veterinary in Mosul University.
Place and Duration of Study: Mosul University, College of science, Biophysics department, IRAQ, between January 2014 and April 2014.
Methodology: Mice were exposed to 100,150 and 200 μW/cm^2 for 60 days. Then mice were divided into 4 groups each of group contain 5 animals. Group I: considered as control group were placed in cages without exposure. Group Π: Mice group were exposed with microwave 100 μW/cm^2 for 60 days. Group Ш : Mice of this group received 150/cm^2 for 60 days. Group IV : Mice of this

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group received 200 μW/cm^2 for 60 days. After exposure testis were obtain from each male group and experiment carried out on the sperm.

Results: The results in control group without exposure the parameters as No of sperm cells in epididymis, Percentage of living sperm cells, Percentage of dead sperm cells, Percentage of normal sperm cells and Percentage of abnormal sperm cells respectively recorded with, 28.96 a ± 0.99, 87.81 a±0.82, 12.19 d±0.81, 86.66 a±0.83 and 13.44 d±0.73 respectively. The first group exposed to 100 μW/cm2 and the results of the same parameters with 15.60 b±0.49, 46.05 b±0.44, 53.96 c±0.43, 44.97 b±0.55 and 55.03 c±0.55 recpectively showed a significant decrease ($p<0.05$) in No. of sperm cells in epididymis, Percentage of living sperm cells and Percentage of normal sperm cells respectively while appeared a significant increase($p<0.05$) in Percentage of dead sperm cells and Percentage of abnormal sperm cells. The second and third groups exposed to microwave radiation at 150 and 200 μW/cm2. Also the results of same parameters recorded with 11.86 c±0.23, 35.90 c±0.69, 64.23 b±0.52, 34.93 c±0.72, 65.28 b±0.46, 6.88 d±0.26, 24.72 d±0.21, 75.28 a±0.21, 23.68 d±0.45 and 76.33 a±0.45 respectively.

Conclusion: The 5GHz of microwave radiation has harmful effect of physiological and histopathological for reproductive system and avoid the exposure for long time about radiation from communication That have this same frequency as Wi –Fi or any communication from internet.

Keywords: EMR; power density; microwave radiation; sperm cells; frequency; mice; male reproductive system; necrosis; testis; seminiferous tubules.

1. INTRODUCTION

The exposure of humans to the electromagnetic radiation (EMR) at very low frequencies between 50-60 hertz continuously, may cause harmful effects on fertility and reproduction [1]. There is no strict indication that refers to exposure to extremely low frequency (ELF) which have an effect on revolution and reproduction of mammals. The noticeable effect could be due to the heat produced as a result of exposure to the ELF [2]. It is well known that male fertility is sensitive and rapidly affected by heat. Studies conducted on experimental animals proved the same effects when they were exposed to ELF fields at certain heat levels. Because of the sensitivity of the testis to heat, concern has been directed to the effects of ELF in gonadal functions. In 1962 a study were carried out on mice, which were exposed to electromagnetic radiation at 9.3 gigahertz (GHz) frequency and power density of 100 milliwatt/cm^2 with specific absorption rate (SAR) of 45 watt /kg for 4-5 minutes daily, for 5 days weekly. In 59 weeks, it led to testicular degeneration at 40 % of the exposed animals and 8% in contrast to the control ones [3].

Researches refer to lately the effects of exposure to electromagnetic radiation ELFon the number of sperms and their nature. As it recorded a significant decrease in the number of the sperms of mice epididymis associated with significant increase in the percentage of abnormal sperms, when the rats were exposed to frequency 9.45 GHz with a specific absorption rate 1.2 Watt/kg for along period [4]. There were a decrease in the sperm tubule diameters in rat testis exposed to frequencies between 890 -915 GHz for a month [5].

