Research Paper Medical Science

Effects of Chronic Exposure to Electromagnetic Waves at 930 MHz Frequency on Oxidative Stress in Plasma and Some Organs of Wistar Rats

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ABSTRACT

The increasing use of mobile phones and the high number of associated base stations constitute a considerable source of electromagnetic waves. These may affect biological systems, by increasing lipid peroxidation and altering antioxidant activities, thus leading, to oxidative stress. To test this hypothesis, we studied the effect of exposure from 3 months to radiation of 930 MHz produced by a wave generator connected to a communication system on the plasma, brain, liver, kidneys and muscles of the thigh. 60 Wistar rats were divided into 6 groups, according to sex (males and females) and the duration of exposure (controls, exposed 1h and 2h). The levels of Malondialdehyde (MDA) and catalase (CAT) were determined in different tissues. The liver glycogen, glucose and plasma cortisol were dosed and osmotic fragility of erythrocytes was studied in control and exposed rats to electromagnetic waves. The statistical analysis of results was performed by ANOVA. The results were considered statistically significant if the probability $p < 0.05$. We observed that the rates of MDA and cortisol increased significantly ($p < 0.05$), while that of catalase decreases significantly ($p < 0.05$) in rats exposed 2h to electromagnetic waves compared to controls. This exposure also causes a destocking glycogen and an increase in glycaemia. At the level of red blood cells, it induced hemolysis by a significant increase ($p < 0.05$) of osmotic fragility in exposed 2h to electromagnetic waves compared to controls in both sexes. It also seems that females are more affected by the action of electromagnetic waves than males. We may therefore conclude that the electromagnetic waves emitted by mobile phones can induce oxidative damage to red blood cells and in various tissues studied.

KEYWORDS Electromagnetic waves, MDA, catalase, cortisol, glycogen, glucose, osmotic fragility, oxidative stress.

Introduction

The use of mobile phones has increased rapidly around the world over the past of 15 years. It is estimated that there are over 6.8 billion of GSM used according to the International Telecommunication Union (2013). This rapid growth of wireless telecommunications has increased the scientific interest on the eventual biological effects of electromagnetic fields (EMF) emitted by mobile phones and their impact on human health. In addition, the results of studies on the potential risks of exposure to electromagnetic fields have received a wide public attention (Valberg, and al., 2007), but their interpretation is not simple due to the various methodological difficulties (Ahlbom, and al., 2004).

Among the various areas of scientific interest over the last years, we find the oxidative stress induced by electromagnetic radiation in biological systems (Brocklehurst, and McLauchlan, 1996 ; Lacy-Hulbert, and al., 1998 ; Dasdag, and al., 2004 ; Ilhan, and al., 2004 ; Ozguner, and al., 2005 ; Zmyslony, and al., 2004). This oxidative stress is a biochemical condition characterized by an imbalance in the presence of relatively high levels of toxic reactive species, mainly reactive oxygen species (ROS) and the antioxidant defense mechanisms (Moller, and al., 1996 ; Ozben, 2007). At the level of the organism, there are antioxidant systems, of enzymatic or non-enzymatic nature (Droge, 2002 ; Mates, and al., 1999 ; Powers, and Lennon, 1999), which have different activities and which are able to protect tissues by retarding or inhibiting the oxidation of oxidizable substrates (Halliwell, and Gutteridge, 1984).

The enzyme systems are considered as the first line of defense of our body against ROS:

- The superoxide dismutase (SOD): catalyzes the dismutation of superoxide anion O_2 to hydrogen peroxide H_2O_2.
- The catalase: responsible for the elimination of H_2O_2 by a transformation in H_2O and O_2.
- The glutathione peroxidase: acts synergistically with SOD and accelerates the dismutation of H_2O_2 to H_2O and O_2 (Matés, and al., 1999 ; Powers, and Lennon, 1999).
- The glutathione reductase: regenerates the glutathione reduced (GSH) from GSSG by NADPH.

