Modulator Role of Grape Seed Extract on Erythrocyte Hemolysis and Oxidative Stress Induced by Microwave Irradiation in Rats

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Abstract

This study was conducted to investigate firstly, the oxidative stress and erythrocyte hemolysis induced by exposure to 2450 MHz continuous wave microwave (MW), which is the carrier of signals emitted by cellular phones. Secondly to evaluate the possible protective role of grape seed extract. Male rats were randomly divided into four groups, the first group was considered as control, the second group was exposed to microwave at frequency 2450 MHz alone, the third group was supplemented by grape seed extract (GSE 200 mg/kg b.wt) and the fourth group was exposed to MW but was also supplemented by (GSE 200 mg/kg b.wt) before irradiation. The level of Malondialdehyde (MDA), an index of lipid peroxidation (LPO), was estimated and used as a marker of oxidative stress. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were expressed to evaluate the changes of antioxidant status. Also erythrocyte osmofragility test was assessed to determine rate by which red blood cells will undergo hemolysis. The level of malondialdehyde significantly elevated and the levels of antioxidative enzymes significantly decreased, in addition the erythrocyte hemolysis rate increased in the MW group compared to the control group. Oral administration of GSE exhibited marked amelioration of LPO, antioxidative enzymes, and rate of hemolysis which returned to normal values during the course of GSE treatment. It could be concluded that GSE has potent antioxidant effect that may be able to compensate MW-induced oxidative changes in the blood tissue by strengthening the antioxidant defense system. [New York Science Journal 2010; 3(6):66-73]. (ISSN 1554 – 0200).

Key Words: Microwave, Grape seed extract, Osmotic fragility, Oxidative stress.

Introduction

Microwave radiation is a type of non-ionizing electromagnetic radiation present in the environment, and is a potential threat to human health. Today, non-ionizing radiation has increasingly been used in industry, commerce, medicine, and for private purposes, especially in mobile telephone usage. Although average exposure levels are low compared to exposure limits, there is a growing public concern about the potential hazard of exposure to these frequencies for human health (Breckenkamp et al. 2003, ICNIRP 2004, Jauchem 2008).

It is well known that low frequency microwaves include the more harmful fractions. Yet the design of new high frequency machines has also increased the harmful fractions. Reports of hypertension, headaches, memory failure, brain damage, dementia, abortion, and breast cancer have been related to exposure to strong microwaves (Radovanobicic et al. 1994). High frequency microwaves increase the temperature of human tissue, which is especially harmful to reproduction and fertilization organs and to blood forming cells (Trosic 2001, Trosic et al. 2004).

It is also known that microwaves generate free radicals that accelerate the aging process in human tissue, and promote adult chronic diseases and cancer. Waters et al. (2007) reported that microwaves generate harmful oxygen radicals that lead to DNA damage. Bavincoba (1993), moustafa et al. (2001) also observed that 2.45 GHz microwaves produce an increase in lipid peroxide. With the introduction of cellular telephones, even more public attention has been drawn to the possible bioeffects of low-level exposure to radiofrequency and microwave radiation (rf/MW). Many epidemiological studies have addressed the possible links between exposure to rf/MW fields and excess risk for cancer (Tice et al. 2002, Vijayalaxmi 2004).

A part of a larger investigation designed to determine biological indicators of microwave radiation after whole-body exposure of rats were the studies of (Trosic et al. 2002 , Garaj-Vrhovac et al. 2009) which described the incidence of cytogenetic damage as assessed by the micronucleus assay, and incidence of DNA damage as assessed by comet assay. Grape seed extract (GSE) contains a number of polyphenols including proanthocyanidins and procyanidins (Bagchi et al. 2003, Thomas et al. 2009). Recent studies reported that Proanthocyanidins exhibited potent antioxidant properties in both lipid peroxidation (Ariga 2004, Fing et al. 2005) and oxidative cell death (Li et al. 2004). Also grape seed proanthocyanidins extract (GSPE) has multiple health benefits due to antioxidant/antiradical activity due to a high degree of oxygen free radical scavenging potential (Devi et al. 2006).

