

## The Investigation of the protective effects of hydro-alcoholic extract of sea buckthorn (*Hippophaerhamnoides L.*) in spermatogenesis of rat after exposure of Wi-Fi radiation

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### ABSTRACT

Male infertility is a distressingly common condition affecting about 1 in 20 of the male population. In a majority of the cases, the male partner produces sufficient numbers of spermatozoa to achieve fertilization but there are functional defects in these cells that prevent conception from occurring. In this study, the effects of Wi-Fi radiation on spermatogenesis, testes and testosterone of large white male rats were studied, and also hydro alcoholic extract of sea buckthorn fruit were used as a protective agent against the effects of Wi-Fi radiation, and the comparative study was done. For this study 60 white male laboratory rats weighing 200-250 g were selected then tested in good conditions of light and food. The mice were randomly divided in terms of Wi-Fi Radiation and received gavage Sea buckthorn into 6 out of groups of 10. It was found that in the groups that had received Wi-Fi radiation, waves did not affect sperm count, testes weight, testosterone and testicular tissue, but it caused a significant decrease in fertilized sperm motility, and sea buckthorn extract could work as a protective agent against the radiations. According to the results of this research, it can be the probability that Sea buckthorn extract, having antioxidant substance can prevent destructive effects of waves that cause free radicals and having vitamin E improves sperm quality.

**Keywords:** Spermatogenesis, Wi-Fi radiation, Sea Buckthorn fruit

### INTRODUCTION

There has been continuing public anxiety about the potential health consequences of wireless communication devices using radiofrequency (RF), despite the fact that authorities have set safety boundaries on the subject to protect the community against RF exposure [1, 2]. Therefore, the World Health Organization (WHO) advised further research to be conducted in this field. Animal studies involving rats have been conducted to analyze the effects of Wi-Fi signals on health parameters and stress markers [3]. The environmental 1802.11.g wireless device (also called Wi-Fi device or wireless internet access device or WIAD) has higher frequency ranges in general and longer exposure times than wireless phones are used [4]. Thus, the level of health hazards associated with Wi-Fi device might be different from and possibly higher than, Mobile Phones. In addition, Wi-Fi devices usually expose the whole body to RF, unlike Mobile Phones which

generally irradiates some parts of the body, e.g. cranium, more than others. Exposure of animals to RF electromagnetic radiation (EMR) may lead to a variety of changes in tissues. The observed changes vary depending on the wireless exposure characteristics, the studied species, and the histological methodology used for the detection of the effects [5-6].

In recent years, the use of portable computers (laptops, connected to local area networks wirelessly also known as Wi-Fi) has increased dramatically. Laptops have become indispensable devices in our daily life, offering flexibility and mobility to users. People using Wi-Fi may be exposed to radio signals absorbing some of the transmitted energy in their bodies. Portable computers are commonly used on the lap [7-8], therefore it can expose the genital area to radio frequency electromagnetic waves (RF-EMW) as well as high temperatures [8, 9]. Infertility is a common worldwide condition that affects more than 70 million couples in their

reproductive age [10]. It has been suggested that male fertility has declined during the past several decades [11]. Such decline has been attributed to direct or indirect exposure to certain environmental factors such as RF-EMW [12]. Extremely low frequency magnetic field can initiate a number of biochemical and physiological alteration in biological system of different species [13-14]. Many of these effects have been associated with free-radical production [15, 16]. Free radicals are causative factors of oxidative damage of cellular structures and molecules such as lipids, proteins, and nucleic acid [17].

In this context, it is significant that spermatozoa are uniquely sensitive to oxidative stress for a variety of reasons. First, these cells are largely devoid of the cytoplasm that is found in somatic cells houses, the antioxidant enzymes offer a first line of defense against free radical attack [18]. Second, these cells possess abundant targets for the induction of per oxidative damage including polyunsaturated fatty acid and DNA [19-20]. Third, these cells are professional generators of reactive oxygen species, that appear to emanate largely from the sperm mitochondria and, possibly, plasma membrane NAD(P)H oxidases [21, 22].

