

Full Length Research Paper

Effects of cell phone radiation on the levels of T₃, T₄ and TSH, and histological changes in thyroid gland in rats treated with *Allium sativum* extract

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Accepted 3 July, 2013

Recent increase of hormonal disorders in human may be correlated to the increased exposure to electromagnetic fields produced by various instruments, including cell phones. Because of its position in the body, thyroid gland is one of the organs most likely to be exposed to cell phone radiation. The aim of this study was to assess the adverse effects of cell phone radiation along with the protective effects of garlic on pituitary thyroid hormones and histology of thyroid gland in rats. Five groups of rats were used: control, sham (exposed to 900 MHz wavelength), experimental 1 (receiving garlic extract), and experimental 2 and 3 (receiving both extract and microwaves). After a month, rats were weighed and serum levels of TSH, T₄ and T₃ were measured using ELISA kits. Thyroid glands were also removed and their probable histological changes were studied. The mean body weight in the sham group showed a statistically significant decrease compared to the control group, whereas, an increase was seen in the experimental group 3 compared with sham ($P < 0.05$). In addition, the concentrations of T₄ and T₃ hormones were lowered while TSH increased significantly in all groups compared to the control. Furthermore, histological examination of thyroid revealed a reduction in the number of cubic cells and disorder among them, as well as a reduction in the amount of follicular fluid and follicular diameter in groups exposed to radiation and received garlic extract. Although, microwaves can cause weight lost, presence of allicin and vitamins A and B in garlic can compensate some of this weight lost. In addition, both microwaves and garlic extract have a considerable effect on thyroid gland, reflected both in its secretion and in its morphology. These changes might be attributed to induction of heat and non-specific stresses, alterations of cortisol level, production of ROS and activities of various enzymes. However, some of these overlapping effects are synergistic and others are antagonistic, and thus, garlic consumption is not always beneficial in reducing deleterious effects of cell phones.

Key words: Electromagnetic radiation, cell phone, garlic, thyroid hormone.

INTRODUCTION

Electromagnetic (EM) spectrum has a broad frequency range including very low frequencies, radio frequencies (radio waves), radar waves, microwaves, infrared radiation, visible light, ultraviolet radiation, X-rays and gamma rays. These waves are intentionally or unintentionally produced by various common devices, such as, refrigera-

tors, freezers, television, radio, microwave, photocopiers, computer monitors, halogen lamps, printers and diathermic machines (Baharara et al., 2004). Microwave radiation, as a part of electromagnetic spectrum, has a frequency ranging from 300 MHz to 300 GHz (Banik et al., 2003; Baharara et al., 2004; Hemayatkhah Jahromi et al.,

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2012). This area of electromagnetic spectrum is used in cell phones, and the frequency of waves emitted from cell phones is between 900 MHz to 1GHz (Baharara et al., 2004).

According to some studies, EM waves emitted from a cell phone even with the power density (Pd) lower than the permitted limit ("1mw/cm²") could lead to a wide range of disorders, such as headache, heat sensation in ears, memory loss and fatigue (Hocking, 1998; Sandstrom et al., 2001). In order to explain the interaction between EMF and living organisms, two different mechanisms have been proposed: thermal and non thermal mechanisms (Ferreri et al., 2006).

Exposure to magnetic fields generated by cell phones influences different organs and organ systems by either thermal or non thermal mechanisms. EMF 900 MHz and its effects on the nervous and endocrine systems have been studied extensively. It has been reported that exposure to magnetic fields increases fat breakdown and glycogenolysis; it also elevates the levels of some hormones such as glucagon, cortisol and thyroxin in mice (Aghdam Shahryar et al., 2009).