For the non-enzymatic systems and contrary to the antioxidant enzymes, most of these components are not synthesized by the body and must be supplied by the diet. In this category of antioxidants we find the oligoelements, the reduced glutathione (Packer, and al., 1997 ; Powers, and Lennon, 1999), the ubiquinone (Packer, and al., 1997 ; Powers, and Lennon, 1999), the cytochrome c (Skulachev, 1996) and the vitamins E and C (Packer, and al., 1997 ; Evans, 2000).

The ROS can constitute an important factor in tissue injury. Therefore, if they are not trapped, these species may lead to damage of lipids, proteins and DNA (Jajte, and al., 2002 ; Agrawal, and al., 2001).

The term ROS includes the free radicals of oxygen: superoxide anion (O_2), hydroxyl radical (OH) but also certain non-radical oxygen derivatives, whose toxicity is important, such as the hydrogen peroxide (H_2O_2). The first targets of ROS are the lipids, in particular those present in the membranes rich in polyunsaturated fatty acids (PUFA) due to their high degree of unsaturation (Hulbert, 2005 ; Pamplona, and al., 2000).

The lipid peroxidation which increases due to the reduction of antioxidant defense systems (Sarban, and al., 2005) generates highly reactive lipid peroxides and induces a modification of the fluidity, the permeability and the excitability of the membranes (Hong, and al., 2004). It also provides a wide variety of products that can react with the proteins and the DNA (Marnett, 1999).

Among the products formed during the lipid peroxidation: the Malondialdehyde (MDA), which is considered as a reliable marker of the oxidative stress in tissues (Draper, and Hadley, 1990 ; Serel, and al., 2004). The cortisol which is a hormone secreted by the adrenal gland is also a well-established indicator of stress (Radon, and al., 2001).

The carbohydrate metabolism appears to be affected by the action of the electromagnetic waves that inhibit the glycogenesis and accelerate the glycogenolysis (Boguslaw, and al., 1999). The magnetic field is known to be strongly glycogenolytic in the rats (Chernysheva, 1990 ; Gorczynska, and Wegrzynowicz, 1991) that which causes a decrease of liver glycogen and increased of plasma glucose (Tsuji, and al., 1996). The cortisol active in the liver the gluconeogenesis enzymes, for producing glucose that is released into the blood, to increase the blood glucose (Lecavalier, and al., 1989).

The objective of this study is to evaluate the effects of exposure to one or two hours for 3 months to electromagnetic waves (930 MHz) emitted by a system described below (Figures 1 and 2) on some markers of oxidative stress in rats through a dosage of the catalase, the Malondialdehyde in plasma and some organs (brain, liver, kidneys and muscles of the thigh), the liver glycogen, the cortisol and the plasma glucose as well as the erythrocyte osmotic fragility in both sexes.

Materials and methods

All the procedures mentioned below have been developed in our laboratory: Physiopathology and Molecular Genetics laboratory in Casablanca Ben M'Sik Science Faculty.

Animals

The use of the animals was done in accordance with the manuals of laboratory animals (National Academy of Science of Washington, 1996, Guide for the care and the use of animals laboratory). 60 male and female rats of Wistar strain aged from 29 to 36 days, of the body weight between 60 and 100 g, in good health and belonging to the Faculty of Scienc-

es Ben M'Sik, were used in this experiment. The temperature of the pet store was maintained at 22 ± 2 °C, the humidity around 50 % and the circadian rhythm was 12 h light and 12 h dark. Any observed variation of temperature or illuminance is corrected as soon as possible.

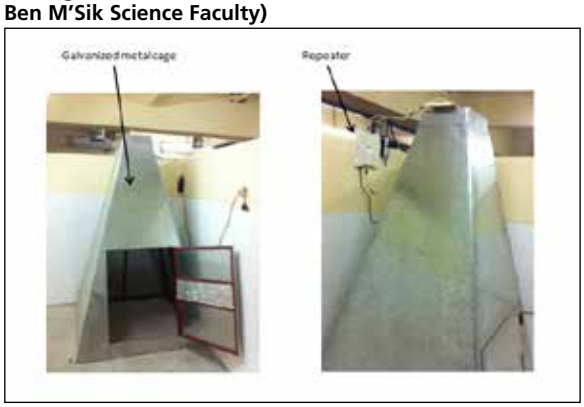
The animals were fed with the standard diet: N° GPF81 of INAAM society, Casablanca, Morocco. The food is distributed daily at the same time. The tap water for drinking is available ad libitum. The bedding cages are changed three times per week. The rats are identified by a registered number by a permanent marker on the proximal tail part.

