



Effects of cell phone radiation on estrogen and progesterone levels and ovarian changes in rats treated with garlic (*Allium sativum* L.) hydro-alcoholic extract

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ARTICLE INFO

Type: Original Research

Topic: Physiology-Pharmacology

Received August 12th 2013

Accepted September 27th 2013

Key words:

- ✓ Electromagnetic wave
- ✓ Estrogen
- ✓ Garlic
- ✓ Ovary
- ✓ Progesterone

ABSTRACT

Background & Aim: Electromagnetic waves can damage sex organs and cause hormonal disorders, whereas garlic is known as reducing risk factors for various diseases. The aim of this study was to investigate the probable effects of mobile phone radiation and consumption of garlic on estrogen and progesterone levels and ovarian changes.

Experimental: Five groups of rats (n = 8) were used which groups included control, sham (exposed to 900 MHz wavelength), receiving garlic extract, and receiving both extract and microwaves. After a month, rats were weighed and serum levels of estrogen and progesterone were measured. Ovaries were also removed, weighed and their probable histological changes were studied.

Results & Discussion: The mean body weights in different groups showed no significant changes, whereas a significant increase was seen in serum level of progesterone in experimental groups-II and III. Also, there were no observable histological changes in the ovaries of different groups.

Recommended applications/industries: Although microwaves can cause weight lost in mails and presence of allicin and vitamins A and B in garlic can compensate some of this weight lost, this isn't the case in females. In addition, both microwaves and garlic extract have less effect on female reproductive system, reflected only in the serum progesterone concentration.

1. 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 (Ferreri *et al.*, 2006). 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 (Verschaeve & Maes, 1998; Banik *et al.*, 2003; Baharara *et al.*, 2004; Hemayatkhah Jahromi *et al.*, 2012). Microwave spectrum is used in mobile phones and mean frequency they emit is about 900 MHz to 1 GHz (Verschaeve & Maes, 1998; Hyland, 2000; Baharara *et al.*, 2004; Hemayatkhah Jahromi *et al.*, 2012).

Skull exposure to microwave may damage central nervous system, including the hypothalamus- pituitary axis, while direct exposure of 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, they can affect the reproductive activities temporarily or permanently (Rowley *et al.*, 1974; Shalet *et al.*, 1976; Amanda & Stephen, 1993).

The evidence for the harmful effects of mobile phone on fertility are still equivocal and biological effects of electromagnetic field (EMF) emitted from these devices are controversial as well (Sarookhani *et al.*, 2011). Results a study of female rat gonads by Baharara *et al.* (2004) indicated that long-term cell phone radiation changes reproductive endocrine system, alters the ultra-structure of oocytes and reduces the rate of successful mating (Baharara *et al.*, 2004). Moreover, in a study by Ozguner *et al.* (2005) on histopathology of testes exposed to RF field, reported a decrease in testosterone level. In contrast, the effects of GSM (Global system for mobile communications, originally Group Special Mobile) 1800 MHz radiation on serum testosterone level, steroidogenic capacity of Leydig cells and histology of reproductive organs have been investigated and results showed a higher level of testosterone with no histological changes in the organs examined (Forgacs *et al.*, 2006). Similarly, results a research on the effects of 950 MHz electromagnetic field on the adrenal and sex organs of mail rabbits in 2010, it was found that there were abnormalities in the levels of testosterone and FSH, likely to affect reproductive functions (Sarookhani *et al.*, 2011).

On the other hand, recent scientific studies have been focusing on the use of plant products as therapeutic agents (Sarkar *et al.*, 2006). Garlic is one of these plant products, traditionally used for its cytotoxic, antitumor, antifungal, antibacterial, antiviral and anti protozoal properties (Sarkar *et al.*, 2006).

As a member of the Aliaceae family, *Allium sativum* or garlic (Ulbricht *et al.*, 2010), contains various substances including minerals, carbohydrates, proteins, fats and vitamins (Kemper Kathi., 2000; Haciseferogullari *et al.*, 2005; Cobas *et al.*, 2010). 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 (Kemper Kathi., 2000; Khalid & Gordon, 2006; Lanzotti, 2006; Cobas *et al.*, 2010). Some of these sulfur compounds are aliin, allicin, ajoene, allylpropyl disulfide, diallyltrisulfide, sallylcysteine, vinylthiines, S-allylmercaptocystein, etc (Kemper Kathi., 2000; Khalid & Gordon, 2006; Sarkar *et al.*, 2006).