Low frequencies (50-60) Hz have many effects on male multiplication, since [6] recorded decrease in malondialdehyde of elongated spermatids of the exposed rats to 50 Hz of ELF for 28 days. It has been noticed that the exposure of adult male rats to 50 Hz for 90 days had an effect on fertility of female rats [7]. This was proven by [8], they recorded a decrease in number and sperm mobility of exposed rats to 50 Hz for two weeks time . There was also a decrease in the average of testis weight of the exposed rats to the same frequency for four week duration. In the case of ELF effect on man exposed to this field in terms of fertility and reproduction, the study performed by [9], showed that the exposure of 21 men to microwave radiation at 3.6 -10.0 GHz during their work for 1-17 years. About 74% of them showed changes in sperm production such as the decrease in sperm number and motile sperm number and the natural ones as well. But they did not refer to any difference in 17- ketosteroids level in comparison with the control. They noticed Azoospermia in men exposed for 14 years. A survey was carried out among the Danish soldiers working in mobile ground – air projectile units which uses radar systems, they found that the average density of sperms was very low compared with the control and this was supported by the previous studies [10]. This was

also proven by the investigation of [11], that there was no significant differences in the weight of testis, prostate, adrenal gland pituitary gland and thyroid gland in rats exposed to 2.9 GHz, with power density of 10 m W/cm 2 for 6 weeks. Therefore, this research aimed to study the effect of ELF radiation at doses 100,150 and 200 microwatt/cm^2 on exposed testes of mice for 60 days at 6 hours daily to 5 GHz.

2. MATERIALS AND METHODS

White mice were used in this study Mus musculus Blub/c which range between 2-3 months of age and 23 -30 gm of weigh. Healthy mice was obtained from the college of veterinary in Mosul University and were kept in plastic cages supplied with plastic metal lids (20x30x15 cm dimensions). Steps were taken to ensure good hygiene. Both the control (sham exposure) and the microwave radiation exposure groups'. ambient temperature and humidity throughout the experiment were maintained at 26°C. ±2 and 35% ± 5%, respectively, and changing the sawdust weekly. Feeding mixture which consist of 35% wheat, 34% corn 20% soybean, 10 % protein and 1% dried milk. They were also supplied with water along the time of experimentation [12].

2.1 Work System

The system is made of source of generating microwaves aimed at the mice with 5 GHz frequency, with different power density in microwatt units/cm^2. The instrument were connected to the internet with six hours exposure daily, it is known that the power density is provided from a change of software program due to power of device with computer from internet which represent power supply as shown in Fig. 1.

The power meter was used to determine the power density using the job radiation power density level meter microwave. This instrument was used to measure the density power of electromagnetic waves in W/m^2 units. This instrument revealed the radiation dose levels of electromagnetic radiation waves within the microwave frequencies used in telecommunication which include blue tooth, Wi-Fi, electricity generating station, fixed towers of mobile phone and energy towers rather than the measurement of radiation dose released from television and computer screen and video display units (VDU). Also this instrument was able to measure the intensity of sound waves associated with high frequency waves in dB units with positive or negative levels as showen in (2) [13].

This instrument have another function such as the measurement of intensity of electrical field in E (V/m) units, when the instrument was placed closer to the source of generating the electromagnetic radiation waves rather than the ability to draw a figure showing the relationship between power density and sound waves intensity of the same frequency at a certain point between the detector and the source. The detector have other uses such as the power density of radiation dose of the microwave released from the other environment as a type of radiation contamination and also to discover the radiation leak from microwave ovens.

