

Effects of cell phone radiation on testosterone levels and testicular changes in rats treated with garlic (*Allium sativum* L.) hydroalcoholic extract

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ABSTRACT

Electromagnetic waves can damage sex organs and cause hormonal disorders, and garlic is known to reduce risk factors for various diseases. The aim of this study was to investigate the probable effects of mobile phone radiation and the consumption of garlic on testosterone levels and testicular changes. Five groups of rats were used: control, sham (exposed to 900 MHz wavelength), experimental group 1 (receiving garlic extract), and experimental groups 2 and 3 (receiving both extract and microwaves). After a month, the rats were weighed and their serum testosterone levels were measured. The testes were also removed, weighed, and their probable histological changes were studied. The mean body weight in the sham group showed a significant decrease, whereas an increase was seen in experimental group 3 compared with the sham ($P < 0.05$). Mean plasma testosterone levels in experimental groups 2 and 3 were decreased. Similarly, a decrease was seen in the number of Leydig cells in the testes of experimental groups. Although microwaves can cause weight loss, the presence of allicin and vitamins A and B in garlic can compensate for some of this weight loss. Microwaves and garlic extract are reflected both in the number of Leydig cells and in serum testosterone concentration. These changes might be attributed to the induction of heat, non-specific stresses, and apoptosis. However, some of these overlapping effects are synergistic and others are antagonistic; thus, garlic consumption is not always beneficial in reducing the deleterious effects of cell phones.

Keywords: De electromagnetic wave, garlic, mobile phone, Testis, testosterone.

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Introduction

Since the use of cell phone technology has grown throughout the world in recent years, the tendency for determining its potential harmful impacts on human health has also increased significantly (1). The spectrum of electromagnetic (EM) waves is very wide, but microwave frequency and wave length range from 300 MHz to 300 GHz and 1 mm to 1 m, respectively (2, 3, 4, 5). Microwave spectrum is used in mobile phones, and the mean frequency they emit is about 900 MHz to 1 GHz (2, 3, 5, 6).

Skull exposure to microwave radiation may damage the central nervous system and the hypothalamus-pituitary axis, while direct exposure of the abdomen, pelvis, or spine might affect the gonads, leading to interference in the function of sex hormones and infertility. Gonads are very sensitive to electromagnetic waves, and depending on the amount of radiation, reproductive activities can be temporarily or permanently affected (7, 8, 9).

The evidence for the harmful effects of mobile phone on fertility are still ambiguous, and the biological effects of EMF emitted from these devices are controversial as well (10). In a study on the histopathology of testes exposed to RF fields, Ozguner *et al.* (2005) reported a decrease in testosterone levels (11). The effects of GSM (global system for mobile communications, originally Group Special Mobile) 1800 MHz radiation on serum testosterone levels, the steroidogenic capacity of Leydig cells, and the histology of reproductive organs have been investigated, and results showed higher levels of testosterone with no histological changes in the organs examined (12). Similarly, in a 2010 research on the effects of 950 MHz electromagnetic field on the adrenal and sex organs of male rabbits, abnormalities were found in the levels of testosterone and

FSH which were likely to affect reproductive functions (10).

Recent scientific studies have focused on the use of plant products as therapeutic agents (13). Garlic is one of these plant products, traditionally used for its cytotoxic, antitumor, antifungal, antibacterial, antiviral, and antiprotozoal properties (13).

As a member of the *Liliaceae* family, *Allium sativum*, or garlic (14), contains various substances including minerals, carbohydrates, proteins, fats, and vitamins (15, 16, 17). The vitamins found in garlic include A, various kinds of B, such as riboflavin, thiamine, nicotinic acid, and vitamins C and E. Studies suggest that the biological and pharmacological effects of garlic are mainly due to its sulfur compounds (16, 17, 18, 19), including aliin, allicin, ajoene, allylpropyl disulfide, diallyltrisulfide, sallylcysteine, vinyl dithiines, S-allylmercaptocystein, and others (13, 17, 19).

In recent years, there has been a tendency among researchers to attempt to treat disorders by replacing chemical drugs with natural plant components, because of the high costs of drugs, their potential side effects, and restrictions in their use (20). Although cell phone use is widespread and some of its inevitable deleterious effects on the body have been documented, no attempt has been made to reduce these effects through diet or use of herbs. Since garlic has some health benefits and microwaves have some adverse effects on the same area of human health, this study investigates the effects of cell phone radiation and the consumption of hydroalcoholic extract of garlic on testes. The importance of fertility and the vital role of testes in reproduction as well as the ubiquity of cell phone use are sufficient enough to urge that such studies be

performed and mobile phones be made safer.