It appears that hormones and their receptors are the first line of health to be adversely affected in cell phone (both present types and next generation) users (Sultan et al., 2010). Since cell phones are usually placed near the thyroid gland during their use, it is one of the organs most likely to be exposed to cell phone irradiation (Lauer et al., 2013; Mortavazi et al., 2009). In an investigation of changes in thyroid hormones and TSH following cell phone use, higher than normal levels of TSH, decreased T4 concentration and normal level of T3 were observed (Mortavazi et al., 2009). Investigated effect of 900 MHz electromagnetic radiation on TSH and thyroid hormones in rats showed a reduction in the serum levels of TSH, T4 and T3 (Koyu et al., 2005).

On the other hand, recent scientific studies have been focusing on the use of plant products as therapeutic agents (Sarkar et al., 2006; Mahmoodi et al., 2011). Garlic is one of these plant products, traditionally used for its cytotoxic, antitumor, antifungal, antibacterial, antiviral and anti protozoal properties. Furthermore, in the ancient Indian medicine (Sanhita Sushruta), garlic is recommended for the treatment of hemorrhoids, rheumatism, dermatitis, abdominal pain, cough, leprosy, etc (Sarkar et al., 2006).

As a member of the *Liliaceae* family, *Allium sativum* or garlic (Ulbricht et al., 2010), contains various substances including minerals, carbohydrates, proteins, fats and vitamins (Hacisferogullari et al., 2005; Cobas et al., 2010; Kemper, 2000). 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, 2000; Khlid and Gordon, 2006; Lanzotti, 2006; Cobas et al., 2010). Some of these organo-sulfur com-

pounds are allicin, ajoene, allylpropyl disulfide, diallyl trisulfide, sallylcysteine, vinylthiines, S-allylmercaptocystein, and others (Kemper, 2000; Sarkar et al., 2006).

In recent years, there has been a tendency among researchers in attempting to treat disorders by replacing chemical drugs with some natural plant components, because of their high costs, their potential side effects, as well as restrictions of their use (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 not been any 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 the thyroid gland. The position of this vital organ in the body and the ubiquity of cell phones are sufficient enough to urge the performance of such studies and make the mobile phone use safer.

MATERIALS AND METHODS

Soaking method (maceration) was used to prepare garlic extract (Tatara et al., 2005). 40 wistar rats with mean 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 to 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.

Animals were randomly divided into five groups of eight 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 electromagnetic field (EMF) and cages were surrounded by aluminum foil to focus waves and limit the EMF to the interior of the cages (Figure 1). 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 TSH, T4 and T3 were measured using ELISA kits (Made by Biosouece Europe). Thyroid glands were also removed, prepared using classical method of hematoxylin and eosin staining and studied by light microscope. The results were examined by SPSS software, one-way ANOVA and Tukey's test and the significant difference was sat at (P<0.05).

RESULTS

Results of body weight are shown in Table 1, which indicates a significant decrease in the mean body weight of sham group in respect to the control, and an increase in the experimental group 3 in respect to the sham group

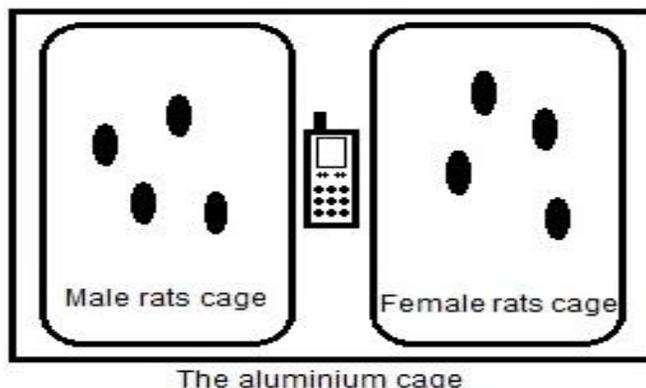


Figure 1. Schema of cellular phone EMFs exposure condition. The mobile set was placed in the middle of the cage containing the male and female rats.

Table 1. Mean body weight and serum levels of T3, T4 and TSH in different groups.