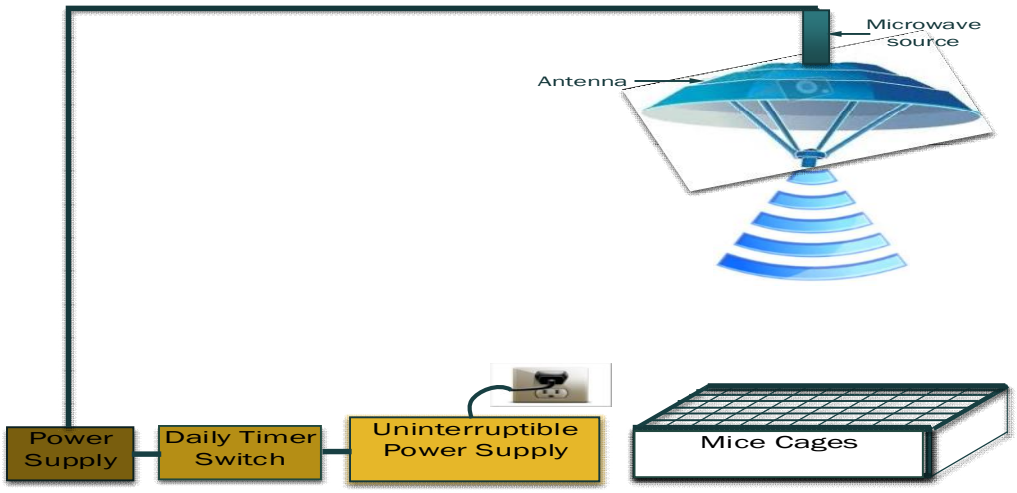

Fig. 1. Work set up

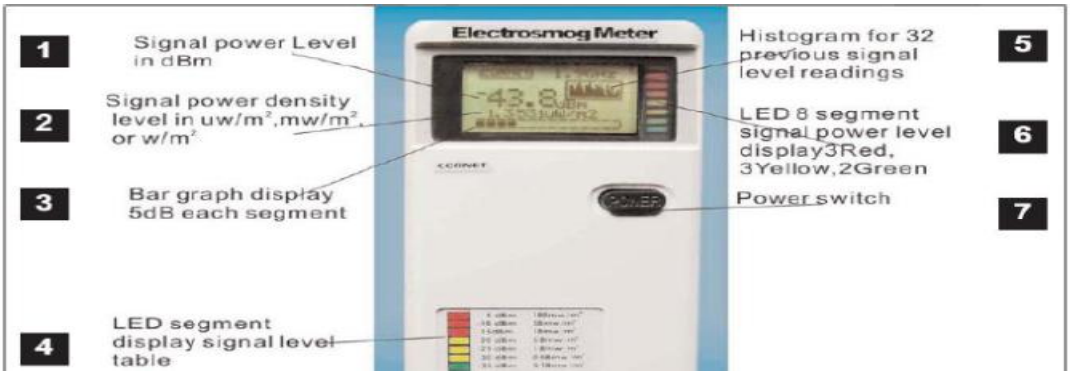

Fig. 2. Electrosmog power meter [13]

2.2 Study of Sperm Density and Percentage of Dead and Abnormal Sperms

Animals were anesthetized using ether after irradiation, then they were dissected and the testis and epididymis were taken out. The semen was extracted from the epididymis [14] by squeezing the contents of the epididymis tail after dissecting them in a clean watch glasses as contianer, then the following test were applied:

2.2.1 Sperm concentrations

The percentage of living and dead sperm were assessed and also the percentage of abnormal sperm according to [15] method.

2.2.2 Experimental design

Twenty mice were used in the experiment, they were divided and given the chosen doses as follow:

1- First group (control) include 5 male mice not exposed to the electromagnetic radiation field (control). Second group include 5 mice they were exposed to 100 μW/cm 2
2- Third group include 5 mice and exposed to 150 μW/cm 2
3- Fourth group include 5 mice exposed to 200 μW/cm 2
4- Group (2-4) , the number of male mice are 15 used for 60 days at a rate of 6 hours daily.

The work system was prepared at 5 GHz frequency as in Fig. 1 with different exposure of power density (100, 150 and 200 μW/cm 2) on white male mice for 60 days and at period 6 hours daily.

2.3 Statistical Analysis

One way analysis of (ANOVA) after variance was used in this experiment. Differences were determined by Duncan Include significant level was determined at p≤ 0.05 [16].

3. RESULTS

All results were included in Table 1.

Table 1. All results with mean value, significant value and standard division

Percentage of abnormal sperm cells	Percentage of normal sperm cells	Percentage of dead sperm cells	Percentage of living sperm cells	No of sperm cells in epididymis	Measurement / Groups
13.44 d±0.73	86.66 a±0.83	12.19 d±0.81	87.81 a±0.82	28.96a ± 0.99	Control
55.03 c±0.55	44.97 b±0.55	53.96 c±0.43	46.05 b±0.44	15.60 b±0.49	100 μW/cm2
65.28 b±0.46	34.93 c±0.72	64.23 b±0.52	35.90 c±0.69	11.86 c±0.23	150 μW/cm2
76.33 a±0.45	23.68 d±0.45	75.28 a±0.21	24.72 d±0.21	6.88 d±0.26	200 μW/cm2

*Mean ± S.D. ** p= 0.05 *** n= 5 **** T= 20;*

Means and standard deviations are reported in Tables 1. **Level of significance (p<0.05).as a,b,c,d between groups; * Number of animals per group; ****Total number of animals used*