Materials and Methods

This is a laboratory and completely randomized research. This study was approved by the Committee of Ethics of Shahid Sadoughi University of Medical Sciences. The soaking method (maceration) was used to prepare garlic extract (21, 22). 40 wistar rats with an average body weight of 200 ± 10 g and aged 80 to 90 days were used in this study. In order to adapt to the new environmental conditions, all animals were kept in the Animal House of Kazeroon Islamic Azad University for one week before entering the trial. They were placed in special cages under standard conditions of $23-25^{\circ}$ C and a 12/12-hour light-dark cycle. The rats had unlimited access to food and water, and all moral principles regarding the use and treatment of animals were taken into consideration.

Animals were randomly divided into 5 groups of 8 including the control (left untreated), sham group (exposed to wavelengths of 900 MHz), experimental group 1 (receiving 400mg/kg garlic extract), experimental group 2 (receiving 200mg/kg extract plus 900 MHz waves), and experimental group 3 (receiving 400mg/kg extract plus 900 MHz waves). Groups

receiving radiation were exposed 12 times a day, each time for a duration of 10 minutes. A Nokia 1200 cell phone was used to make EMF and cages were surrounded by aluminum foil to focus waves and limit the electromagnetic field to the interior of the cages. During wave exposure, the cell phone was set in different modes, including call, missed call, and turned-on mode (without real talk). After the first round of irradiation, animals of experimental groups 2 and 3 received the extract followed by 11 rounds of daily exposure.

At the end of the experiment (one month duration), the animals were weighed, blood samples were collected, and serum levels of testosterone were measured using ELISA kits (Biosouece Europe). Testes were also removed, prepared using the classical method of *hematoxylin* and *eosin* staining, and studied by light microscope. The results were examined by SPSS software and ANOVA/Tukey tests, and the significant difference was set at <0.05 ($P < 0.05$).

Results

Results of body weight and serum testosterone levels are shown in Table 1. As seen, a significant decrease in the mean body weight of the sham group and an increase in that of experimental group 3 occurred (Figure 1 and Table 1).

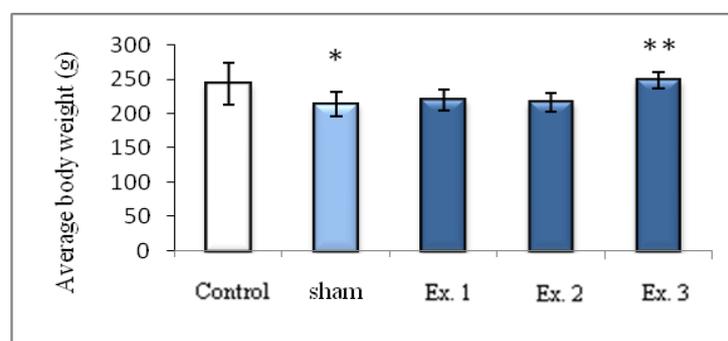


Figure 1. Mean body weight differences among various groups exposed to cell phone radiation and/or received garlic extract. * significant difference.

Table 1. Mean body weight and serum levels of testosterone in different groups.

Groups	Weight (g; \pm SE)	Testosterone (nmol/l; \pm SE)
Control	243.7 \pm 30.44	5.1 \pm 0.17
Sham	217.7 \pm 17.67 *	4.7 \pm 0.12
Experimental 1	220.5 \pm 15.13	4.8 \pm 0.16
Experimental 2	216.5 \pm 14.07	4.1 \pm 0.36 *
Experimental 3	248.7 \pm 12.17 **	4 \pm 0.1 *

Difference with control: Statistical significance ($P < 0.05$) as compared to control (*) or as compared to sham treated (**).