Group	Weight (g)			T3 (ng/dl)			T4 (mic/dl)			TSH (micIU/dl)		
	Mean±SE	P*	P**	Mean±SE	P*	P**	Mean±SE	P*	P**	Mean±SE	P*	P**
Control	243.7± 30.44			2.3± 0.10			5.1± 0.05			3.7± 0.06		
Sham	217.7± 17.67	*		2.0± 0.07	*		4.7± 0.10	*		4.0± 0.09	*	
Ex. 1	220.5± 15.13			1.8± 0.07	*		4.7± 0.05	*		4.0± 0.09	*	
Ex. 2	216.5± 14.07			1.9± 0.11	*		4.4± 0.10	*	**	4.2± 0.07	*	**
Ex. 3	248.7± 12.17		**	1.6± 0.04	*	**	4.1± 0.04	*	**	4.7± 0.03	*	**

*Compared to control; **compared to sham; * Indicates a significant difference.

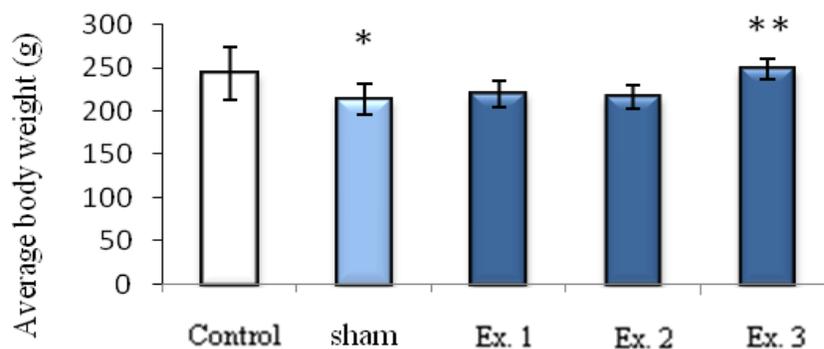


Figure 2. Mean body weight differences among various groups exposed to cell phone radiation and/or received garlic extract. * Indicates a significant difference.

(Figure 2 and Table.1). Mean serum levels of T3 in the sham group and experimental groups 1, 2 and 3 showed a significant decrease compared to the control (Figure 3 and Table 1). Similarly, a significant decrease was observed in the mean serum levels of T4 in these groups (Figure 4 and Table 1). In contrast, mean serum levels of TSH in the sham group and experimental groups 1, 2 and 3 were significantly increased (Figure 5 and Table 1).

Figure 6 show morphological changes in thyroid gland in different groups. As seen, there is a disordering among

cubic cells, a reduction in the number of cubic cells and follicles, and a decrease in the amount of follicular fluid and diameter compared to control.

DISCUSSION

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

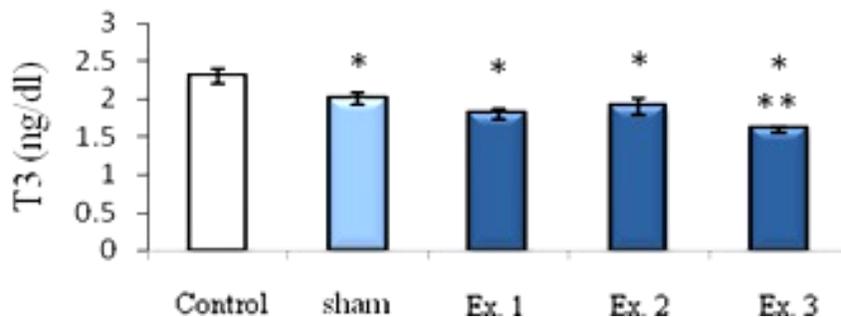


Figure 3. Mean serum levels of T3 hormone among various groups exposed to cell phone radiation and/or received garlic extract. * indicates a significant difference.