In addition to antioxidant/antiradical of (GSPE) activity, were shown to possess many biological properties including the inhibition of DNA damage (Balu et al. 2006), prevent chromosomal damage in human lymphoblastoid cells (Sugisawa et al. 2004), prevent oxidative injury by modulating the expression of antioxidant enzyme systems (Ariga 2004), modulation of lipid metabolism and inhibition of low-density lipoprotein oxidation (Devi et al. 2006, Balu et al. 2006).

The aim of this study was to test the hypothesis that the exposure to 2450 MHz MW microwave radiation induces oxidative stress in enzyme activities of antioxidative system and erythrocyte hemolysis, and to investigate the protective effect of grape seed extract in rats.

Materials and Methods

Animal Model

Adult male Wistar rats (11 weeks old, and average body weight 250 g) obtained from the Laboratory Animal Production Unit of National Research center were used in the study. The experimental group of animals (N = 40) were kept in an environment of controlled temperature (24–26ºC), humidity (55–60%), and received standard laboratory food and water ad libitum, with alternating 12 h light and dark cycles. They were under responsibility of veterinary in compliance with the Guide for the Care and Use of Laboratory Animals for Scientific Purposes. The experimental protocols were approved by the Ethics Committee of National Research Center, Egypt. Animals were kept for 1 week before the start of the experiment. The untreated sham exposed and the exposed animals were kept in the same conditions.

Microwave Exposure System

The experimental groups were exposed to 2450 MHz continuous waves MW for 1 h daily for 30 days and every day at the same hour. Animals were placed in individual Plexiglas cages and exposed to the MW oven (550 Watt, 2450 MHz Imperial V- 8505T Model). The device was modified to supply nonthermal conditions by water–cooled coils. Thus, the experiments carried out at 37°C (±1 °C). The power density of the field within the individual cages was measured with an EM Radiation Monitor, (Wandel & Golterman GmbH & Co., Germany) at 'average mode' option. Mean total-body specific absorption rates were estimated according to standard procedures (ICNIRP 1998) (2 W/kg) and average power density (0.251 mW/cm2).

Experimental design

The animals were classified into four groups, 10 rats in each:

Group 1: control (untreated sham exposed) group. Group 2: microwave irradiated group. Group 3: rats were orally supplemented with grape seed extract by means of stomach needle at dose 200 mg/kg b.wt. Group 4: rats were orally supplemented with grape seed extract at dose 200 mg/kg b.wt. half hour before MW irradiation.

The grape seed extract (GSE) was composed of 89.3% proanthocyanidins, 2.24% moisture, 1.06% protein, 0.8% ash and 7.0% gallate ester (Agli et al. 2004). The obtained GSE was dissolved in water.

Blood Sampling

Animals were anesthetized and whole blood samples were collected by cardiac puncture at the end of experimental period in heparinised tubes containing lithium heparin as anticoagulant. After collection, blood was divided into many samples according to experimental groups.

Analytical Procedures

Blood lipid peroxidation was measured as the amount of malondialdehyde (MDA) formed employing thiobarbituric acid as described by Yoshioka et al. (1979) using a spectrophotometer (Shimadzu UV-1601, Japan). Activity of the antioxidant scavenger enzymes glutathione peroxidase (GSH-Px), Superoxide dismutase (SOD) and catalase (CAT) were measured spectrophotometrically according to the methods of Paglia and Valentine (1967), Klamt et al. (2002) and Aebi (1984) respectively.

Osmofragility test was carried out within two hours of collection of blood. The osmotic fragility of the membrane can be measured by placing the red blood cells in hypotonic salt solutions, the osmotic pressure exerted by the diffusion of water into the cells, makes them first swell and then hemolyse. The osmotic fragility measures the capacity of the cells to withstand hypotonicity and resist hemolysis, which is determined by their volume to surface area ratio (Mazeron et al. 2000). red blood cells were added to varying concentrations of buffered sodium chloride solution NaCl buffered to pH 7.4 and kept at

25°C. The amount of hemolysis in each saline concentration was then determined by reading the absorbance of supernatant at 450 nm using a double beam UV/VIS spectrophotometer model-240 manufactured by shimadzue - Japan. This procedure was followed for the treatment of each blood sample collected from the animals

Statistical analysis

All the experimental data were expressed as the means ± SE. Each experiment was

carried out at least three times. Statistical significances of differences between two groups were determined using Student's t-test. All P values were two-sided, and $P < 0.05$ was considered statistically significant.