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 (Mahmoodi *et al.*, 2011). 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 female reproductive system in particular, ovaries. The importance of fertility and vital roles of ovaries in reproduction as well as, the ubiquity of cell phone use is sufficient enough to urge detailed studies of the physiology, cell biology, and molecular effects of microwaves. These studies offer the potential to enhance our understanding of garlic and the optimal use of cell phones. Therefore, the goal of present research was to investigate the probable effects of mobile phone radiation and consumption of the extract of garlic on estrogen and progesterone levels and ovarian changes.

2. Material and methods

2.1. Plant material

Soaking method (Maceration) was used to prepare garlic extract (Lachance, 1997; Tataru Marcin *et al.*, 2005).

2.2. Animals

40 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 one week before entering into the trial. They were placed in special cages under standard

conditions of 23-25 °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.

2.3. Treatments

Animals were randomly divided into five groups of eight, including control (left untreated), sham group (exposed to wavelength of 900 MHz), the experimental group-I (receiving 400mg/kg garlic extract), Experimental group-II (receiving 200mg/kg extract plus 900 MHz waves), and experimental group-III (receiving 400mg/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 II and III received the extract followed by 11 rounds of daily exposure.

2.3. Measurements

At the end of the experiment (lasting a month), animals were weighed, blood samples were collected and serum levels of estrogen and progesterone were measured using ELISA kits (Made by Biosouece Europe). Ovaries were also removed, prepared using classical method of hematoxylin and eosin staining and studied by light microscope.

2.4. Statistical analysis

The results were examined by SPSS software and ANOVA/Tukey tests and the significant difference was sat at $P < 0.05$.

3. Results and discussion

Results of body weight and serum estrogen and progesterone levels are shown in Table 1. As seen, there are no significant differences in the mean body weights of various groups (Fig. 1 and Table 1). In addition, mean serum levels of estrogen in the experimental groups showed a decrease compared to control, but this decrease wasn't significant (Fig. 2 and Table 1). Conversely, the levels of progesterone elevated significantly in experimental groups II and III

(Fig.3 and Table 1). Finally, microscopic examination of ovarian sections revealed no visible morphological changes among different groups (Fig. 4). As seen, there are no adverse effects on corpus luteum groups receiving extract and exposed to microwaves (Fig. 4). Since mobile phones are wide spread and generally kept close to the body, they are considered as the main source of EM radiation that an average person is exposed to. Indeed, the whole body can act as an efficient antenna for absorption of EM radiation. Therefore, radiation emitted from a cell phone can reach all parts of the body and penetrate into the living tissues, and influence the body at the cellular level (Sarookhani *et al.*, 2011).

In fact, magnetic fields (MFs) can enhance fat breakdown and glycogenesis (Aghdam shahryar *et al.*, 2009). It also increases body metabolism, body temperature and activity of the sweat glands (Russelreiter, 2007). In addition, the results by Atilla Ilhan *et al.* (2004) indicated that 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 lost (Ilhan *et al.*, 2004). In contrast, our studies indicated that male and female rats respond differently to EM waves, and that mails 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 and Fig. 1), while this treatment can cause weight loss in mail rats and administration of garlic extract, especially high dose, can prevent weight loss caused by radiation (in the press). The study by Lotfi *et al.* (2009) indicates that plasma cholesterol and triglyceride levels decline in mail rodents following exposure to 900 MHz radiation emitted from cell phones leading to weight lost (Lotfi & Aghdam shahryar, 2009). The observed weight loss in mails might be related to the decline in testosterone level. This hormone is involved in weight gain, especially in the production of muscle (Srinivas-Shankar & Frederick, 2009). Indeed, testosterone and/or its derivatives are used in body building by athletes (Te-Chi *et al.*, 2009). As a probable site for deleterious effects of microwaves, hormone testosterone is almost absent in females.

