OXIDATIVE STRESS IN RATS EXPOSED TO MICROWAVE RADIATION

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Abstract. The present study has been conducted to evaluate the effects of non-thermal microwaves (NTMws) and their late effects on hemoglobin (Hb) and other organs such as liver and kidney. In the present work, 40 Swiss male albino rats were distributed into four groups. Group 1 was used as a control. Groups 2 and 3 were exposed directly to microwave irradiation of frequency 3.5 GHz (low power) for periods of one and two months respectively. Like group 3, Group 4 was watched for 50 days after that exposure. Temperature inside the laboratory ranged between 25 °C to 27 °C. The liver function tests such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, albumin and total proteins and so the kidney function tests such as urea and creatinine were measured. The antioxidant reduced glutathione (GSH) and the oxidative stress parameter malondialdehyde (MDA) were measured. The biophysical parameters such as electrical conductivity and intrinsic viscosity of Hb were measured. All investigated liver enzymes were significantly increased compared with the control. Urea and Creatinine were significantly increased compared with the control. The applied microwave irradiation also caused a significant increase in the plasma lipid peroxidation marker (malondialdehyde, MDA) while a significant decrease in glutathione concentration was observed. Results consequently suggest that the redox potential of glutathione (GSH) and nicotinamide dinucleotide (NADH/NAD) were disturbed as a result of the exposure. The electrical conductivity of Hb was significantly increased in the microwave exposed rats compared with the control. The value of electrical conductivity of Hb reaches its maximum value following the increase in duration of microwave exposure. The significant increase in electrical conductivity of Hb molecule may be due to the increase in its surface charge density which increases charge transfer through the medium. The intrinsic viscosity of Hb was significantly increased compared with the control. Delayed effect studies proved that the microwave irradiation may cause injuries to the blood generation system and other tissues.

Key words: microwaves radiation, hemoglobin, electrical conductivity, oxidative stress.

INTRODUCTION

In spite of previous studies, knowledge about the adverse effects of RF/MW radiation on human health, or the biological responses to RF/MW radiation

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exposure, is still limited. Many consumers, industrial products and applications used some forms of electromagnetic energy. One type of electromagnetic energy that is of increasing importance worldwide is radiofrequency (RF) energy, including radio waves and microwaves, which are used for providing telecommunications, broadcast and other services.

The expanding use of RF technology has led some people to speculate that "electromagnetic pollution" is causing significant risks to human health from environmental RF electromagnetic fields. Effects of radiofrequency energy and electromagnetic fields on biological systems have become more important in our world today. They become increasingly existent in the environment, medicine, research and industry.

Modern lifestyle of human was dependent on electrical appliances such as televisions, computers, microwave ovens, mobile telephone and many other devices in his life. These devices operate at frequency of 50/60 Hz, which emit electromagnetic fields of few orders of millitesla. It was reported that this ELF EMF affects the various biochemical processes. Various surveys [5, 19, 27] and epidemiological studies [26, 43, 44] have been carried out to find the effects of these low frequency electromagnetic fields. Several studies have been carried out to investigate the effects on DNA [23, 35], enzyme activity [2, 3, 4, 11, 32, 33] and cells [6, 25]. Hemoglobin and other enzymes play a vital role in the biological processes; and also cell communication is facilitated by these biocatalysts. Any alteration in the activity of these enzymes may affect their functions.

Lai and Singh described effects of MWs on the rat brain cells measured by using a microgel electrophoresis assay [28]. These effects were significantly blocked by treatment of rats either with the spin-trap compound N-tert-butyl-α-phenylnitrone or with melatonin that is a potent free radical scavenger and antioxidant [29]. These data suggested that radicals might be involved in the effects of MWs. Oktem *et al*. [37] exposed rats to MWs from GSM900 mobile phone with and without melatonin treatment. Malondialdehyde (MDA), an index of lipid peroxidation, and urine N-acetyl-beta-d-glucosaminidase (NAG), a marker of renal tubular damage, were used as markers of oxidative stress-induced renal impairment. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate the changes of antioxidant status. In the MW-exposed group, while tissue MDA and urine NAG levels increased, SOD, CAT, and GSH-Px activities were reduced. Melatonin treatment inhibited these effects. The authors concluded that melatonin might exhibit a protective effect on mobile phone-induced renal impairment in rats [20, 37].