The rats were divided into six groups of five rats according to the sex (males and females). The duration of exposure to electromagnetic fields (controls, exposed 1h and 2h) at a frequency of 930 MHz for 3 months on workdays concerned different groups of rats. During the experiment, the rats (5 per group) were housed inside a pyramidal sheet chamber in cages.

Exposure system to electromagnetic fields
The simulation of environmental of GSM coverage was performed by using a pyramidal structure having a ground base of 1.2 m x 1.2 m and a height of 2 m, the disposal of structure is vertical with a top opening which allows having an omnidirectional antenna allowing a homogeneous radiation of the electromagnetic field.

The radiation was generated by a repeater of 900–1800 MHz that allows reproducing the GSM signal, received from the GSM network and concentrating the radiation inside the test device, which plays the role of a Faraday cage, avoiding the interference with other external signals that can distort the envisaged study. The principle consists to reproduce of an environment similar to the real radiation, while ensuring that is studied only the radiation from GSM network.

Figure 1: The experimental device for the production of the signal GSM in the 900-1800 MHz band (Casablanca Ben M'Sik Science Faculty)



We used an amplifier ANDREW (PN: S-2-CPEUS-L-N, LEW POWER SP LIPPER, 800-2500 MHz).

The repeater has two antennas:
- A receiver external antenna which can capture the GSM signal emitted by the nearest GSM station.
- An internal antenna transmitting at a power equivalent to -40 dbm and which can be positioned and oriented according to the desired needs.

The sealing of the device was tested and the signal level was measured by a trace post (Sony Ericsson Phone), with TENS software that performs the measurement in the 2G band 900 MHz. The temperature inside room was measured by a thermometer (digital interior – basic function).

During the exposure to the electromagnetic waves, the rats moved freely in a Plexiglas cage of 40 cm × 30 cm × 10 cm. The cage average power density is about 100 μW/cm2 (19.4 V/m). In the cell, the cage was placed in the level where an input power could generate the maximum field on the entire surface of the cage. Depending on the cell size, the level of the position of the cage, the input and output power, and the SAR (Specific Absorption Rate) were calculated.

Assays performed
The principle of our work was to assay some markers of oxidative stress in control and exposed one or two hours rats to electromagnetic waves in both sexes.

At the end of the experiment, the rats were anesthetized by intraperitoneal injection of ethyluretane at 10 %, then decapitated in accordance with International rules for ethical. The blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA), a portion was kept at 4 °C for studying the osmotic fragility of the erythrocytes and another was centrifuged for 10 minutes at 3000 rpm to recover the plasma which was distributed then aliquoted and rapidly frozen at -20 °C, a view to subsequent analysis (dosage of glucose, cortisol, CAT and MDA).

Immediately after collection of blood, the organs (brain, liver, kidneys and muscles of the thigh) were removed, rinsed with physiological saline solution, numbered, dated and preserved at 4 °C for determination of CAT and MDA and liver glycogen.

A- Determination of plasma cortisol:
The plasma cortisol is measured by the AxSYM Cortisol method (Abbot AxSYM system). This is an immunoassay by a Fluorescence Polarization Immunoassay (FPIA). The reagents AxSYM Cortisol used are: Cortisol Fluorescein Tracer, anti-Cortisol antiserum and the pretreatment solution. This is a technical competition assay. The antibodies (anti-Cortisol) and the Cortisol Fluorescein Tracer is added to the reaction mixture and incubated. The substance to be assayed enters into competition with the tracer to occupy the antibody binding sites.

B- Assays of tissue and plasma enzymes (CAT and MDA):
We assayed two enzymes (CAT and MDA) often associated with oxidative stress in plasma and tissue level to assess possible damage associated with exposure to electromagnetic waves from mobile phones.