3.1 No of Sperm Cells in Epididymis

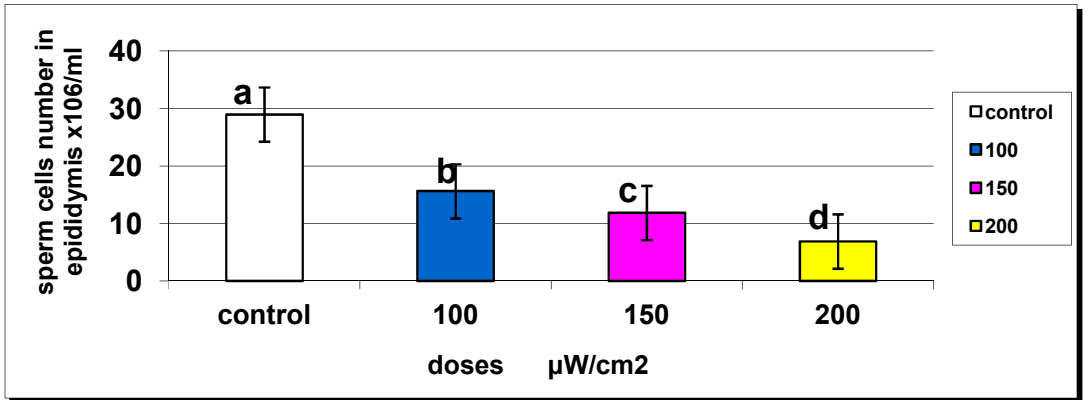

Fig. 3. Show the effect of microwave radiation 100,150 and 200 µW/cm^2 for 6 hours daily exposure to 5 GHz for 60 days on no of sperm cells in epididymis with Standard error mean

3.2 Percentage of Living Sperm Cells

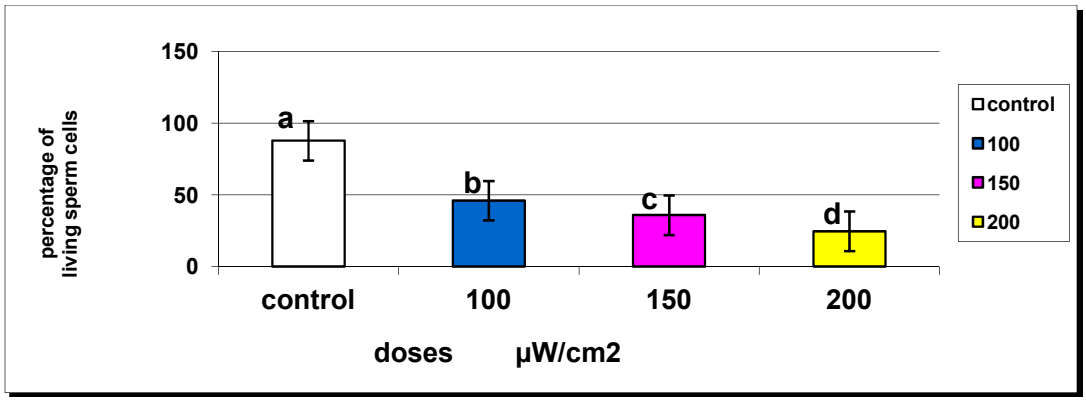

Fig. 4. Shows the effect of microwave radiation at dose of 100,150 and 200 µW/cm^2 for 6 hours dailybasis at 5 GHz for 60 days on the percentage of living sperm cells Standard error mean