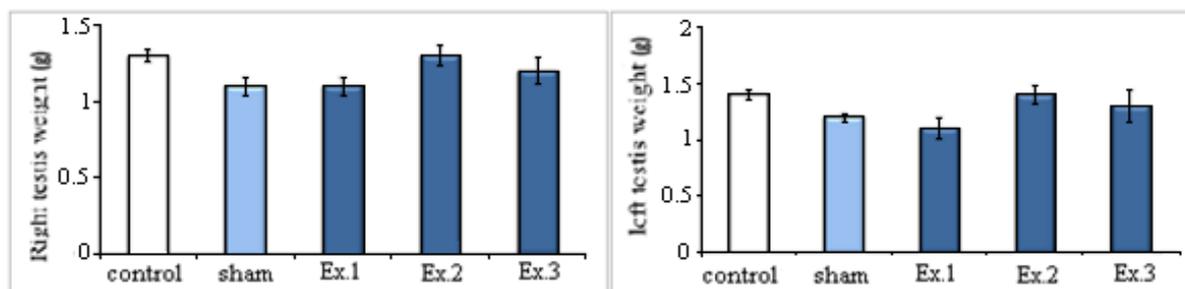


Figure 2. Mean testicular weight differences among various groups exposed to cell phone radiation and/or received garlic extract.

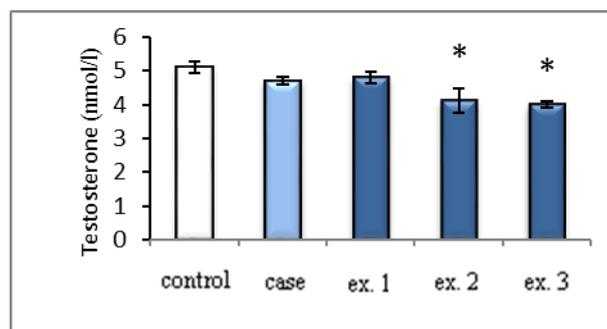


Figure 3. Mean serum levels of testosterone hormone among various groups exposed to cell phone radiation and/or received garlic extract. * indicates a significant difference.

No significant differences in the mean testicular weights of the various groups were noted (Figure 2). Mean serum levels of testosterone in experimental groups 2 and 3 showed significant decreases compared with the control (Figure 3 and Table 1). Finally, Figure 4 shows the morphological changes in testicular tissues observed among different groups. As seen, the number of Leydig cells and sperm counts in groups receiving the extract and exposed to microwaves were reduced (Figure 4).

Discussion

Since mobile phones are generally held and used close to the body, they are considered the main source of EM radiation to which an average person is exposed. In fact, the whole body could act as an efficient antenna for absorbing EM radiation. Thus, the signals transmitted by a cell phone can reach all parts of the body, penetrate the living tissue, and influence the body at the cellular level (10).