The protein extraction was performed from the plasma and the different organs removed. For organs, after a potterization (maximum speed, 10 round trips) in the ice, the various organs cut into small pieces with the extraction buffer (0.25 mM Saccharose, 10 mM HEPES, 10 mM EDTA) to using an Ultra-turrax; T-25 BASIC to burst the cells. A filtration over 8 layers of cheesecloth was carried out to remove cell debris. The filtrate collected was centrifuged at 4000 revs/mn for 10 min at 4 °C. The supernatant recovered is, used immediately for assays or stored frozen at a temperature of -28 °C until use.

The total protein concentration was determined by the Bradford technique (1976) in the plasma, as well as in the organs homogenate. This method is based on the use of the Bradford reagent (100 mg of Coomassie blue G250, 50 ml of 95% ethanol, 100 ml of 85% phosphoric acid and double distilled water to 1 liter). The Coomassie blue G250 has the property of adsorbing proteins, giving a color ranging from brown for the low concentrations to blue for high concentrations for proteins. The absorbance is measured at 595 nm in a spectrophotometer (Anachem models; 220, 320). The protein concentration is determined using of a calibration curve prepared using the BSA.

The catalase activity and the rate of Malondialdehyde were expressed relative to the total proteins.

1- Determination of the catalase (CAT):
The catalase is almost omnipresent in the peroxisomes of all aerobic cells. Its principal function is to prevent the accumulation of toxic levels of H2O2 formed by products of metabolic processes, principally that of the electron transport pathway. The catalase activity was determined by the Aebi method (1984) based on the property of the enzyme to degrade the hydrogen peroxide H2O2

to H2O and O2. The disappearance of the substrate is then followed by spectrophotometry at 240 nm.

2- Determination of the Malondialdehyde (MDA):
The Malondialdehyde is one of the terminal products formed during the decomposition of polyunsaturated fatty acids by free radicals. Its measurement by the method of Samokyszyn and Marnett (1990) allows the determination of the rate of lipid peroxidation. This method is based on the property of MDA to react with thiobarbituric acid to regenerate a pink chromophore absorbing at 535 nm.

C- Determination of liver glycogen and blood glucose:
1- Liver Glycogen:
After extraction of glycogen in the liver, the assay was performed with respect to a reference range (made with the commercial glycogen to 3 mg/ml).

10 g of fresh liver was weighed, cut into small pieces and then poured into 100 ml of boiling distilled water for 2 min. After grinding in a mortar, the sample was submitted to boil for 5 minutes and filtered. 5 drops of hydrochloric acid were added to the filtrate. A new filtration of the neoformed precipitate was carried. The recovered supernatant was mixed with 2 to 4 times its volume of 95% alcohol to precipitate the glycogen. The pellet obtained after centrifugation was dissolved in 10 ml of water and the presence of glycogen was tested by adding one drop of iodine water in 2 ml that gives a brown color in the presence of glycogen. After addition of iodine water and distilled water, the absorbance is measured at 470 nm in a spectrophotometer (Anachem models; 220, 320). The concentration of glycogen is deduced from a calibration curve.

2- Blood Glucose:
The glucose determination is performed for quantifying the glucose in the serum of control rat or exposed to electromagnetic waves. For its realization, we used an enzymatic method with glucose oxidase / peroxidase (biosystems). The principle resides in the oxidation of glucose by the glucose oxidase inducing the formation of gluconic acid and hydrogen peroxide. The hydroxy 4 aminophenazone sodium present in the reagent is oxidized by the hydrogen peroxide, in the presence of peroxidase to form a stable red quinone complex. The pink coloration developed is proportional to the glucose concentration. The absorbance of the standard and the sample were determined compared to the white at 500 nm.

D- Analysis of the osmotic fragility of erythrocytes from whole blood:
The osmotic fragility test (Oladele, and al., 2003 ; Oyewale, 1993) was performed in control rats and exposed to electromagnetic waves for 1h or 2h after an incubation period of 30 mn at 37 °C, of µl of whole blood into tubes numbered 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, containing 10 ml of NaCl solutions respectively to 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9 g/l. The tubes were after centrifuged at 1500 x g for 10 mn and the supernatant absorbance (OD) at 540 nm was measured. The percentage of hemolyzed erythrocytes (H %) was calculated using the following formula (Faulkner, and King, 1970) :

H % = (OD blood in NaCl /OD blood in water) x 100

The concentration of NaCl causing a 50% hemolysis of red blood cells (H50) was determined.