When the biological systems were exposed to an external magnetic field with very large strength relative to the biomagnetic field of the cells, a disturbance in their metabolic function is expected which leads to death of the cells or increases their cell division, Fadel *et al*. [9, 10] and Shin-Ichiro *et al*. [42].

Ghannam *et al*. [13] reported that the dielectric relaxation of hemoglobin molecule from exposed rats to 2 mT (50 Hz) magnetic field for a period of 15 and 30 days (8 h/day) indicate conformational changes, in addition to the less stabilization of Hb molecule. This result was supported by the pronounced increase in relaxation time and Cole-Cole parameters of hemoglobin molecule which indicates increase in its molecular diameter and change from spherical to non-spherical form, in addition to the redistribution of charges within and/or on the surface of the macromolecule.

The liver and kidney function tests are very important because the public hold the mobile phone in the nearest of liver and kidney in the lower abdomen part. So the aim of the present work is to investigate the functional properties of the liver and kidney as well as the oxidative stress of hemoglobin of rats exposed to 3.5 GHz of microwave radiation.

MATERIAL AND METHODS

ANIMAL PROTOCOL

In the present work, 40 Wister male albino rats were used, with body weight range 120–180 g. Rats were placed in plastic cages with dimensions 30×110×10 cm (10 rats in each cage) and housed in an animal house at about 25 °C.

MICROWAVE IRRADIATION

The whole body of each rat was exposed to modulated microwave irradiation with frequency 3.5 GHz (low power) emitted from MW transmitter (Poadhead Gar and ET Co, Cleveland, OHIO, USA). It has the maximum available frequency of 10 GHz with power density of about 0.50 W/m^2. The whole body average specific absorption rate (SAR) was calculated to be 1.0 W/kg. The animals were divided into four groups namely 1, 2, 3, and 4. Each group was composed of 10 animals.

During microwave irradiation, the rats of each group were placed in plastic cages at room temperature 25±0.5 °C and positioned in the wave guide device operating at 10 GHz, at a distance of 5 cm from the MW. The power density of the field was measured with a EM Radiation Monitor, types EMR-20 and 8.2 (Wandel and Golterman GmbH&Co., Germany), set to average mode. Mean total body specific absorption rates were estimated according to a radiation dosimetry handbook [8].

Group 1 was used as a control group. Groups 2 and 3 were exposed for 4 and 8 weeks respectively. Group 4 was exposed for 8 weeks (as group 3) and was left for 50 day post exposure and used for late effect study.

COLLECTION OF BLOOD SAMPLE

Following the end of exposure, each animal was slightly anaesthetized with ether, and then blood samples were collected by heparinated capillary tubes from the eye vein in heparin containing tubes and used for biochemical and hematological analysis.

BIOCHEMICAL ASSAYS

Some liver function tests such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin and bilirubin and so kidney function test such as urea were measured by using kits obtained from Roche, Germany, in the clinical laboratory centre of National guard hospital, King Saud Health Science University, Riyadh, Saudi Arabia.

Plasma creatinine and total protein levels were measured by the spectrophotometric method according to [24, 30] respectively.

ANTIOXIDANT AND OXIDATIVE STRESS PARAMETERS

Malondialdehyde (MDA)

Plasma Malondialdehyde MDA concentration was determined by using the method described by Draper and Hadley [9, 15] based on TBA reactivity. Briefly, 2.5 mL of 10% trichloracetic acid and 0.5 mL of plasma were added into tubes and mixed. After incubating for 15 min at 90 °C and cooling with cold water, the mixture was centrifuged at 3000 rpm for 10 min. Two milliliters of supernatant were taken and 1 ml of 0.675% TBA was added. The tubes were sealed and incubated at 90 °C for 15 min and then cooled to room temperature. The optical density was measured at 532 nm by a spectrophotometer.

Erythrocyte reduced glutathione (GSH)

Intra-erythrocyte GSH was determined with a colorimetric assay using Bioxytech GSH-400 kit (Oxis International, Portland, OR, USA) based on a two step reaction: thioethers formation followed by a β-elimination under alkaline conditions. Thioethers obtained are transformed into chromophoric thiones which have a maximum absorbance wavelength at 400 nm.

