

Effects of Garlic (*Allium sativum* L.) Hydroalcoholic Extract on Estrogen, Progesterone and Testosterone Levels in Rats Exposed to Cell Phone Radiation

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Article information	Abstract
<p>Article history: Received: 7 Aug 2013 Accepted: 15 Sep 2013 Available online: 8 Dec 2013 ZJRMS 2014; 16(12): 19-24.</p> <p>Keywords: Electromagnetic wave Estrogen Garlic Progesterone Testosterone Rat</p> <p>*Corresponding author at: Department of Physiology, Kazeroon Branch, Islamic Azad University, Kazeroon, Iran. E-mail: behnazhajiun@gmail.com</p>	<p>Background: The aim of this study was to investigate the probable effects of radiation and consumption of garlic on estrogen, progesterone and testosterone levels.</p> <p>Materials and Methods: In this experimental study, 5 male and 5 female groups of rat were used: control, sham (under exposed), experimental 1 (receiving garlic extract), and experimental 2 and 3 (receiving both extract and microwaves). After a one month, rats were weighed and serum levels of hormones were measured.</p> <p>Results: In male the mean body weight in the sham showed a significant decrease, whereas, an increase was seen in the experimental 3 compared with sham. Also, mean plasma testosterone levels in experimental 2 and 3 were reduced. Estrogen showed this decrease in all groups. Also in all groups progesterone showed increase. In female the mean body weights in different groups showed no significant changes, whereas a significant increase was seen in serum level of progesterone in experimental 2 and 3.</p> <p>Conclusion: Although, microwaves can cause weight lost, presence of allicin and vitamins A and B in garlic can compensate some of this weight lost. Microwaves and garlic extract have fewer effects on female reproductive system, reflected only in the serum progesterone concentration. Also they reflected in the number of Leydig cells and serum testosterone and estrogen concentration. The differences observed in the responses of male and female to cell phone radiation might be attributed to the position of gonads in the body and sensitivity of testis to heat.</p> <p>Copyright © 2014 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

As 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 [2-4]. Microwave spectrum is used in mobile phones and mean frequency they emit is about 900 MHz to 1 GHz [2, 4, 5].

Gonads are very sensitive to electromagnetic waves, and depending on the amount of radiation, they can affect the reproductive activities temporarily or permanently [6-8].

The evidence for the harmful effects of mobile phone on fertility are still equivocal and biological effects of electromagnetic fields (EMF) emitted from these devices are controversial as well [9]. In a study of female rat gonads, Baharara et al. showed that long-term cell phone radiation changes reproductive endocrine system alters the ultra-structure of oocytes and reduces the rate of successful mating [2].

In a study on histopathology of testes exposed to radio frequency field (RF), Ozguner et al. reported a decrease in testosterone level [10].

Similarly, in a research on the effects of 950 MHz electromagnetic field on the adrenal and sex organs of male rabbits in 2010, it was found that there were

abnormalities in the levels of testosterone and FSH, likely to affect reproductive functions [9].

On the other hand, recent scientific studies have been focusing on the use of plant products as therapeutic agents [11, 12]. Garlic is one of these plant products, traditionally used for its cytotoxic, antitumor, antifungal, antibacterial, antiviral and anti protozoal properties [11].

As a member of the Liliaceous family, *Alliums sativum* or garlic [13], contains various substances including minerals, carbohydrates, proteins, fats and vitamins [14-16]. Vitamins found in garlic include vitamin A, various kinds of vitamin B, such as riboflavin, thiamine, nicotinic acid, and vitamins C and E. Among many different compounds found in garlic, studies suggest that biological and pharmacological effects of this plant are mainly due to its sulfur compounds [15-18]. Some of these sulfur compounds are aliin, allicin, agoene, allylpropyl disulfide, diallyltrisulfide, sallylcysteine, vinylidithiines, S-allylmercaptocystein, and others [11, 17, 18]. Because of their high costs, their potential side effects and restrictions of their use, in recent years, there has been a tendency among researchers in attempting to treat disorders by replacing chemical drugs with some natural plant components [12]. Although, cell phone use is wide spread and some of their inevitable deleterious effects on the body have been documented, there has been little attempt 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, in this study we tried to investigate the effects of cell phone radiation along with the consumption of hydro alcoholic extract of garlic on reproductive system. These studies offer the potential to enhance our understanding of garlic and the optimal use of cell phones.