Plants possess a variety of phytochemicals like antioxidants and cytokines that help in the alleviation of the pathophysiological symptoms of a number of free radical mediated diseases and in overcoming environmental adversities of both physical and biotic origin. Many of the plants exploited in *Ayurvedea*, the Indian system of Medicine, have been reported to exhibit radio protective ability and among them *Hippophaerhamnoides* have been the most impressive [23].

*Hippophaerhamnoides* commonly known as a Sea buckthorn, a nitrogen fixing plant (2-5 m) belongs to Elaeagnaceae family and the fruits are reported to be a rich source of vitamins and vitamin C in particular and fruit oil is rich in vitamin E, K and carotenoids, flavonoids and tannins [24].

## MATERIALS AND METHODS

To perform this test, 60 male rats from Sprague-Dawley race, weighing 200-250 g, were grouped randomly, from the Laboratory Animal Center of Shiraz University of Medical Sciences.

### **Grouping of Animals**

The rats were randomly divided into 6 groups of 10 which were housed in cages measuring 40×20×15 cm, 5 mice per cage. Each group had a quintuple cage. All mice were weighed and numbered. Throughout the experiment, the lighting condition was regarded as 12 hours of light (6 am to 6 pm) and 12 hours of darkness, and the temperature was  $23 \pm 2^\circ\text{C}$ . All groups received the same diet. Among 6 groups, group 1 was set as a true control group (did not receive any radiation), group 2 was set as the sham group (received 2ml/kg distilled water by gavage for 48 days), group 3 was set as the experimental group 1 (received 2ml/kg hydroalcoholic *Hippophaerhamnoides* (Elaeagnus angustifolia) extract by gavage for 48 days), group 4 was set as the experimental group 2 (the rats were placed within Restrainer and were exposed to Wi-Fi radiation 4 hours a day for 48 days). Group 5 was set as the experimental group 3 (the rats received 2ml/kg hydroalcoholic *Elaeagnus angustifolia* extract by stomach tube then they were placed within Restrainer and were exposed to Wi-Fi radiation 4 hours a day for 48 days), Group 6 was set as the Wi-Fi control group (the rats were placed within the Restrainer 4 hours daily for 48 days without any radiation).

### **Elaeagnus Angustifolia Extract**

#### **Method of Giving Elaeagnus Angustifolia Extract**

Experimental groups 1, 2 and 3, received 70 mg per 1kg a day by gavage. It should be mentioned that the dose was dissolved in 2 ml of distilled water.

### **Wi-Fi Radiation Technique**

#### **Equipment used to give radiation**

EMF Device (Electro Magnetic Field) or dosimeter, used in this experiment, was from Holaday company, HI-3604 model. The apparatus was used for measuring and recording the electric and magnetic fields. To perform this test, Irancell WiMAX desktop modem, WIXFMM-130 model (internal modem equipped with Wi-Fi) was used. WiMAX is a digital wireless system, which used a very large area covered by WiMAX towers, the city and industrial zones and strategic areas and provides high-speed Internet for organizations and businesses as well as residences and any other places at any point of its coverage. In this experiment, we used a laptop (ASUS N43J). The laptop was used to connect to the internet and download different material at the time of Wi-Fi radiation.