3.3 Percentage of Dead Sperm Cells

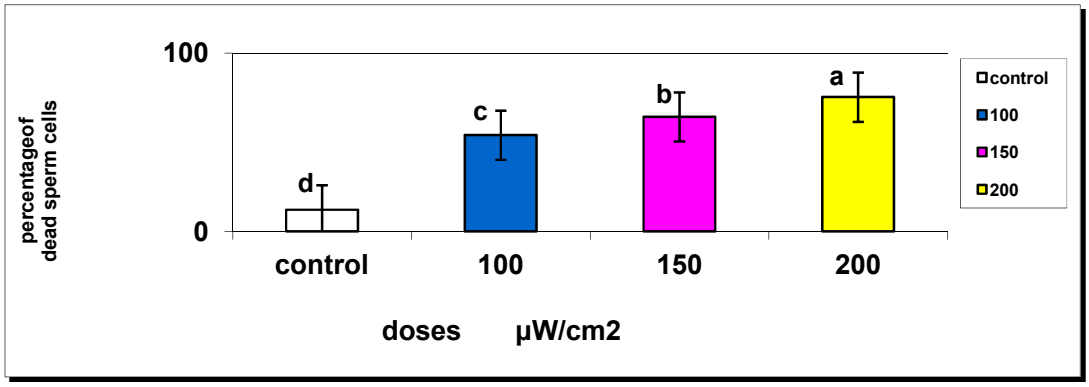

Fig. 5. Shows the effect of microwave radiation at the doses 100, 150 and 200 µW/cm^2 at 6 hours on daily basis at 5 GHz on a period of 60 days on the percentage of dead sperm cells standard error mean

3.4 Percentage of Normal Sperm Cells

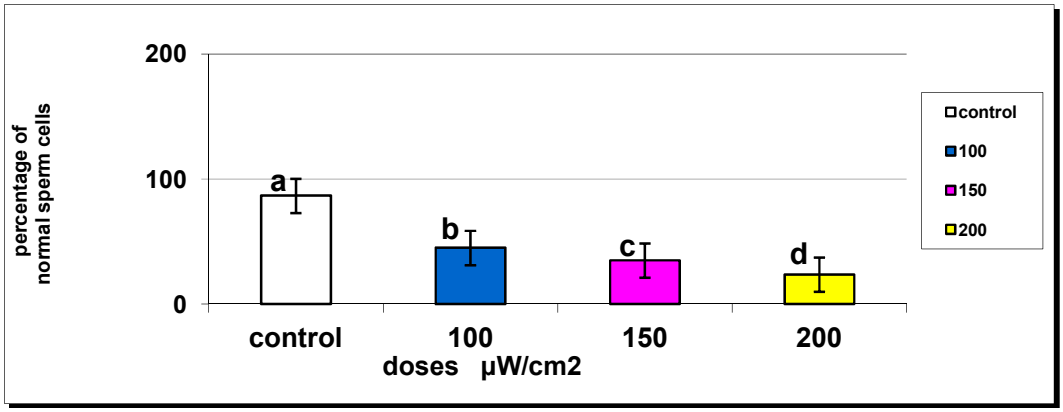

Fig. 6. Shows the effect of microwave radiation at 100, 150 and 200 µW/cm^2 doses for 60 days at 6 hours daily with 5 GHz on the percentage of normal sperm cells Standard error mean

3.5 Percentage of Abnormal Sperm Cells

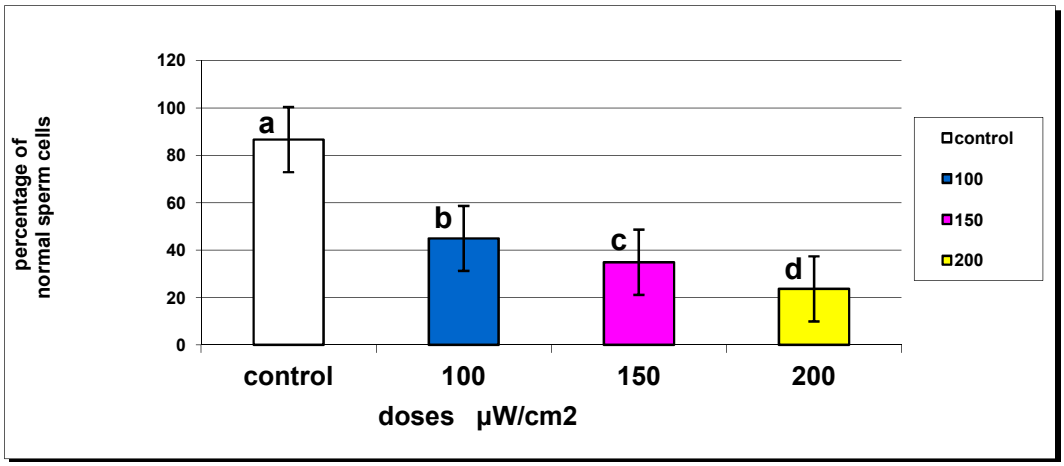

Fig. 7. Shows the effect of microwave radiation at the doses of 100, 150 and 200 µW/cm^2 and for 60 days at 6 hours daily with 5 GHz on the percentage of abnormal sperm cells