BIOPHYSICAL MEASUREMENTS

Electrical conductivity of Hb

Static electrical conductivity was measured using a conductivity meter type digimeter L21 aqualytic automatic compensator temperature. Measurements were

performed at constant frequency (1500 Hz in the range of 0 to 200 μSiemens/cm). The conductivity meter was calibrated before measurements using standard solution [7].

Intrinsic viscosity

The intrinsic viscosity η_{in} was calculated by the following equations:

$$\eta_{in} = \lim \eta_{red} / C \quad (1)$$

$$\eta_{red} = \eta_{sp} / C \quad (2)$$

$$\eta_{sp} = \eta_{rel} - 1 \quad (3)$$

$$\eta_{rel} = \eta/\eta_0 \quad (4)$$

where η, η_0 are the viscosities of Hb and distilled water as a function of density and flow time at room temperature; η_{sp}, η_{red} and η_{rel} are the specific, reduced and relative viscosities; C is the concentration of diluted hemoglobin [38].

Statistical analysis

Statistical analysis was performed to obtain the mean and standard error. The data analysis was performed using the SPSS-10 package (release 3, SPSS Inc., Chicago III). ANOVA test was used to compare between the means of the different parameters of the four studied groups. A difference was considered significant at probability $p < 0.05$.

RESULTS

Table 1 showed that the activities of all examined liver and kidney function tests significantly increased following the increase in duration of exposure to microwave irradiation compared with the control. Serum bilirubin, albumin and total protein levels were also affected by exposure to microwave irradiation (Table 1). The levels of serum bilirubin, albumin and total protein significantly increased following the increase in duration of exposure to microwave irradiation compared with the control.

Concerning the oxidative state, the results showed that the lipid peroxidation marker malonaldialdehyde (MDA) (Table 2) was significantly increased following the increase in duration of exposure to microwave irradiation compared with the control. On the other hand, the concentration of GSH (Table 2) was significantly decreased following the increase in duration of exposure to microwave irradiation compared with the control.

Table 1

Liver and kidney function tests of microwave exposed rats compared with the control

Group / Parameter	Group 1 control	Group 2 (4 weeks)	Group 3 (8 weeks)	Group 4 Delayed effect
ALT (U/L)	30.52±5.7	42.13±8.1	58.46±9.0*	84.12±10.6*
AST (U/L)	35.10±5.9	40.10±7.9	60.00±9.5*	73.23±9.7*
ALP (U/L)	138.66±11	156.25±12	165.00±13.4*	189.30±14*
Bilirubin (mg/dL)	0.442±0.1	0.502±0.1	0.658±0.2*	0.953±0.2*
Albumin (g/dL)	3.925±0.29	4.111±0.3	4.610±0.33*	4.950±0.39*
Total Protein (g/dL)	5.125±0.7	5.981±0.75	6.835±0.8*	7.913±0.8*
Creatinine (mg/dL)	0.93±0.16	1.4±0.18	2.6±0.25*	3.9±0.4*
Urea (mg/dL)	28.5±6.58	43.5±7.51	59.5±8.51*	72.23±9.01*

*$p < 0.05$. The data are represented as mean values ± standard error.

Table 2

Oxidative and antioxidant parameters of microwave exposed rats compared with the control

Group / Parameter	Group 1 control	Group 2 (4 weeks)	Group 3 (8 weeks)	Group 4 Delayed effect
Plasma MDA (μmol/L)	1.7±0.21	2.10±0.3*	3.53±0.42*	4.80±0.54*
GSH (mmol/L)	4.1±0.5	3.3±0.48*	2.4±0.39*	1.96±0.29*

*$p < 0.05$. The data are represented as mean values ± standard error.

Table 3 illustrates the electrical conductivity and intrinsic viscosity of hemoglobin of microwave exposed rats compared with the control. Electrical conductivity and intrinsic viscosity of hemoglobin were significantly increased following the increase in duration of microwave irradiation compared with the control.

Table 3

Electrical conductivity and intrinsic viscosity of Hb of microwave exposed rats compared with the control

Group / Parameter	Group 1 control	Group 2 (4 weeks)	Group 3 (8 weeks)	Group 4 Delayed effect
Electrical Conductivity (μS/cm)	40±0.23	53±0.39*	73±0.51*	93±0.63*
Intrinsic Viscosity (Poise)	3.5±0.08	5±0.1*	6.5±0.16*	8.4±0.20*

*$p < 0.05$. The data are represented as mean values ± standard error.