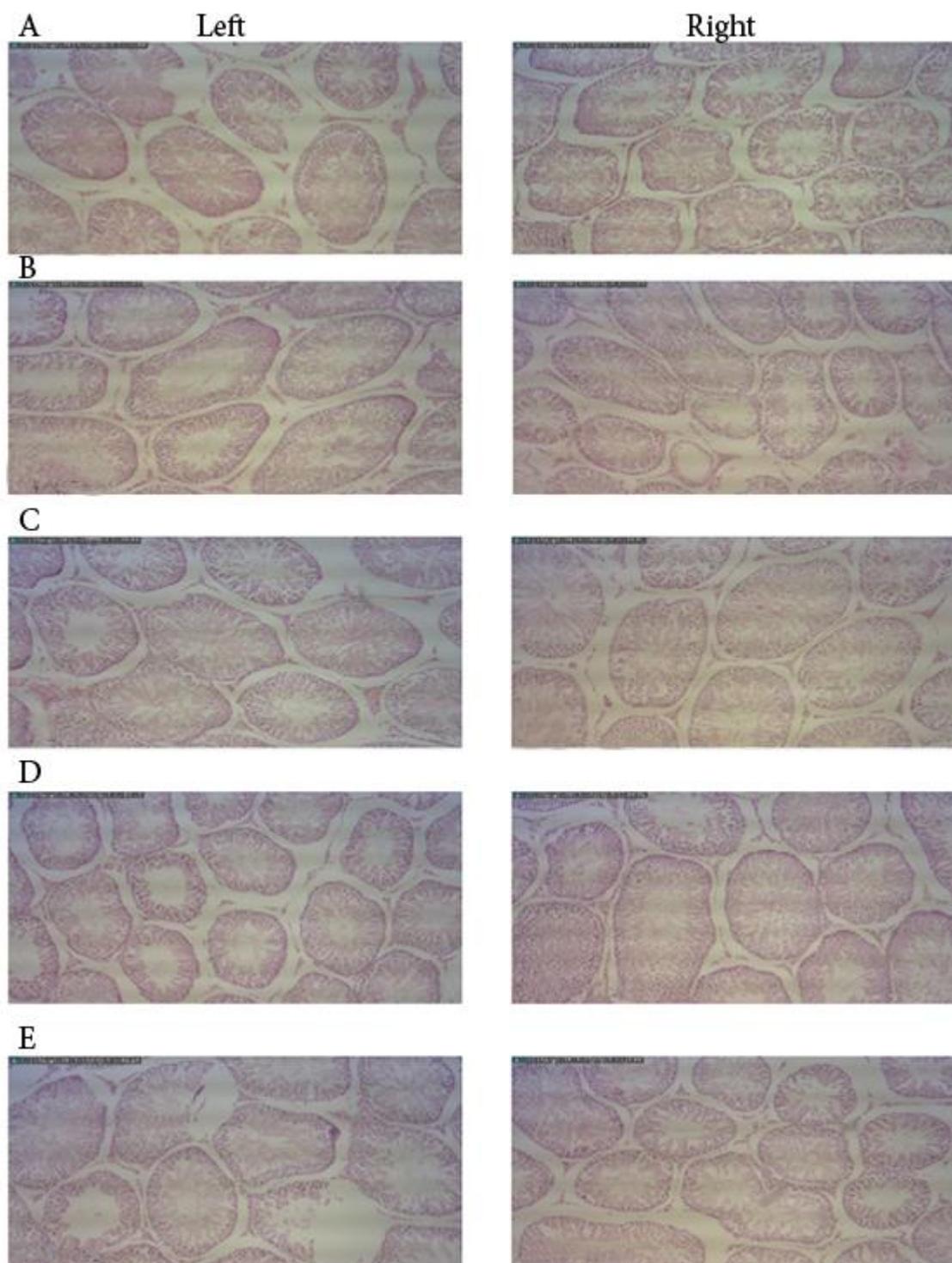


Figure4. Cross sections of testicular tissues in different groups exposed to cell phone radiation and/or received garlic extract. A: control, B: sham (exposed to wavelength of 900 MHz), C: experimental 1 (receiving 400mg/kg garlic

Our study showed that EM waves can cause weight loss and that the administration of garlic extract, especially in high doses, can prevent weight loss caused by radiation (Table 1 and Figure 1). These results are in agreement with the results of Atilla Ilhan et al. (2004), who showed that exposing rats to microwave frequencies at 900 MHz (used in cell phones) caused weight loss. According to their findings, exposure to microwave frequencies can cause oxidative stress in animals and decrease their antioxidant activities, leading to weight loss (23). The study of Lotfi et al. (2009) indicated that plasma cholesterol and triglyceride levels declined in rodents following their exposure to 900 MHz radiation emitted from cell phones leading to weight loss. Their results agree with the results of the present study (24).

Magnetic fields (MFs) can enhance fat breakdown and glycogenesis (25). They can also increase body metabolism, body temperature, and sweat gland activity (26). The adverse effect of microwaves on cell division might be another reason for weight loss (3).

As seen in Table 1 and Figure 1, garlic extract (especially in high doses) can prevent weight loss caused by microwaves. Although it has been suggested that garlic influences body weight through lowering cholesterol and lipids levels, consumption of this plant does not necessarily lead to weight loss (27). The presence of the vitamin B family (especially thiamine) in garlic can stimulate the appetite (19; 28), and vitamin B6 helps the body to convert carbohydrates and fat into energy (28). It facilitates digestion and activates stomach acid secretion.

Moreover, garlic contains vitamin A (29), which is considered an important growth factor in animals as its absence in mice can lead to stunted growth and weight loss. This

vitamin can be converted to retinoid that can induce fat storage in the form of triglycerides and cause weight gain (30). Similarly, it has been reported that the consumption of garlic juice can inhibit body weight reduction in diabetic rats (31). Thus, one can conclude that the presence of vitamins A and B in garlic can compensate for some of the weight lost caused by exposure to radiation.