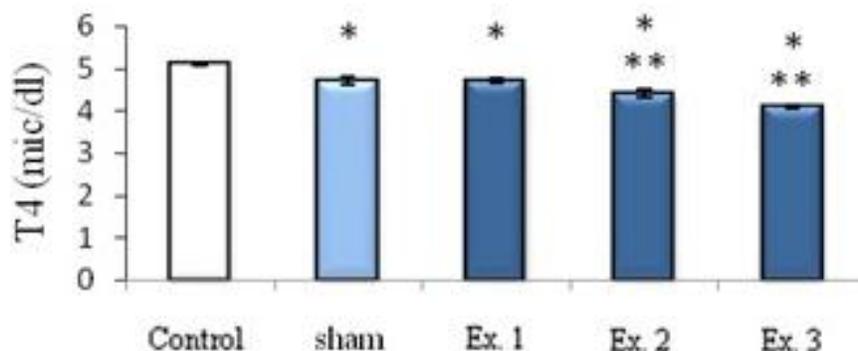


Figure 4. Mean serum levels of T4 hormone among various groups exposed to cell phone radiation and/or received garlic extract. * Indicates a significant difference.

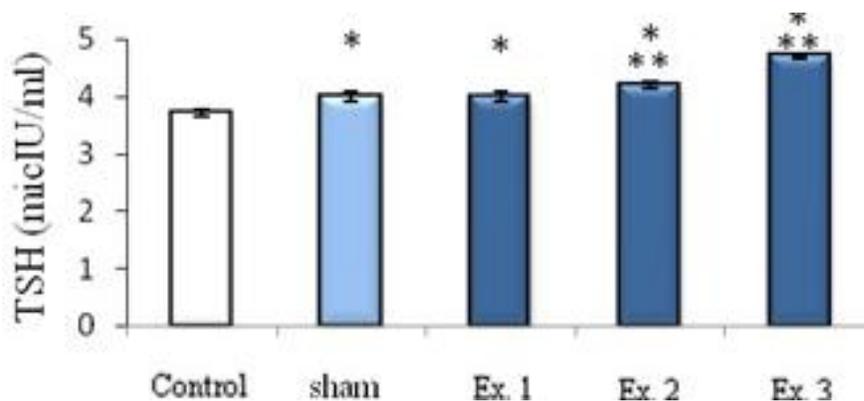


Figure 5. Mean serum levels of TSH hormone among various groups exposed to cell phone radiation and/or received garlic extract. *Indicates a significant difference.

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 (Sarookhani et al., 2011). It is possible to say that the deleterious effects of electromagnetic microwaves are generally exerted through elevation of body temperature (Thalau et al., 2003; Bagher et al., 2009) and creation of free radicals (Rollwitz et al., 2004; Bagher et al., 2009).

Our study show that EM waves can cause weight loss

and that administration of garlic extract, especially high dose, can prevent weight loss caused by radiation (Table 1 and Figure 2). Exposure of rats to microwave frequencies at 900 MHz (used in cell phones) can cause oxidative stress on these animals and decrease their antioxidant activities, leading to weight loss (Ilhan et al., 2004). In contrast, it has been reported that consumption of garlic juice can inhibit body weight reduction in diabetic rats (Musabayane et al., 2006) and can impose anti mutagenic effects against gamma radiation, possibly through in-