DISCUSSION

Our results showed that ALT and AST were significantly increased following the increase in the duration of microwave exposure. Both alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as reported, are specific liver enzymes that increase in hepatic diseases and toxic damage of liver cells [38]. It was found that these enzymes were significantly increased under the effect of 50 Hz magnetic field which also enhanced the concentration of alkaline phosphatase [31, 40]. On the other hand, the elevation in the levels of serum bilirubin, albumin and total protein, observed in the present study, may result from the damaged cells which leak into circulation after exposure to magnetic field [36]. It was also found that in vivo exposure to a pulsed magnetic field at 1.5 mT caused significant changes on plasma proteins in rats [20]. This observation supports the hypothesis that the state of physiological equilibrium of a biological system is crucial to its response to a potentially effective electromagnetic field [41].

Our results showed that the kidney function tests such as urea and creatinine were significantly increased following the increase in the duration of microwave exposure. Similar results by Oktem *et al*. were recorded [37].

Electromagnetic fields may affect biological systems by increasing free radicals, which mainly enhanced lipid peroxidation and changed the antioxidant activities of human blood thus leading to oxidative stress.

The lipid peroxidation level was significantly increased while a significant decrease in reduced glutathione content was recorded. These results indicated that there is an association between the exposure to microwave radiation and the oxidative stress through distressing redox balance leading to physiological disturbances. Therefore, we speculated that the changes induced by microwave irradiation may have cascade effect on the biochemical processes.

The observed increased level of total lipid peroxidation, in this study, was an indication to a high production of free radicals during the microwave exposure. These free radicals are highly oxidative moieties which directly affect the lipid membrane producing oxidative products such as GSSG and lipid peroxidation that increased also in many diseases and in tissues poisoned by a variety of toxins [14].

The results of this study also showed that a significant decrease in plasma GSH, accompanied by an increase in plasma lipid peroxidation marker Malondialdehyde after exposure to microwave radiation. GSH depletion could be related to its involvement in the detoxification of the deleterious effects of the increased free radicals produced within the cell [17].

It was also observed that when free hydroxyl radicals increased, the activities of antioxidant enzymes decreased resulting in a decrease in GSH/GSSG ratio. This was accompanied by an increase in the content of lipid peroxidation resulting in an oxidative stress [12]. Reduced glutathione depletion renders the animal more susceptible to free radicals-mediated damages, especially the damage induced by

cellular lipid peroxidation. It has been observed that reduced glutathione depletion is accompanied by an increase in the amount of total lipid peroxidation in experimental animals due to the deflator of GSH [34].

Electrical conductivity can provide significant information about the molecular arrangement of the red blood cells as well as the electrical conduction mechanism. The electrical conductivity of hemoglobin of rats exposed to microwave irradiation was significantly increased compared with the control (Table 3). The significant increase in electrical conductivity of Hb indicated to a partial charging of the hemoglobin (Hb) molecules and caused partial changes in the structural properties of Hb, which may affect their properties and so the RBCs physiological function [1].

Exposure to ELF magnetic field has reversible effects on N–H in plane bending and C–N stretching vibrations of peptide linkages and changes the secondary structures of α-helix and β-sheet in proteins [22]. Accordingly as a response to microwave radiation interaction, the exposure of animals to microwave irradiation was mediated by biochemical changes that could alter the state of biological structure and function.

Hassan *et al.* [18] showed that exposure to magnetic field promoted hemoglobin oxidation to metHb with the resultant broken hydrogen bonds between hydrophobic non-polar groups. This led to the unfolding of the globular protein with the formation of new groups exposed to the surface beside the polar hydrophilic groups leading to the increase of electrical conductivity.

The significant increase in the intrinsic viscosity of Hb may be due to the unfolding of Hb molecule or to the increase in the size of Hb [13, 16, 18].

CONCLUSION

The microwave radiation affects the oxidative state of the liver, kidney and hemoglobin macromolecule. The delayed effect of microwave irradiation on the rats was significantly higher than those of fractionated doses of the same type of radiation. On the basis of present results, it can be concluded that exposure to acute doses of microwave radiation is more hazardous than that produced by fractionated doses of the same type of radiation.

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