Materials and Methods

In this experimental study, soaking method (Maceration) was used to prepare garlic extract [19]. Sixty Wistar rats with average body weight of 200 ± 10 g and 80 to 90 days old were used in this study. In order to adapt to new environmental condition all animals were kept in the Animal House of Kazeroon Islamic Azad University for 1 week before entering into the trial. They were placed in special cages under standard conditions of $23-25^{\circ}\text{C}$ and 12 h of light and 12 h of dark cycle. They had unlimited access to food and water, and all moral principles on using and treating animals were taken into consideration.

Animals were randomly divided into 5 male and 5 female groups of 6, including control (left untreated), sham group (exposed to wavelength of 900 MHz), the experimental group 1 (receiving 400 mg/kg garlic extract), experimental group 2 (receiving 200 mg/kg extract plus 900 MHz waves), and experimental group 3 (receiving 400 mg/kg extract plus 900 MHz waves). Groups receiving radiation were exposed 12 times a day, each time 10 min. 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 sat in different modes, including call, missed call and turn 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 (lasting a month), animals were weighed, blood samples were collected and serum levels of estrogen, progesterone and testosterone were measured using ELISA kits (Made by Biosouece Europe). The results were examined by SPSS-19.0.1 software and ANOVA/ Tukey tests and the significant difference was sat at <0.05 .

Results

Results of body weight and serum estrogen, progesterone and testosterone levels are shown in table 1. In male there is a significant decrease in the mean body weight of sham group and an increase in the experimental group 3 in respect to the sham group ($p < 0.01$) (Table 1). Also, there were no significant differences in the mean testicular weights of various groups (Fig. 1). As seen, in female there are no significant differences in the mean body weights of various groups (Table 1).

In addition, in male mean serum levels of testosterone in the experimental groups 2 and 3 showed a significant decrease compared to control ($p < 0.01$). But there were no significant differences in serum levels of testosterone in female (Table 1). In male, mean serum levels of estrogen in the sham group and experimental groups 1, 2 and 3 showed a significant decrease compared to control; and in female this decrease in experimental groups compared to the control was no significant ($p < 0.01$) (Table 1). In contrast, in male rats mean serum levels of progesterone in the sham group and experimental groups 1, 2 and 3 were significantly increased, and in female mean serum of progesterone compared to the control increase in group experimental 2 and 3 ($p < 0.01$) (Table 1).

Table 1. Results of body weight and serum estrogen, progesterone and testosterone levels in different groups

Groups	Weight (g)		Estrogen (ng/mL)		Progesterone (ng/mL)		Testosterone (nM/L)	
	Male	Female	Male	Female	Male	Female	Male	Female
Control	243.7 \pm 30.44	227.3 \pm 5.23	0.44 \pm 0.02	1.53 \pm 0.08	3.3 \pm 0.11	10.8 \pm 0.1	5.1 \pm 0.17	0.85 \pm 0.01
Sham	217.7 \pm 17.67*	228.5 \pm 3.33	0.35 \pm 0.01*	1.48 \pm 0.09	3.7 \pm 0.06*	11.5 \pm 0.2	4.7 \pm 0.12	0.82 \pm 0.02
Ex. 1	220.5 \pm 15.13	219 \pm 6.32	0.33 \pm 0.01*	1.28 \pm 0.06	4 \pm 0.06*	11.3 \pm 0.14	4.8 \pm 0.16	0.78 \pm 0.01
Ex. 2	216.5 \pm 14.07	215.6 \pm 6.79	0.33 \pm 0.02*	1.4 \pm 0.08	3.9 \pm 0.09*	11.9 \pm 0.37*	4.1 \pm 0.36 *	0.79 \pm 0.01
Ex. 3	248.7 \pm 12.17**	244.8 \pm 3.56	0.33 \pm 0.01*	1.23 \pm 0.04	4 \pm 0.01*	11.8 \pm 0.28*	4 \pm 0.1 *	0.87 \pm 0.02

*mean \pm SE: Difference with control. * indicates a significant difference, ** $p < 0.05$: Difference with sham. Ex: experimental group.
Ex: Experimental group

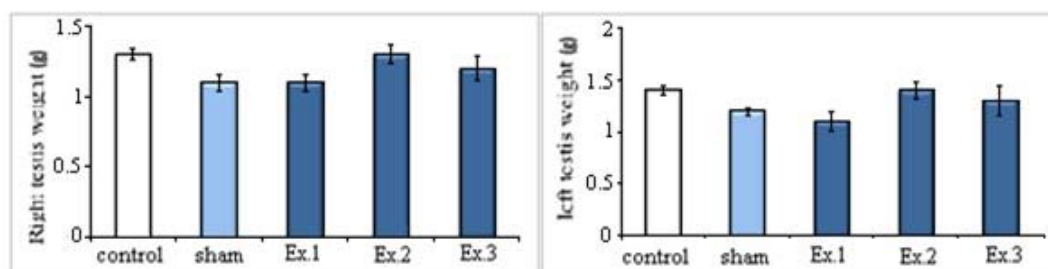


Figure 1. Mean testicular weight differences among various groups exposed to cell phone radiation and/or received garlic extract