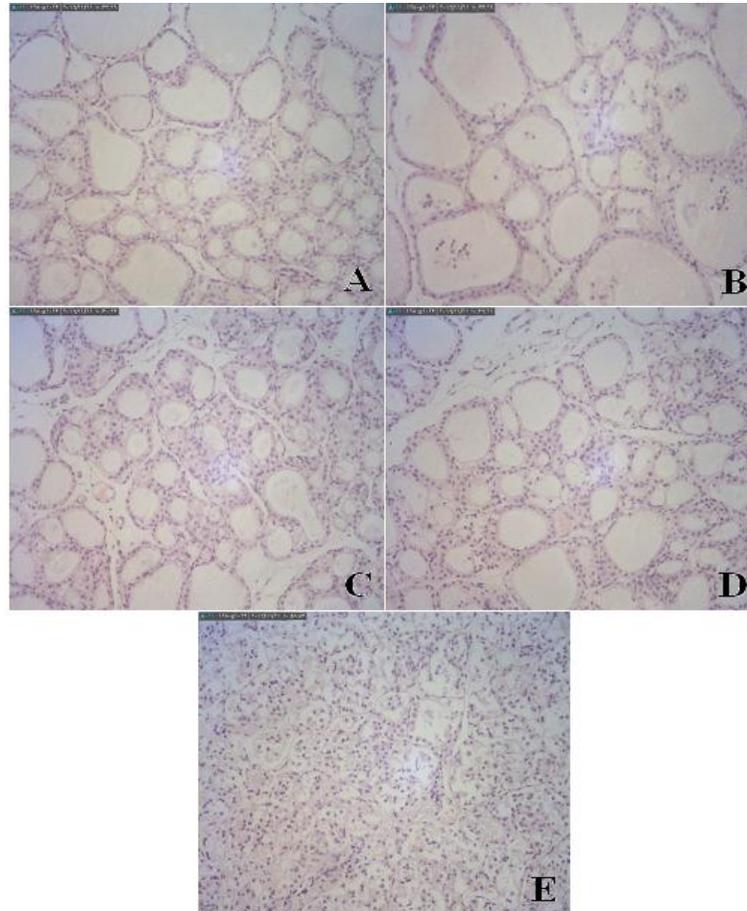


Figure 6. Profile layout of thyroid tissues in different groups exposed to cell phone radiation and/or received garlic extract. A, control; B, sham; C, experimental 1; D, experimental 2 and E, experimental 3. (Magnification = 70X).

hibition of free radicals (Singh et al., 1996). Garlic contains a significant amount of *thiosulfanats* or allicin (Jessica et al., 2011) and pharmacological studies have shown that allicin can trap free radicals, and cause an inhibition of lipid oxidation, inhibition of platelet aggregation and stimulation of fibrinolysis resulting in blood lipid reduction (Shahrani et al., 2007).

It has been indicated that, plasma cholesterol and triglyceride levels decline in rodents following exposure to 900 MHz radiation emitted from cell phones leading to weight lost (Aghdam and Lotfi, 2009). However, it deviates from the results of some previous study that no significant changes were observed in the body weight of mice at the end of experiment (Sang Lee et al., 2004). Nevertheless, many studies have shown that MF And EMF can reduce total cholesterol and triglyceride levels in human and laboratory animals and that these effects were more significant in long-term exposure to radiation and can be related to an increase in body lipid metabolism. Moreover, these changes may due to the reversible accumulation of triglycerides in the liver or its precursors

after acute exposure to 900 MHz microwaves, because magnetic fields can enhance fat breakdown and glycogenesis (Aghdam Shahryar et al., 2009). It also increases body metabolism, body temperature and activity of the sweat glands (Russel and Reiter, 2007).

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 (Saba et al., 2011). Presence of vitamin B family (especially thiamine) in garlic can stimulate the appetite (Newall et al., 1996; Khalid and Gordon, 2006) and vitamin B6 helps the body to convert carbohydrates and fat into energy (Newall et al., 1996). It makes digestion easier and activates stomach acid secretion.

On the other hand, garlic contains vitamin A (Corzo et al., 2007) 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 (Bakkali, 2008). Thus, one can conclude that presence of allicin and vitamins A and

B in garlic can compensate some of the weight lost caused by exposure to radiation.

The results of this study also showed a considerable effects of both microwaves and garlic extract on thyroid gland, reflected both in its secretion and in its morphology. Interference in thyroid functions may occur through an increase in the level of TSH following excessive use of cell phones, leading to a decline in the levels of T4 and T3 (Table 1 and Figures 3 to 5). Apparently, hypothalamus or pituitary glands play no direct roles in this interference, because if there were any adverse effects on these organs, TSH levels would have been lower than normal (Mortavazi et al., 2009). The strongest and most consistent effects of such irradiation on the endocrine systems would be the induction of heat in tissues and elevation of temperature, or it could be attributed to some non-specific stresses associated with irradiation (Naduvil Narayanan et al., 2010).