3.6 Histological Changes of Testis

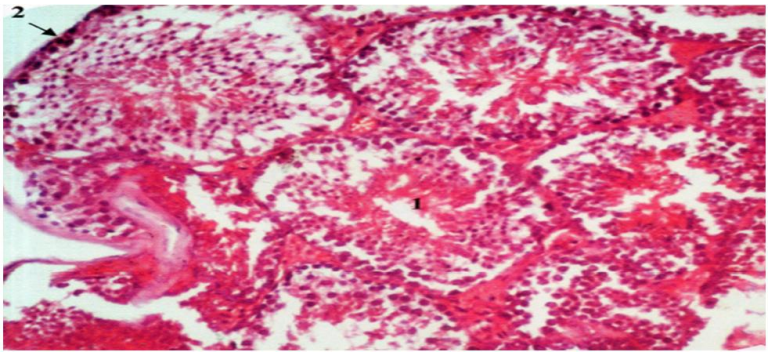

Fig. 8. C.S in testis of control group showing 1- seminiferous tubules 2- testis capsule. Stain H&E. 10X

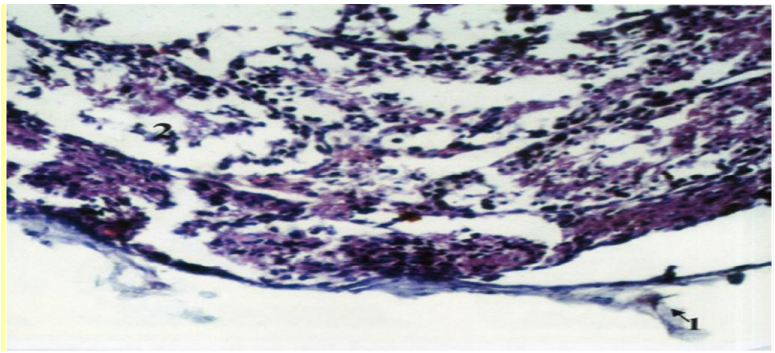

Fig. 9. c.s in testis of mice exposed to 100 µW/cm2 showing 1-speration of testis capsule 2-necrosis in seminiferous tubules. Stain H&E.10X

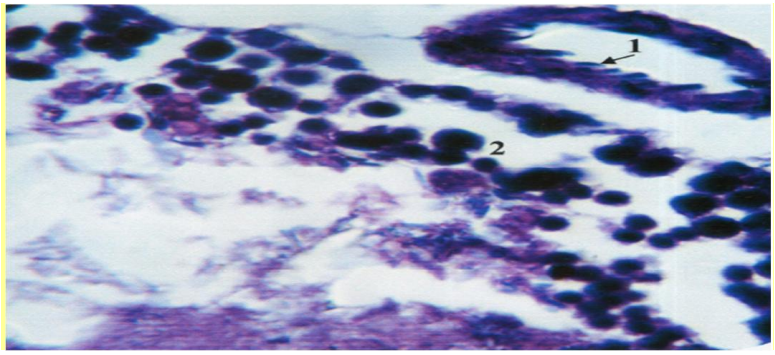

Fig. 10. C.S in seminiferous tubules of mice which exposed to 150 µW/cm2 showing 1-Magnifical of nucleus cells of blood vessels 2-Necrosis in seminiferous tubules. Coating H&E. 40X

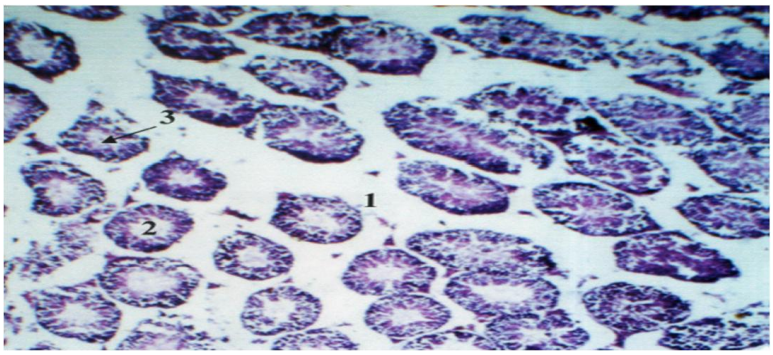

Fig. 11. C.S in seminiferous tubules of mice which exposed to 200 µW/cm2 showing 1-Necrosis of arulear connective tissue 2- appearances of vacuoles of star shape in seminiferous tubules.3-contraction of same seminiferous tubules in mice stain H&E. 10X