### Sorting the rats to receive Wi-Fi radiation

Before sorting the rats, the electric and magnetic waves of the environment should be recorded by the dosimeter and because this experiment was performed in the central laboratory of animals at Shiraz University of Medical Sciences, the device was moved to the center and waves were recorded as follows:

•Background environment before turning on WiMAX and Laptops:

Magnetic field: 14mA/m and the electric field (0.5 V/m)

•Laptop on, Modem off:

Magnetic field /mind the electric field (10 V/m)

•Laptop on without downloading and using internet, Modem on:

Magnetic field: 19 mA/ mind the electric field (11.5 V/m)

•Laptop on when downloading, Modem on:

Magnetic field: 67 mA/mind the electric field (17.4 V/m)

Therefore, the waves were distributed in the environment spherical, the modem device was placed at the center and the rats were placed in there strainer and arranged in a circle around the modem. Since two groups were going to be exposed (groups 4 and 5) to Wi-Fi radiations, one group was placed below and the other one was placed on it as you can see in the figure. As the irradiation time was 4 hours a day so after 2 hours we moved up the groups in order to receive the same signal (Figure1).



**Figure1:** Arrangement of rats inside the restraining around the Wi-Fi modem.

On day 50 (48 days after giving the extract and Wi-Fi radiation and 49 days after giving the busulfan), the rats were anesthetized in order to test hormone levels (testosterone) with 2 cc syringe. 2 ml of blood were drawn from the hearts of animals and poured in a clot test tube. Then rat was dissected. In order to prepare the sperm and then counting, one centimeter from the left vas deference was isolated, placed in the 2.5 ml HBSS solution (Hank's Balanced Salt Solution) with 37°C of heat. Then by using the Jennifer (25) method, a drop of the solution was poured onto the Neubauer chamber, after placing a cover glass on it, houses number 1,3,7,9 (houses for counting white blood cells) were used to count the sperms. After counting the average number was calculated from the above mentioned houses. Finally left testicular tissue was removed for histological study and placed in 10% formalin.

### RESULTS

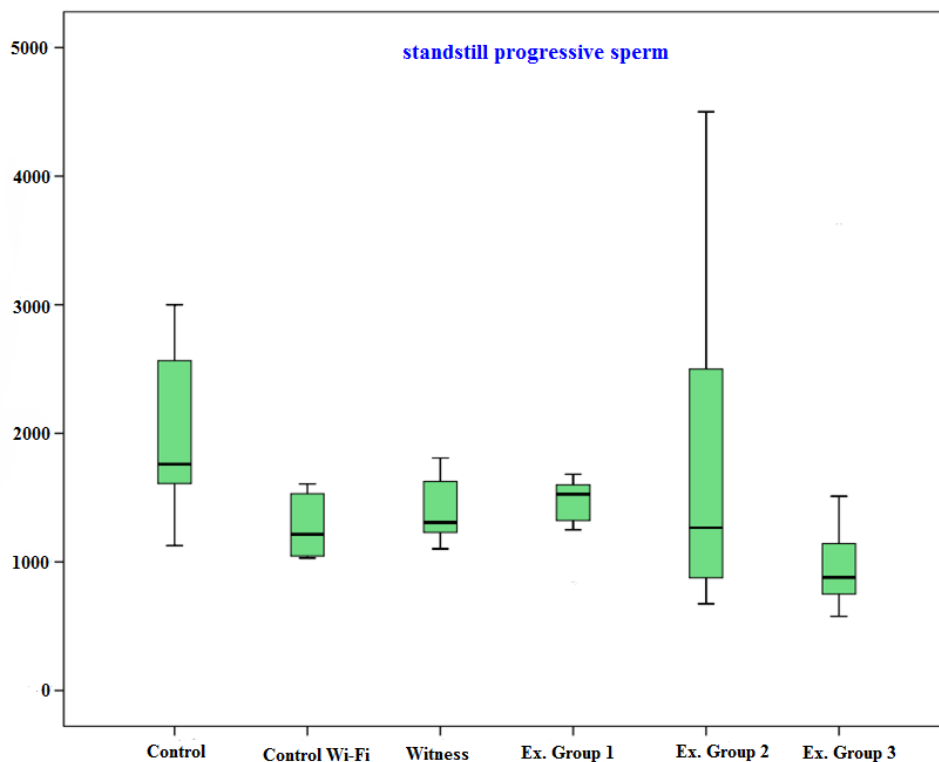
Wi-Fi radiation had no adverse effects on sperm and testosterone and testicular tissue and no significant differences were observed between the irradiated groups and control groups. But in regards to the fertile sperm mobility it was shown that the no index entries found. Wave reduced mobility and it had significant differences with the control group. The use of *Elaeagnus Angustifolia* extract in Group 2, which had received the radiation with the extract simultaneously, had a significant difference with Group 3 that was only irradiated. So we can conclude that *Elaeagnus Angustifolia* extract improves sperm motility and fertility. Statistical analysis Standstill progressive sperm were shown in Table 1 (*t*-test and one-way ANOVA;  $P < 0.05$ ). Three experimental groups compared with the control group showed no significant difference at ( $P < 0.05$ ), [Experimental