Results

The data of the present study revealed that exposure to frequency 2450 MHz, 0.25mW/cm^2 continuous waves MW for 1 h daily for 30 days induced significant decrease in antioxidant enzymes activities of glutathione peroxidase (GSH-Px) (P< 0.001), catalase (CAT) (P< 0.05) and superoxide dismutase (SOD) (P< 0.001) when compared to control group as illustrated in (Table 1). Such decrease was accompanied with significant increase in malondialdehyde level which indicates lipid peroxidation degree (MDA) (P< 0.001), (Fig. 1).

Regarding the data recorded in (Table 1) , on comparing with microwave irradiated group, it was observed that administration of grape seed extract protector either prior to MW radiation exposure or to normal groups significantly increased the activities of (GSH-Px) (P< 0.05, P< 0.001), (CAT) (P< 0.05, P< 0.05) and (SOD) (P< 0.001, P< 0.001) respectively. The data in (Fig.1), revealed a significant decrease in the level of malondialdehyde on administration of grape seed extract protector prior to MW radiation exposure (P< 0.05) and GSE group (P< 0.001) as compared with MW irradiated group.

Table 1. Effect of grape seed extract (GSE) on glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) activities in normal and rats exposed to microwave (MW) radiation.

Groups	GSH-Px U/ min / ml	CAT µmol / min /ml	SOD Nmol / ml
Control	43.52 ± 2.19	9.81 ± 1.08	1.16 ± 0.06
MW Radiation	30.41 ± 3.01[a]	5.12 ± 0.72[a]	0.49 ± 0.02[a]
GSE protector	50.60 ± 4.71[b]	10.49 ± 1.67[b]	1.03 ± 0.05[b]
MW Radiation + GSE protector	39.11 ± 2.03[b]	8.03 ± 1.56[b]	0.76 ± 0.03[a,b]

Each value represent a mean of 10 rats ± SE
[a] significantly different from control group
[b] significantly different from MW irradiated group

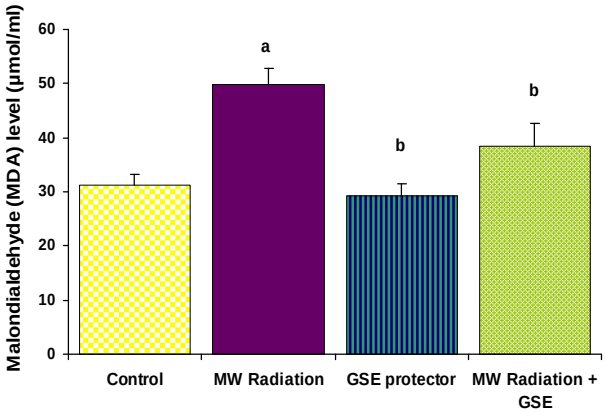

Figure 1. Effect of grape seed extract (GSE) on malondialdehyde level (MDA) in rats exposed to microwave (MW) radiation.

Data are represented as mean of 10 rats± SE
[a] significantly different from control group
[b] significantly different from MW irradiated group

Fig. 2 showed the results of osmotic fragility measurements for the RBCs collected from animals of the different groups, where the percentage of hemolysed cells was plotted as a function of the concentration percentage of NaCl. For analysis of these results, the curves were differentiated and plotted as a function of NaCl concentration percentage as shown in (Fig. 3).