4. DISCUSSION

In Fig. (3) there was a significant decrease at ($p< 0.05$) in the number of sperm cells in epididymis of the mice exposed to microwave radiation at 5 GHz for 6 hours daily for 60 days when the 100 , 150 and 200 microwatt/cm ^{2} dose were used ,compared with the control treatment.

This comes in agreement with [17] who studied the effects of electromagnetic radiation fields at 50 GHz on the number of sperm cells in epididymis, weight of testis and secondary sexual organs in male mice. They found a significant decrease in the number of sperm cells in epididymis and a decrease in the number of sperm cells in the semen tubules in rats

exposed for 1 or 2 hours. Another study carried out by [18] showed the effect of electromagnetic field at 2.45 GHz in germ cell spermatogenesis of male rats which were exposed for 1-2 hours/day for 8 weeks , the quantitative analysis of leydig cells in the testis show significant increase of sexual hormone in serum(testosterone) . Also there was a significant decrease in the number spermatecytes in the semen tubules of the exposed rats in comparison with the control . [19], found that the exposure of rabbits to EMR fluctuated frequencies at low intensity led to a significant decrease in semens tube diameter. The study done by [20] found that the continuous exposure to the electromagnetic field at very low frequencies lead to programmed killing of semen cells in rats of Balb/c strain. Whereas no significant effect was found on body weight and testicular organs. These results refers to possibility of programmed cell killing of the spermatogenetic cells, in mice because of the continuous exposure to the EMR frequencies at 60 Hz [21]. The World heath organization [22] warns from the over exposure to electromagnetic fields which would lead to destroy the sperms. This will amount to strong decrease in sperm speed of these persons, in comparison to those who have moderate exposure. The change in semens quality of those who are over – exposed to electromagnetic fields belong to the effect of radiation which cause a severe injury on the nucleic acid which in turn have an effect on the testis cells which produce the testosterone hormone, or the tubes producing the semens. Wdowiak [23] concluded through his research by exposing both mice testis to the waves of the mobile phone at 900 MHz which caused a decrease in their fertility by causing a trophy of testis tubules . Yan [24] emphasized that the waves released from the mobile phone caused a significant decrease in the number of sperms in mice. Figs. (4-6) showed a significant decrease at ($p< 0.05$) in the percentage of living and normal sperm cells through the exposure to the microwave radiation at 5 GHz frequency for 6 hours along a period of 45 days at the doses 100, 150 and 200 microwatt/cm^2. While the percentage of dead and abnormal sperm cells shown in Figs (5-7) an increase was noticed at ($p< 0.05$) when they were exposed to the same treatment before and the Figs. (3-7) represent with the following information: - Values expressed by average ± experimental error - The different letters on each rectangular means significant differences at less than 0.05% level.- Number of animals is 5 per each group. This is in agreement with [25], the results of study revealed that the radio electromagnetic field with short wave at three frequencies (1.6, 2.4, 3.2) MHz at power density 16 millwatt/cm^2 have a harmful effects in sperm formation and testis functions as there was a decrease noticed in sperm cells number at the head of epididymis and an increase in the percentage of the dead and abnormal sperm cells in the tail of epididymis a compared with a decrease in the percentage of living sperm cells. This results was in agreement with that of [4], the exposure of rats to the radio electromagnetic field at 9.45 GHz caused a decrease in the number of sperm cells in the epididymis and an increase in dead sperm cells percentage . There were a negative effect in the sexual efficiency of the exposed person to frequencies between 3.6- 10 GHz. They recorded a decrease in the number of sperm cells motile and and other abnormalities led to changes in the functions of sperms **(**obtained normal sperm cells. Also there were no sperm cells been formed in the persons who are exposed for 14 years [9]. Hjollund [10] showed that there was a significant reduction in the average density of sperm cells found in the soldiers exposed to the radar waves. This was in agreement with [26] who found that the mobile phone waves when used at 2 hours daily for 45 days imposed on rats 70 days ago. Abnormalities in sperms at the head, neck and tail, rather than loss in testis weight and number of living sperms fertility hormones and eventually affect the average of reproduction in men and also affect the speed, the number, and the number of abnormalities occurred in sperms [27]. These harmful effects which resulted from radio electromagnetic field was owing to the thermal load or to the oxidative stress or to the direct effect on endocrine glands. The studies proved that thermal or heat sensitivity of testis tissue in mammals resulted from exposure to different frequencies [28]. In 1980 another study conducted by Cairnie and leach concerning the effect of heat or temperature on testis through exposing mice to a water bath at 32-43°C. for 4 hours, when they discovered a damage in testis tissue after two hours of exposure to the water bath at 41°C for 30 minutes at 43°C which caused lowering in sperm number in the epididymis after 30 minutes exposure. There was a similarity between the results caused by the radio electromagnetic field and that caused by heat on testis [29]. Absorption of energy of the electromagnetic field by the body led to raise the testis temperatures and the sperm production in mammals decreases when exposed to temperature a bit higher than the normal