group 1 (hydroalcoholic-*elaegnus angustifolia* extract): ( $P < 0.05 = 2.961e-012$ ), experimental group 2 (Wi-Fi radiation): ( $P < 0.05 = 1.284e-010$ ), and experimental group 3 (hydroalcoholic-*elaegnus angustifolia* extract + Wi-Fi radiation): ( $P < 0.05 = 1.128e-008$ ). Also, There was a significant difference between experimental (group III) and control Wi-Fi ( $P < 0.05 = 2.038e-009$ ), (showed in Figure 2). Statistical analysis and Chart box - whisker of rapid progressive sperm were shown in Table 2 and showed in figure 3 ( $t$ -test and one-way ANOVA;  $P < 0.05$ ). Three experimental groups compared with the control group showed no significant difference at  $P < 0.05$ , [Experimental group 1 (hydroalcoholic-*elaegnus angustifolia* extract): ( $P < 0.05 = 2.078e-007$ ),

experimental group 2 (Wi-Fi radiation):  $P < 0.05 = 2.106e-007$ , and experimental group 3 (hydroalcoholic-*elaegnus angustifolia* extract + Wi-Fi radiation): ( $P < 0.05 = 2.521e-006$ )]. Also, There was a significant difference between experimental (group I, II, III) and control Wi-Fi, [Experimental group 1 (hydroalcoholic-*elaegnus angustifolia* extract): ( $P < 0.05 = 2.960e-005$ ), experimental group 2 (Wi-Fi radiation):  $P < 0.05 = 2.243e-005$ , and experimental group 3 (hydroalcoholic-*elaegnus angustifolia* extract + Wi-Fi radiation): ( $P < 0.05 = 1.124e-004$ )]. Showed in figure 4, categorization of change in Experimental group (hydroalcoholic-*Elaegnus Angustifolia* extract + Wi-Fi radiation) compared with the control and Wi-Fi radiation.