Statistical analysis of results
The results are presented as mean ± standard error. The statistical analysis was performed by Excel program and comparing of the means between different groups was performed by using the ANOVA test. The results were considered statistically significant if the probability p is less than 5 % ($p < 0.05$).

Results
It should be noted that the experimental conditions of our study were performed according to the standards, in order to minimize animal stress, whose the results can be only due to the exposure to the electromagnetic waves.

A- Plasma cortisol:
A significant increase ($p < 0.05$) of plasma cortisol in both sexes was observed in the animals exposed to the electromagnetic waves compared to the controls. This increase is most important in the rats exposed for 2 h at these microwaves (Table 1).

Table 1: Rates of plasma cortisol of the rats control and exposed to electromagnetic waves for 1h and 2h; * = P < 0.05 (significant difference)

Groups	Sex	Levels of cortisol (µg/dl) ± standard deviation
Controls	Females	1,9333 ± 0,2728
	Males	1,7333 ± 0,3383
Exposed 1h	Females	2,7667 ± 0,3383
	Males	2,0667 ± 0,2186
Exposed 2h	Females	3,2333 ± 0,3180 *
	Males	3,0333 ± 0,5239 *

B- Tissue and plasma enzymes (CAT and MDA):

1 - The catalase (CAT):
A significant decrease ($p < 0.05$) in catalase activity due to the increase of the duration of the exposure of rats to the electromagnetic waves compared to the controls was observed in this experimental study in plasma, liver, kidneys, brain and muscles of the thigh in both sexes (table 2).

Table 2: Catalase activity in various tissues of the rats control and exposed to electromagnetic waves for 1h and 2h; * = P < 0.05 (significant difference)

Groups	Sex	Catalase activity (µmol/mg) ± standard deviation				
		Plasma	Liver	Kidneys	Brain	Muscles of the thigh
Controls	Females	5,6351 ± 0,4624	5,0512 ± 0,4274	5,3744 ± 0,4588	5,76 ± 0,4764	5,5276 ± 0,4425
	Males	5,0154 ± 0,4021	4,8934 ± 0,4549	4,9007 ± 0,4055	5,1383 ± 0,4372	4,3085 ± 0,4017
Exposed 1h	Females	4,9214 ± 0,3841	4,4265 ± 0,4024	4,5389 ± 0,4027	4,368 ± 0,3771	4,05 ± 0,3482
	Males	4,3162 ± 0,3594	3,9238 ± 0,3468	4,0407 ± 0,3573	4,0474 ± 0,3299	3,6016 ± 0,3375
Exposed 2h	Females	3,8451 ± 0,3284 *	3,8138 ± 0,3368 *	3,5913 ± 0,3169 *	3,7338 ± 0,3095 *	3,4944 ± 0,3277 *
	Males	3,2548 ± 0,2951 *	3,2264 ± 0,2834 *	3,2899 ± 0,3091 *	3,0574 ± 0,2877 *	3,3292 ± 0,2927 *

2 - The Malondialdehyde (MDA):
The increase in the duration of exposure of rats to electromagnetic waves of 1 to 2 hours compared with controls leads to a significant increase in MDA levels ($p < 0.05$) in all tissues studied and in both sexes male and female (Table 3).