Study of other biological effects of EM waves includes the increase level of ornithine carboxylase enzyme that plays some roles in tumor enhancement, changes in thyroid hormone levels and behavior. Other studies are indicative of the induction of reactive oxygen species (ROS) by EM waves which play an important role in damaging cells (Sinha, 2008). In addition, EM waves can cause stress and increase serum cortisol level. Stress can alter thyroid functions through an increase in endogenous cortisol production, because elevation of glucocorticoid secretion constitutes an obstacle to the conversion of T4 into T3 (Aghdam Shahryar et al., 2009). Our findings are in agreement with the results of Mortavazi et al. (2009), but they are inconsistent with Sinha (2008) survey. These differences may due to the differences in the dose and duration of exposure to radiation.

Likewise, the effects of garlic extract on observed hormonal changes is well justified. This plant contains flavonoids (Eteng and Aletan, 2012), which inhibit the activities of enzymes antithyropoxidase (TPO) and liver deiodinase as the key enzyme in the biosynthesis of thyroid hormones and bring about some changes in thyroid functions (Ferreira et al., 2006). Another compound present in garlic is coumarin cis (Abebe, 2002) that influences thyroid functions via blocking the conversion of T₄ to T₃ (Patton et al., 1989).

Garlic can affect TSH level in different ways. It can mediate TSH production through calcium - phosphatidyl inositol as secondary messenger (Ulianich et al., 2004), since it contains calcium and magnesium (Khalid and Gordon, 2006). Garlic is also known as an inhibitory factor in the production of cortisol, and this could be considered another reason for the observed changes in the serum level of TSH, because reduced cortisol level has an elevating effect on the secretion of TSH.

The observed alterations in the levels of T₃ T₄ and TSH are accompanied by the histological changes in thyroid gland. Our histological study indicates a reduction in the number of cubic cells, an irregularity in the arrangement

of these cells, and a decline in the number of follicles, amount of follicular fluid and their diameters in the groups exposed to EM waves and received garlic extract (Figure 6B and C). When both factors were administered simultaneously, tissue degradation rate and destruction of follicles increased (Figure 6D and E), and at the same time, led to a significant reduction in the rate of thyroid hormone production (Table 1 and Figures 3 and 4). These signs of thyroid malfunction may be the results of increased caspase-dependent apoptosis pathways (Esmekaya et al., 2010).

In short, it appears that, body exposure to microwave radiation emitted by mobile phones can cause pathological changes in the thyroid gland leading to changes in its secretion and its structural alteration. Moreover, some of these changes are in the same area where garlic components can influence. However, some of these overlaps are synergistic and others are antagonistic, and thus, garlic consumption are not always beneficial in reducing deleterious effects of cell phone use.

Conclusion

This study highlights some adverse effects of microwaves and benefits of garlic in making cell phone use safer. Although, microwaves can cause weight lost by enhancing fat breakdown and glycogenesis, presence of allicin and vitamins A and B in garlic can compensate some of this weight lost through an increase in appetite, digestive processes and body's metabolism. In addition, both microwaves and garlic extract has a considerable effects on thyroid gland; reflected both in its secretion and in its morphology. These effects could be attributed to induction of heat in tissues and non-specific stresses associated with irradiation, changes in the levels of cortisol, reactive oxygen species, calcium - phosphatidyl inositol and various enzymes, such as ornithine carboxylase, anti thyroperoxidase and liver deiodinase. In spite of these, consumption of garlic is not recommended to prevent the deleterious effects of cell phone radiation on thyroid gland.

ACKNOWLEDGMENT

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

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