**Table 1.** Statistical analysis of standstill progressive sperm in different groups

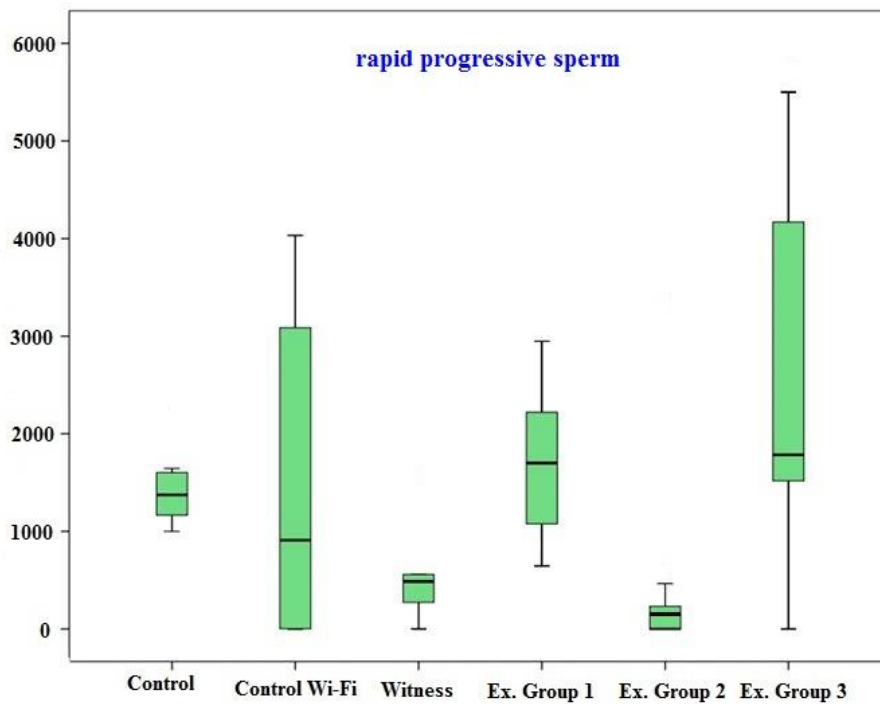
	Control	WiFi Control	Witness	Experimental group 1	Experimental group 2	Experimental group 3
Valid	10	10	10	10	10	10
Missing	0	0	0	0	0	0
Mean	39	26	28	29	36	24
Median	38	27	28	27	34	25
Error of mean	4.5	2.4	2.1	1.7	7.6	6.4
Variance	20.25	5.8	4.4	2.9	58	41
Minimum	26	21	21	18	19	17
Maximum	52	32	33	36	58	32
Rande	26	21	22	18	39	15



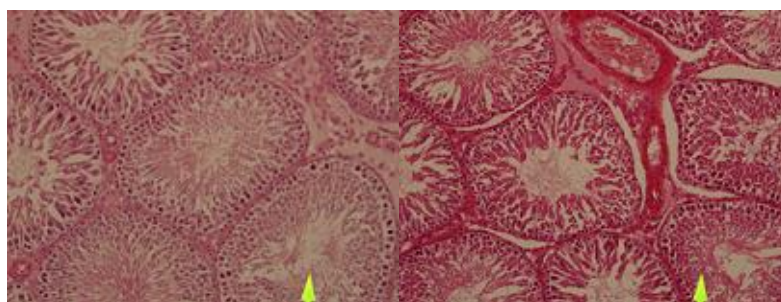
**Figure 2.** Chart box - whisker for the percentage of sperm with no movement in the different groups.

**Table 2.** Statistical analysis of rapid progressive sperm in different groups.

	Control	WiFi Control	Witness	Experimental group 1	Experimental group 2	Experimental group 3
<b>Valid</b>	10	10	10	10	10	10
<b>Missing</b>	0	0	0	0	0	0
<b>Mean</b>	13.32	16.06	5.29	16.90	4.07	24.24
<b>Median</b>	14.1	15.7	6.1	16.8	6.2	22.2
<b>Error of mean</b>	2.20	8.28	1.92	2.36	3.33	6.77
<b>Variance</b>	4.84	68.56	3.68	5.57	11.08	45.83
<b>Minimum</b>	10.7	11.2	4.9	13.5	3.2	18.8
<b>Maximum</b>	22	19.4	7.2	20.5	8.1	29.3
<b>Rande</b>	11.3	8.3	2.3	7	4.9	10.5



**Figure 3.** Chart box - whisker for the percentage of sperm with rapid move in different groups.



**Figure 4.** Experimental group (hydroalcoholic-Elaeagnus Angustifolia extract + Wi-Fi radiation) compared with the control and Wi-Fi

**DISCUSSION**

Electromagnetic waves can lead to infertility or fertility decline in a person and since the use of these radiations such as Wi-Fi that is a microwave radiation, is growing to higher levels, we decided to do a comparative study in this field. Nowadays the most current Wireless

technology uses 802.11.g protocol which is used in many wireless electronic systems. This system includes small video monitors, Wi-Fi mobile phones, and TV devices. The 802.11.g radio device has unique specifications in the frequency range, output power and exposure time which may have negative effects on human

health. This device uses 22100 MHz of bandwidth which is higher than the frequencies used by GSM (Global System for Green Communications) or 3G mobile phones (third generation mobiles)[25, 26].