Table 3: Rate of MDA in different tissues of the rats control and exposed to electromagnetic waves for 1h and 2h; * = P < 0.05 (significant difference)

Groups	Sex	MDA levels (µmol/mg) ± standard deviation				
		Plasma	Liver	Kidneys	Brain	Muscles of the thigh
Controls	Females	0,0334 ± 0,0031	0,0385 ± 0,0034	0,389 ± 0,0031	0,0333 ± 0,0034	0,0375 ± 0,0032
	Males	0,0332 ± 0,0028	0,0361 ± 0,0032	0,375 ± 0,0029	0,0363 ± 0,0033	0,0314 ± 0,003
Exposed 1h	Females	0,048 ± 0,0043	0,048 ± 0,0042	0,421 ± 0,004	0,0462 ± 0,0041	0,047 ± 0,0041
	Males	0,0442 ± 0,0039	0,0404 ± 0,0038	0,418 ± 0,0041	0,0385 ± 0,0035	0,0435 ± 0,0041
Exposed 2h	Females	0,0573 ± 0,005 *	0,0534 ± 0,005 *	0,507 ± 0,0052 *	0,0589 ± 0,0053 *	0,0524 ± 0,0051 *
	Males	0,0487 ± 0,0045 *	0,0481 ± 0,0045 *	0,493 ± 0,0042 *	0,0535 ± 0,0048 *	0,0489 ± 0,0043 *

C - Liver glycogen and blood glucose:

A significant decrease ($p < 0.05$) in liver glycogen associated with a significant increase ($p < 0.05$) in plasma glucose was observed during this experimental study in rats exposed to the electromagnetic waves compared to controls, especially exposed 2h and in both sexes male and female (Table 4).

Table 4: Concentration of liver glycogen and plasma glucose levels of the rats control and exposed to electromagnetic waves for 1 or 2 hours; * = P < 0.05 (significant difference)

Groups	Sex	Liver glycogen (mg/ml) ± standard deviation	Plasma glucose (mmol/l) ± standard deviation
Controls	Females	2,98 ± 0,14	4,75 ± 0,3
	Males	2,72 ± 0,15	4,64 ± 0,29
Exposed 1h	Females	2,48 ± 0,09	5,27 ± 0,25
	Males	2,52 ± 0,16	5,12 ± 0,32
Exposed 2h	Females	1,89 ± 0,13 *	6,19 ± 0,27 *
	Males	2 ± 0,1 *	5,88 ± 0,37 *

D - Osmotic fragility of erythrocytes from whole blood:

The curve of the osmotic fragility of the erythrocytes of rats expressing the correlation between the percentage of hemolysis and the salt concentration, showed a sigmoidal configuration, and the response of red blood cells to hemolysis increases significantly ($p < 0.05$) with decreasing the concentration of NaCl in both sexes male and female. The control rats showed a significantly higher resistance of erythrocytes to hemolysis ($p < 0.05$) compared to the rats exposed to the electromagnetic waves for 1 or 2 hours (Figs 2 and 3).

Figure 2: Test of the osmotic fragility of the erythrocytes in male rats exposed to electromagnetic waves; * = P < 0.05 (significant difference)

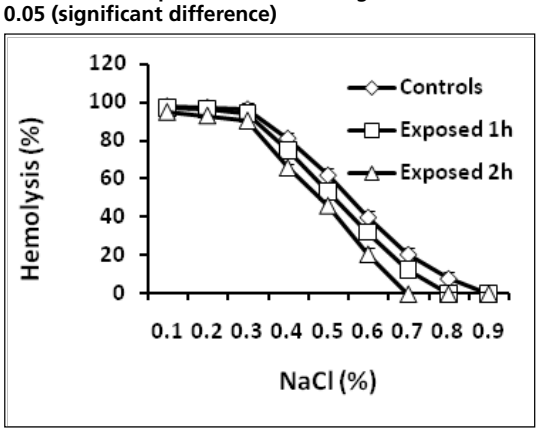
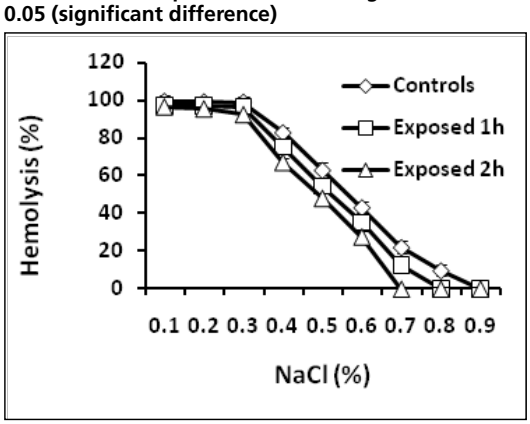


Figure 3: Test of the osmotic fragility of the erythrocytes in female rats exposed to electromagnetic waves; * = P < 0.05 (significant difference)



Discussion

The results of this experimental study show that the exposure of rats to the electromagnetic waves at a frequency of 930 MHz for 3 months, to the duration of 1 or 2 hours, causes oxidative damage assessed by increased lipid peroxidation in studied organs (brain, liver, kidneys, muscles of the thigh and plasma).