temperature of the body. The heat generated through exposure to electromagnetic field causes such effects in testis functions similar to those effects resulted from high temperature [30]. The mice exposed to 2.45 GHz showed an increase in temperature which resulted in exhaustion of primary spermatocyctes [31], with increase in percentages of sperm abnormalities and reduction of fertilization in mice exposed active free radicals affect the lipid and protein layer in the plasma membrane of the sperm rather than changes occur in the DNA . Since the sperm contain lipid materials such as unsaturated multi fatty acid, plasmalogen and sphingomyelins which are susceptible to oxidation causing disturbances in the function of sperm [32,33].

The free radicals are playing their harmful role on phosphor - lipids found in cell membrane leading to formation of malondialdehyde [34]. It is possible to predict of lipids peroxidation by measurement of malondialdehyde [35]. A lipid peroxidation is conceder as essential key for the active oxygen types leading to lose the ability for fertilization as a results of the lack of sperms and a quick loose of energy inside the cell leading to destroy and decrease of sperm activity and increase of abnormal sperms [36], or the reason of these change may be due to the interaction of active oxygen varieties formed as a result of the exposure to electromagnetic field together with energy production and metabolism in sperms leading to lowering the concentration of ATP in the sperms and eventually the loss of sperms and death [37]. Another study by [38] showed that the exposure to the electromagnetic field caused lowering of energy sources such as ATP. It is well known the formation of sperms need a high energy, mobility and their ability for fertilization. The power houses physiologically provide the sperms by energy through glucose and fructose metabolism since the fructose is available in the semen of rats and metabolized and only 10 % of the metabolites will be converted to glucose [39]. The active energy varieties are poisonous to cells and tissue, as they cause histological pathogenic changes in body cells and tissues including testis cells [40].

4.1 Histological Changes of Testis

The changes observed made an effect on the structure of the testis tissue such as degeneration changes in semen tubules represented by necrosis and sloughing of sperm generating cells and sertoli cells, in some semen tubules especially those located under the semen sac. These changes retard the formation and maturation of sperms and eventually increase the percentage of dead sperms and abnormality. Knowing that sertoli cells participate in the formation of sperms and their maturation and at the end they cause an interference of sperm maturation and other changes represented by degeneration tissue. This was in agreement with [32] they used different frequencies on the testis tissue. They showed that these degeneration raised up the temperature due to the exposure to radio electromagnetic field. The reason of for these changes may be due to the effect of radio electromagnetic field in the role or function of mitochondria and through disturbance in energy production ATP. The effect on sodium pump and plasma membrane or through lowering of pH of cells by the increase of lactic acid which cause a reduction in protein production and retarding the formation of plasma membrane which means the occurrence of necrotic [41].

5. CONCLUSION

The 5GHz of microwave radiation has harmful physiological and histopathological effects for reproductive system. Avoid exposure for a long time about radiation from communication gadgets that have the same frequency as Wi – Fi communication or any communication from internet will help a lot.

DISCLAIMER

Some part of this manuscript was previously presented and published in the following conference.
Conference name: Third Scientific Conference of Biology
Dates: 2018
Location: NA
Web Link of the proceeding:
https://www.iasj.net/iasj?func=fulltext&aId=154285

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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