In addition, 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 spermiogenesis and reduction in the number of live fetuses in rats (Soeradi & Tadjudin, 1989; Mevissen & Buntenkotten, 1994; Fernie & Bird, 2000; Baharara *et al.*, 2004). Most of these studies, however, are related to male reproductive functions, and show that microwave radiation can harm testes, reflected in their structures and testosterone level.

In his report, Sultan (2010) stated that mobile phone radiation can cause a decrease in serum testosterone concentration (Sultan *et al.*, 2010). Similarly, in their study, Jelodar *et al.* (2008) reported that the mean testosterone level is reduced in mice exposed to radiation leaked from microwave ovens (Jelodar & Zare, 2008). 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 (Jelodar & Zare, 2008). Moreover, the physiological and morphological impacts of cell phones on testis lead to disorders in seminiferous epithelium, and decrease in germinal epithelium and testosterone level (Ozguner *et al.*, 2005). Conversely, Ozguner (2002) found that EMF causes Leydig cell proliferation, increase in testosterone level and testicular weight, and lowering of testicular germ cells (Ozguner *et al.*, 2002). 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 (Fig. 2 and 3 and Table 1). This is partly in agreement with the study of Baharara (2004) 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 (Baharara *et al.*, 2004). Jahromi *et al.* (2012) also reported that FSH, Estrogen and progesterone levels rise after exposure to radiation (Hemayatkah Jahromi *et al.*, 2012). 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) (Baharara *et al.*, 2004; Hemayatkah Jahromi *et al.*, 2012).

Huuskonen (2001) noticed no significant changes in the levels of estrogen and progesterone after the initial exposure to microwaves (Huuskonen & Saastamoinen, 2001; Baharara *et al.*, 2004). However, they 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 (Hyland, 2000).

In 2010, the effect of garlic oil on the thickness of zygote wall was measured and indicated that garlic oil plays a direct or indirect role in ovarian activity and increases secretion of sex hormones, including estrogen (Kadhim *et al.*, 2012). 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 (Obochi *et al.*, 2009). This might be one reason for the observed increase in progesterone level (Fig. 3).

Based on our results, both garlic and cell phone radiation have no observable effects on ovarian tissues, and primary follicles and corpus luteum appear unchanged in various groups (Fig. 4). These results support the study of Jahromi *et al.* (2012) who stated that the number of primary follicles and corpus luteum show no significant changes after exposure to radiation (Hemayatkah Jahromi *et al.*, 2012). Conversely, microwaves can damage testes, particularly Leydig cells, which are responsible for secretion of testosterone (Carmela, 2004; Jelodar & Zare, 2008). While garlic extract is known as a lowering factor in blood cholesterol levels (in both human and animal) and inhibits cholesterol biosynthesis (Campbell *et al.*, 2001), presence of compounds, such as thiamine and organic sulfur in garlic affect male and female differently. It seems that the production of testosterone and progesterone are not related to cholesterol metabolism (Hammami *et al.*, 2008), rather their levels are dependent on the modulation of steroidogenic enzymes. It has been shown that green garlic can damage Leydig cells, which are responsible for secretion of testosterone (Chakrabarti & Bhattacharyya, 2003; Ebomoyi & Ahumibe, 2010). Likewise, garlic causes a dose-dependent decrease in plasma and intra testicular testosterone concentrations in rats and Leydig cells are known as its target (Hammami *et al.*, 2008). In contrast, garlic extract has no observable pathological effects on ovaries and their secretion (Fig 2, 3, and 4).

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.

4. Conclusion

We showed that cell phone radiation can differently affect male and female reproductive systems, and garlic extract can only partially protect males against microwaves. 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, and they should be more cautious in using cell phones and perhaps, should stay away from mobile relaying ground antenna.

5. Acknowledgment

This article is based on the results of master thesis of Behnaz Hajioun, Physiology Student of Islamic Azad University-Kazeroon Branch. We sincerely appreciate all people who supported her in this project, especially lab staff of KAU. We also thank Mr. Esfandiar Sharifi for his help in translation and editing of this article.

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