From the RF EMR (Radio frequency electromagnetic radiation) and the number of the testicular tissue, the result of this study is that these reproductive organs are more sensitive against the radio waves than other organs. Previous studies on animals have had no significant impact on the sperms, their shapes and morphology. Carnie and Harding investigated the influence of RF (Radio frequency) on mice, and no significant difference were observed in sperm numbers and no increase was found in the percentage of abnormal sperms (in terms of morphology) in animals exposed to microwaves [27].

Ono and his colleagues could not find any mutagenesis effect from the testis of a mouse that its whole body was exposed to radio waves with 2.45Gh of frequency[28]. Since we suspected that the continuous exposure to Wi-Fi devices has a more significant impact than exposure to radiation from mobile phones, we used longer time and more frequent irradiation. It is possible that this situation does not reflect real life, because in our daily life, the duration and intensity of exposure to radio waves is more variable. Since the modern human involvement with technology (Wireless modems, Wireless strategies, home video systems, Wi-Fi mobile phones and so on)exposed many children and adults to radio waves, so this level of exposure to these waves is comparable with the levels observed in this study. According to the results obtained in this study on sperm balance, no significant difference was found between control and experimental groups and the results were consistent with previous studies. However, a significant difference was found in sperm motility between experimental and control groups that were inconsistent with previous results. Oxygen Species is a clear and obvious reason for male infertility and cancer. Reactive oxygen species (ROS) from Spermatozoa and invading leukocytes can cause infertility by the effects on sperm motility. In physiological terms of, ROS is kept at a low level by antioxidant mechanisms of by intracellular Spermatozoa [29].

So the use of *Elaeagnus Angustifolia* extract due to the antioxidant properties can help to

improve the effects of radiation. The results of this study also confirm this theory because in the experimental group 3 which had received the extract with the radiation at the same time, a significant difference was observed in sperm motility in comparison with experimental group 2 and also recovery in motility of fertile sperms. According to previous research regarding the effects of electromagnetic fields in the destruction of the mitochondria, the cell would not be able to provide energy for its activities and on the other hand, damage of the Golgi system by the electromagnetic fields and endoplasmic reticulum, leads to changes in the secretive features of these cells. All of these cases disrupted ladies cells's function and it cannot produce enough testosterone [30, 31, 32]. With regards that until now no research has been done on the impact of Wi-Fi signals on testosterone, the present study showed that there was no significant difference between the studied groups. The increase in cells apoptosis, damaged cells in the testes, spleen, liver, marrow and pancreas have been reported after irradiation for 4-16 hours. In their study in 2012, Halil and et al, histological examinations of the experimental and control groups which received Wi-Fi, were not significantly different [33].The obtained results in the present study were consistent with previous research. Also use of *Elaeagnus Angustifolia* extract made no significant difference between the tested groups (Figure2) However, the previous study on ionizing radiation had shown that this extract restrains the superoxide and hydroxyl radicals [23].

The existence of polyphenols and Tannins might be responsible for the absorption of free radicals induced by different irradiations, which protects spermatogonial cells and differentiate the cells against irradiation damage. *Elaeagnus Angustifolia* extracts treatment causes chromatin compaction that minimizes the damage caused by free radicals and thus protects stem cells from death due to irradiation. The stem cells may be stimulated by certain compounds found in the *Elaeagnus Angustifolia* to be reproduced [34].

Use of *Elaeagnus Angustifolia* before irradiation leads to radioactive protection and attraction of various types of free radicals by Tannins and other polyphenols, also various antioxidants have been reported in different articles [35].

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