Discussion

We showed that, in male the mean body weight in the sham showed a significant decrease, whereas, an increase was seen in the experimental 3 compared with sham. Also, mean plasma testosterone levels in experimental 2 and 3 were reduced. Estrogen showed this decrease in all groups. Also in all groups progesterone showed increase. In female the mean body weights in different groups showed no significant changes, whereas a significant increase was seen in serum level of progesterone in experimental 2 and 3. Since mobile phones are generally held and used close to the body, they are considered as the main source of EM radiation that an average person is exposed to. In fact, the whole body could act as an efficient antenna for absorption of EM radiation. Thus, the signals transmitted by a cell phone can reach all parts of the body and penetrate into the living tissues, and influence the body at the cellular level [9].

Our studies show that male and female rats respond differently to EM waves, and that males appear more sensitive than females. According to our results, exposure of female rats to cell phone radiation has no significant effect on body weight (Table 1), while this treatment can cause weight loss in male rats and administration of garlic extract, especially high dose, can prevent weight loss caused by radiation (in the press). This is in agreement with the results of Ilhan et al. who showed exposure of rats to microwave frequencies at 900 MHz (used in cell phones) can cause 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 [20].

The study of Lotfi and Aghdam-Shahryar indicates that plasma cholesterol and triglyceride levels decline in male rodents following exposure to 900 MHz radiation emitted from cell phones leading to weight loss [21]. The observed weight loss in males might be related to the decline in testosterone level. This hormone is involved in weight gain, especially in the production of muscle [22]. Indeed, testosterone and/or its derivatives are used in body building by athletes [23]. As a probable site for deleterious effects of microwaves, hormone testosterone is almost absent in females. Magnetic fields (MFs) can enhance fat breakdown and glycogenesis [24]. It also increases body metabolism, body temperature and activity of the sweat glands [25]. Moreover, as seen in table 1, garlic extract (especially high dose) can prevent the weight loss caused by microwaves. Although, it has been suggested that garlic influences body weight through lowering of cholesterol and lipids, consumption of this plant does not necessarily lead to weight loss [26]. Presence of vitamin B family (especially thiamine) in garlic can stimulate the appetite [18, 27] and vitamin B6 helps the body to convert carbohydrates and fat into energy [27]. It makes digestion easier and activates stomach acid secretion.

In addition, garlic contains vitamin A [28] which is considered an important growth factor in animals, and 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 [29]. Similarly, it has been reported that consumption of garlic juice can inhibit body weight reduction in diabetic rats [30]. Thus, one can conclude that presence of vitamins A and B in garlic can compensate some of the weight lost caused by exposure to radiation.

On the other hand, the effects of low frequency electromagnetic waves on gonads and fertility have been studied by many researchers. Some of these reports are indicative of reduced fertility, impaired spermatogenesis and reduction in the number of live fetuses in rats [2, 31-33].

Based on results of this study, microwave radiation can have harmful effects on testis which are reflected in its structure and testosterone level (Table 1). The effect of microwaves on testosterone level can be related to their potential influence on secretion of melatonin from the pineal gland [34]. In his report, Meo et al. stated that mobile phone radiation can cause a decrease in serum testosterone concentration [35]. Also, in their study, Jelodar et al. reported that the mean testosterone level is reduced in mice exposed to radiation leaked from microwave ovens [36]. They stated that the decrease in testosterone concentration could be due to the effects of radiation on Leydig cells, pituitary or hypothalamus and alteration of gonadotropin secretion [36].

Under the influence of corpus luteum hormone, Leydig cells secrete androgen. The deleterious effects of microwaves on Leydig cells are not only functional, but rather, they cause structural and pathological changes as well (Table 1). Apoptosis associated with the nuclear damage of these cells can lead to a decrease in testosterone and estrogen production [37].