The effects of low frequency electromagnetic waves on gonads and fertility have been studied by many researchers. Some of their reports are indicative of reduced fertility, impaired spermiogenesis, and a reduction in the number of live fetuses in rats (2; 32; 33; 34). Based on the results of this study, microwave radiation can have harmful effects on testes which are reflected in their structure and testosterone levels (Figures 3 and 4). The effect of microwaves on testosterone levels can be related to their potential influence on the secretion of melatonin from the pineal gland (35). In his report, Sultan (2010) stated that mobile phone radiation can cause a decrease in serum testosterone concentration (36). Also, in their study, Jelodar *et al.* (2008) reported that the mean testosterone level in mice exposed to radiation leaked from microwave ovens had declined (37). They stated that the decrease in testosterone concentration could be due to the effects of radiation on Leydig cells, the pituitary, or the hypothalamus and an alteration in gonadotropin secretion (37).

Under the influence of corpus luteum hormone, Leydig cells secrete androgen. The deleterious effects of microwaves on Leydig cells are not only functional, but they cause structural and pathological changes as well (Figure 4). Apoptosis associated with nuclear damage to these cells can lead to a decrease in testosterone production (38).

Moreover, the decline in serum testosterone

levels may be due to the absence of Leydig cells in sperm producing tubules, resulting from a decrease in the LH level, because LH is responsible for the differentiation of mesenchymal cells into Leydig cells (39). This result agrees with that of Ozguner *et al.* (2005), who showed that the physiological and morphological impacts of cell phones on testes caused disorders in the seminiferous epithelium and decreases in germinal epithelium and testosterone levels (11). Conversely, Ozguner (2002) in his study on testes found that EMF causes Leydig cell proliferation, an increase in testosterone levels and testicular weight, and the lowering of testicular germ cells (40). These results are consistent with our findings.

Ebomoyi *et al.* (2010) reported that garlic consumption can significantly reduce serum testosterone levels (42, 41). Based on this study, garlic can inhibit the manufacture of steroids in two ways: (a) by interfering in the transfer of free cholesterol to the mitochondria of Leydig cells, which is an important step in steroidogenesis, and (b) by disrupting the conversion of cholesterol to testosterone by affecting the activity of key regulatory enzymes in steroidogenesis. Furthermore, green garlic can damage Leydig cells, which are responsible for the secretion of testosterone (43, 41).

Likewise, garlic causes a dose-dependent decrease in plasma and intratesticular testosterone concentrations in rats, and Leydig cells are known to be its target (44). Since testosterone protects germ cells (especially spermatocytes and spermatid) against apoptosis (45,46), its decline after garlic consumption may explain the apoptosis of spermatid and spermatogonia cells by an induced cell death process. While garlic extract is known to lower blood cholesterol levels (in both humans and animals) and

inhibit cholesterol biosynthesis (47), the production of testosterone is not related to cholesterol metabolism (44); rather, it is dependent on the modulation of steroidogenic enzymes. For example, the conversion of cholesterol to biologically active testosterone is a multistep enzyme process (including enzymes that control the transfer of cholesterol from outer to inner mitochondrial membranes) (48).

The consumption of raw garlic has inhibitory effects on the expression of steroidogenic enzymes and markers in Leydig and Sertoli cells, respectively. These changes may induce cell death in germ cells (spermatogonia and spermatid) through apoptosis (49).

According to our results, there were no significant differences in testicular weight among the various groups (Figure 2). Similarly, in 2006, Al-Akhras assessed the effects of EMF with an intensity of 25 mT 50 Hz on reproductive variables and sex hormones in rats and found no significant decrease in testicular weight (50). Although we didn't count sperm in this study, a reduction in the number of sperm was apparent in our micrographs, and this could be a topic for future research.

Conclusions

This study showed that cell phone radiation can cause weight loss by increasing metabolism, fat breakdown, body temperature, and sweat gland activity and that the presence of compounds in garlic, such as thiamine, vitamin A, and organic sulfur, could be used to compensate for some of this weight loss. On the other hand, it was revealed that both microwaves and garlic can change testicular structures considerably, particularly Leydig cells and serum levels of androgens. Even though microwave radiation affects various

tissues via induction of heat generation and temperature rise, the harmful effects of excessive cell phone use are more pronounced in males, because the testes are more temperature sensitive. In addition, garlic extract cannot prevent all hormonal changes in males caused by microwaves.

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Conflict of interest

There is no conflict of interest.

This article isn't under consideration anywhere else in any form, and we have no obligations to anyone or any institutions. Since this article is based on the results of the master thesis of Behnaz Hajioun, all funding was provided by this individual. All experiments were carried out in the labs of KAU. Thus, there are no financial or personal interests that might potentially influence the work presented.

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