It also appears that the female rats are more affected than the males by electromagnetic waves emitted by mobile phones, with a significant increase ($p < 0.05$) in the plasma cortisol levels, the Malondialdehyde, the plasma glucose and the response of cells red to hemolysis. A significant decrease ($p < 0.05$) in the activity of catalase and the liver glycogen were observed significantly in females than males in all tissues studied. This could be related to the particular sensitivity of the females to electromagnetic fields, compared to the males. These results are similar to those of Loomis, and al., (1994) and those of Santini (1998).

The mechanisms by which the electromagnetic fields cause the cellular responses in different tissues (plasma, liver, kidneys, brain and muscles of the thigh) are still unknown, but several hypotheses have been proposed, including the extension of the life of the reactive oxygen species that are causing the macromolecular damages (Harkins, and Grissom, 1994 ; Scaiano, and al., 1994 ; Simko, 2007).

The proximity of the antenna of mobile phones of the abdominal organs has raised concerns about the biological interactions between electromagnetic radiation and the liver and kidneys.

The liver is the organ which consumes the largest amount of oxygen in a stressful situation. We found that the MDA levels increased significantly ($p < 0.05$) and the catalase levels decreased significantly ($p < 0.05$) in exposed to electromagnetic waves compared to controls, particularly in rats exposed for 2 hours. These results are in agreement with those of Dasdag, and al., (2008) who exposed the rats to electromagnetic waves of 900 MHz.

It may also suggest that the long-term exposure to the electromagnetic waves can affect lipid peroxidation in rats. Consequently, the reactive oxygen species may be generated in the liver (Dasdag, and al., 2008).

This could be due to the metabolism of cytochrome P450 which use oxygen to oxidize their substrates and are an important source of formation of the reactive oxygen species (Koyu, and al., 2005).

The present study also showed that the exposure to the electromagnetic waves at a frequency of 930 MHz has a significant effect on oxidative stress in the kidneys of rats, suggesting that the reactive oxygen species were generated in the framework of the experimental conditions used. A significant increase in the MDA ($p < 0.05$) and a significant decrease of the catalase ($p < 0.05$) were observed in exposed for 2 hours to electromagnetic waves compared to controls. These results are in agreement with those reported in other studies in which the rats were exposed to electromagnetic waves of a frequency of 900 MHz (Ozguner, and al., 2005 ; Oktem, and al., 2005.) and of 945 MHz (Yurekli, and al., 2006).

An alteration of the balance oxidative/antioxidant in the kidneys could be partly responsible for the undesirable effects in kidneys causing tissue damages induced by the electromagnetic waves (Oktem, and al., 2005).

The brain can absorb the electromagnetic waves more than other internal organs because the mobile phones are typically used near the brain (Sokolovic, and al., 2008). The results of our study indicated that the electromagnetic waves emitted from our exposure system are associated with increased production of the free radicals and high levels of the lipid peroxidation in the brain.

The significant increase of the MDA ($p < 0.05$) and the significantly decreased of the activities of the catalase ($p < 0.05$) in the brain in this study in exposed for 2 hours to electromagnetic waves compared to controls could be due to high rate of oxidative metabolism activity and a higher concentration of polyunsaturated fatty acids of the membrane of the neuronal cells easily oxidizable (Halliwell, and Gutteridge, 1984).

Our result is in agreement with other studies, which suggest that the exposure of rats to electromagnetic waves at a frequency of 900 MHz in the brain, causes depression of their antioxidant systems due to increased of the lipid peroxidation and formation of the free radicals with an increased of the rate of MDA and decreased of the catalase activity (Dasdag, and al., 2004 ; Ilhan, and al., 2004 ; Dasdag, and al., 2009).