Moreover, the decline in serum testosterone level may due to the absence of Leydig cells in sperm producing tubules, resulted from a decrease in the LH level, because LH is responsible for the differentiation of mesenchymal cells into Leydig cells [38]. This is in agreement with Ozguner et al. that showed physiological and morphological impacts of cell phones on testis cause disorders in somniferous epithelium, and a decrease in germinal epithelium and testosterone level [10]. Conversely, Ozguner et al. in his study on testis found that EMF causes Leydig cell proliferation, increase in testosterone level and testicular weight, and lowering of testicular germ cells [39]. These results are in consistence with our findings.

Ebomoyi et al. reported that garlic consumption can significantly reduce serum testosterone level [40]. Based on this study, garlic can inhibit the manufacture of steroids in two ways: (a) interference in the transfer of free cholesterol to mitochondria of Leydig cells, which is an important step in steroid genesis, and (b) disrupting the conversion of cholesterol to testosterone by affecting the activity of key regulatory enzymes in steroid genesis. Also, green garlic can damage Leydig cells, which are

responsible for secretion of testosterone and estrogen [40-42].

Likewise, garlic causes a dose-dependent decrease in plasma and intra testicular testosterone concentrations in rats, and Leydig cells are known as its target [43]. Since testosterone protects germ cells (especially spermatocytes and spermatid) against apoptosis [44, 45], its decline by garlic consumption may explain the apoptosis of spermatid and spermatogonia cells by an induced cell death process. While garlic extract is known as a lowering factor in blood cholesterol levels (in both human and animal) and inhibits cholesterol biosynthesis [46], the production of testosterone is not related to cholesterol metabolism [43], 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) [47].

Consumption of raw garlic has Inhibitory effects on the expression of steroidogenic enzymes and markers in Leydig and Sterol cells respectively. These changes may induce cell death in germ cells (spermatogonia and spermatid) through the process of apoptosis [41].

On the other hand, the effects of microwaves on female reproductive system are less clear. According to the results of this study, cell phone radiation can elevate progesterone level, while estrogen concentration remains unaffected (Table 1). This is partly in agreement with the study of Baharara who investigated the levels of estrogen and progesterone in female rats exposed to radiation, and showed reduction in the serum concentration of progesterone and no change in estrogen level [2]. Hemayatkah-Jahromi et al. also reported that FSH, estrogen and progesterone levels rise after exposure to radiation [4].

These differences are probably due to the differences in the radiation dose, conditions and the way in which waves are administered. It seems that radiation emitted from mobile phones can alter levels of FSH, LH and progesterone through influencing CNS (especially hypothalamus) and changes in the secretion of gonadotropin-releasing hormone (GnRH) [2, 4].

Huuskonen noticed no significant changes in the levels of estrogen and progesterone after the initial exposure to microwaves [48]. However, he reported reduction in the levels of LH and FSH. This decrease in the levels of FSH and LH can be regarded as the effects of electromagnetic fields on brain and GnRH release [49].

Apparently, garlic extract stimulates the secretion of gonadotropins and ovarian hormones through activation of pituitary gland, promotion of exit from the Golgi cells, cell cycle, and increased ability of binding to estrogen receptors [50]. This might be one reason for the observed increase in progesterone level (Table 1).

The apparent difference in the sensitivity of male and female reproductive systems, particularly gonads to microwaves is probably related to the position of testes and ovaries in the body. While testes are located out of the body within the testicular sac (Scrotum), ovaries are positioned deep in the body within the coelom, protected from the environmental factors [51]. On the other hand, microwaves have long wave lengths and cannot penetrate deep in the body [52]. Hence, ovaries are less accessible to cell phone radiation than testes, and cannot be directly affected by their microwaves. Nevertheless, the observed differences in the sensitivities of testes and ovaries to garlic extract are not clear. Considerable attention should be given to the molecular functioning of the compounds present in garlic, particularly organic sulfur, as new insights on deleterious effects of cell phones will emerge. Such studies shed further light on the molecular functioning of the reproductive system and factors (including microwaves and various food products) effecting it, and provide insight for additional experimentation.

We showed that cell phone radiation can differently affect male and female reproductive systems, and garlic extract can only partially protect males against microwaves. Cell phone radiation can cause weight loss by increasing metabolism, fat break down and sweat gland activity, and presence of compounds, such as thiamine and vitamin A in garlic could be used to compensate some of this weight loss. On the other hand, it was revealed that both microwaves and garlic can considerably change sex hormones. Even though, microwave radiation affects various tissues via induction of heat generation and temperature rise, the harmful effects of excessive use of cell phones is higher in males. These differences are probably due to the location of gonads in the body, because testes are placed out of the body within the testicular sac, and ovaries are positioned within coelom and thus less exposed to cell phone radiation. Thus, it appears that men are more sensitive to microwave irradiation than women.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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