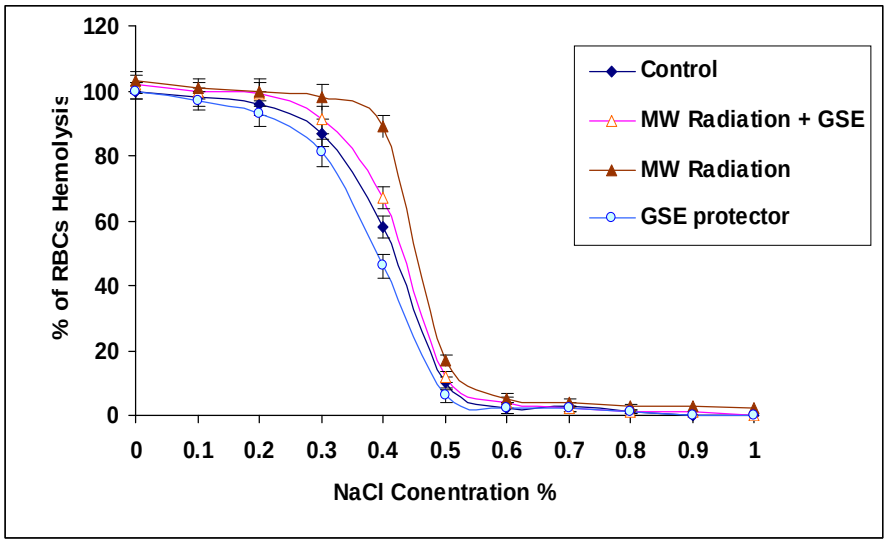

Figure 2. Variation of the hemolysis percentage of the RBCs as a function of the surrounding NaCl concentration percentage in the saline medium for different sample groups of the study. Control (—■—), MW Radiation (—▲—), GSE Protector (—○—) and MW Radiation + GSE protector group (—△—).

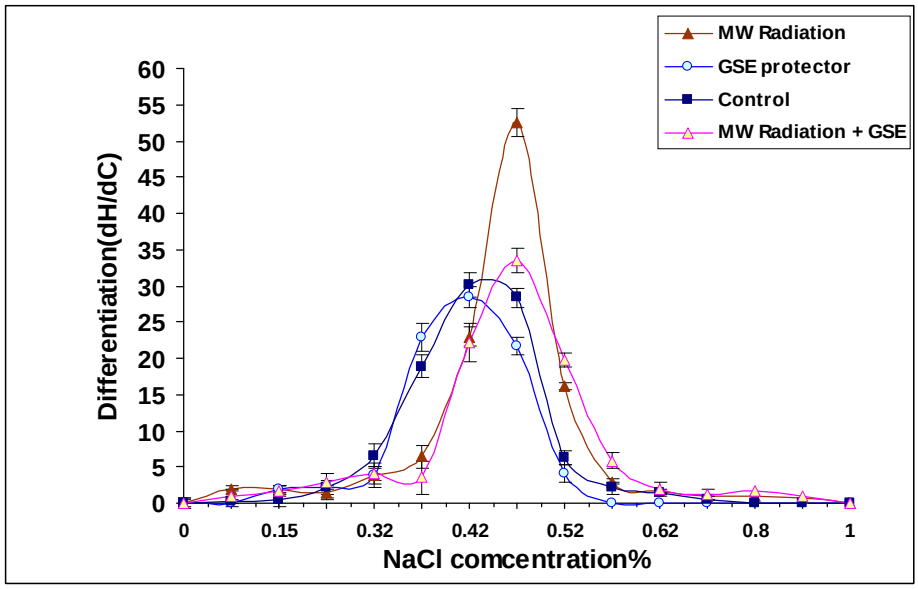

Figure 3. Differentiation curves for different sample groups for Control (—■—), MW Radiation (—▲—), GSE Protector (—○—) and MW Radiation + GSE protector group (—△—).

As a result of this treatment each characteristic plot in (Fig. 2) was represented by a peak in (Fig. 3). From (Fig. 2 and 3), the mean corpuscular fragility (MCF) (the NaCl concentration at which 50% of RBCs are hemolyzed) and

the maximum half width ($W_{h\ max}$), (indicates the elastic range of the RBCs cellular membrane) were calculated as given in (Table 2).