There are two direct and indirect effects of lipid peroxidation on neurons. The direct effects include a loss of fluidity, a decrease in electrical resistance, a depression in the mobility of the proteins of the phospholipid membrane and increased exchange between the membrane bilayers (Richter, 1987).

The indirect effects of the lipid peroxidation may be less apparent, but probably no less damaging. The aldehydes that are produced as a result of the lipid peroxidation are biologically active. One such aldehydes (MDA) leads to the oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of the lipid peroxidation in the brain of rats (Sokolovic, and al., 2008).

The thigh muscles are the tissues with a larger surface of exposure, therefore with more probability to be affected than in other tissues. The analysis of samples of the thigh muscles indicates that the rats exposed to electromagnetic radiation for 2h have high levels of the MDA and low of the Catalase activities compared to the controls. The significant increase of the MDA ($p < 0.05$) can be attributed to the lipid peroxidation and the significant decrease of the activity of the catalase ($p < 0.05$) can be explained by the disruption of the antioxidant mechanisms, which neutralize the free radicals (Halliwell, 1994 ; Winklhofer-Roob, 1994) due to oxidative damage and loss of compensatory mechanisms (Warner, and al., 2004).

In the plasma, our study confirms some of the results reported, where it was observed a significant increase in the MDA levels ($p < 0.05$) and a significant decrease in the catalase activity ($p < 0.05$) (Yurekli, and al., 2006 ; Aydin, and Akar, 2011), and a significant increase ($p < 0.05$) of the plasma cortisol (Lazetic, and a.l, 1997 ; Bouji, and al., 2012) which is a well-established marker of oxidative stress in exposed to the electromagnetic waves at a frequency of 900 MHz compared to controls.

For the osmotic fragility, the control rats showed a resistance of erythrocytes to hemolysis higher than the rats exposed to electromagnetic waves for 1h and 2h. The erythrocytes are highly susceptible to oxidative damage (Bernabucci, and al., 2002). An increase in the generation of free radicals following exposure to electromagnetic waves can cause oxidative damage to membrane lipids and proteins to cause hemolysis (Hanzawa, and Watanabe, 2000).

During this experimental study, were observed a significant decrease ($p < 0.05$) of the concentration of liver glycogen and a significant increase ($p < 0.05$) in the rate of plasma glucose in rats exposed to electromagnetic waves compared to controls. These results are in agreement with other experimental studies that have investigated the effects of the exposure to electromagnetic waves of 850 MHz (Boriraksantikul, and al., 2012a) and 900 MHz (Boriraksantikul, and al., 2012b) on blood glucose.

The hyperglycemia observed after exposure to electromagnetic waves could be explained by structural and functional changes in the pancreas in response to stress caused by these microwaves (Amara, and al., 2006). Indeed, Gorczynska and Wegrzynowicz (1991) have shown that the exposure to electromagnetic radiation causes a reduction of the production of insulin which is a hypoglycemic hormone causing the glycogenesis, and an increase in the production of glucagon which is hyperglycaemic hormone responsible for the glycogenolysis. On the other hand, a significant increase ($p < 0.05$) of plasma cortisol which is a gluconeogenetic hormone caused by the stress of exposure to electromagnetic waves may contribute to hyperglycemia (Lecavalier, and al., 1989).

Conclusion

Our results showed that the exposure to electromagnetic waves emitted from a mobile phone for 3 months can induce:

- An oxidative damages that result in an increase in lipid peroxidation, in oxidative stress, and a decreased of the antioxidant system activity in all tissues studied in both sexes.
- An increase in plasma cortisol, Malondialdehyde, plasma glucose, the response of red blood cells to hemolysis, and a significant decrease of the catalase
- activity and liver glycogen.
- It also appears that the females are more sensitive to electromagnetic waves.

It is therefore recommended within the framework of the protection against the electromagnetic waves to limit the duration of calls per day and use hands free kits as an alternative to keep mobile phones away from the body.

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