Table 2. Red blood cells mean corpuscular fragility (MCF) calculated in g% NaCl, and the corresponding maximum half width ($W_{h\ max}$) for different groups

	MCF (g% NaCl)	$W_{h\ max}$
Control	0.41 ± 0.09	0.172 ± 0.043
MW Radiation	0.49 ± 0.06	0.110 ± 0.039
GSE protector	0.39 ± 0.07	0.197 ± 0.025
MW Radiation + GSE protector	0.43 ± 0.11	0.131 ± 0.036

The results of osmotic fragility test revealed that exposure to microwave radiation caused shift to the right of the hemolysis curve indicating increase in the mean corpuscular fragility (MCF) as shown in (Table 2). The widths at half maximum of these differential plots represent the relative elastic limit of RBCs membrane. The increase of $W_{h\ max}$ indicates the increase of cellular membrane elasticity. The results indicated that both RBCs membrane elasticity and permeability decreased due to exposure of the animals to MW radiation. Administration with Grape seed extract either to normal or pre-exposure to MW radiation improved the values of MCF and $W_{h\ max}$.

Discussion

The biological effects of nonthermal MWs are dependent on such physical parameters as frequency, intensity, polarization, and modulation (Banik et al. 2003, Belyaev 2005, Lai 2005). There is growing evidence that the effects of microwave irradiation are mediated by the formation of reactive oxygen species (ROS) and free radicals, which are highly reactive, removing hydrogen atoms from fatty acids, causing lipid peroxidation and consequently cell death. (Stopczyk et al. 2002, moustafa et al. 2001).

The results obtained in the present study revealed significant acceleration in the oxidation of lipid associated with depletion in antioxidant enzymes contents due to microwave irradiation. It was suggested that the oxidant / antioxidant disequilibrium due to oxidative stress is the main cause of excessive formation of peroxides as MDA (Ilhan et al. 2004). In agreement with our lipid peroxidation and oxidative damage results many authors suggested that UHF-EMF and microwave radiation might also increase free radicals formation, based on the assumption that reactive oxygen species (ROS) are implicated in several types of tissue injury (Zmyślony et al. 2004, Oktem et al. 2005, Yurekli et al. 2006 , Sokolovic et al. 2008).

A number of trials were conducted to assess potential health risk of microwave radiation due to oxidative stress (Stopczyk et al. 2002, Vander Vorst et al. 2006 , Yurekli et al. 2006). The present study showed that exposure to MW with a frequency of 2450 MHz had a significant effect on rat red blood cells suggesting that ROS were generated under the experimental conditions employed. A significant increase was observed in MDA level and significant decrease in antioxidant activities in the exposed group. The change in activities of antioxidant enzymes with MDA levels may be regarded as an indicator of increased ROS production occurring during the exposure period and may reflect the pathophysiological process of the exposure to microwave radiation. These continuously produced ROS are scavenged by SOD, GSH-Px and CAT. Under some circumstances, these endogenous antioxidative defenses are likely to be perturbed as a result of overproduction of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in tissue. It was demonstrated in numerous studies that ROS are directly involved in oxidative damage of cellular macromolecules such as lipids, proteins, and nucleic acids in tissues (Oktem et al. 2005, Simko et al. 2006).

Administration of GSE significantly stimulated the reduced activities of SOD, GSH-Px and CAT in blood tissue over those measured in the MW-exposed and control groups. The potent efficacy of GSE as antioxidant in suppressing lipid peroxidation was reported before (Feng et al. 2005, Balu et al. 2006). It was found that the free radical scavenging capacity of GSE is 20 times more effective than vitamin E and 50 times more effective than vitamin C on a weight/volume basis (Shi et al. 2003). GSE showed potent antioxidant activity by trapping free radicals (hydroxyl , lipid free radicals, free iron molecules and lipid peroxides), delaying fat oxidation, inhibiting the major substance responsible for generating oxygen derived free radicals (xanthin oxidase) and reducing the concentration of H_2O_2 produced by the oxidative stress (Sugisawa et al. 2004). The antioxidant activity of grape extract was evaluated by measuring it's capacity to scavenge hydrogen peroxide and to decrease in the rate of peroxide formation, the antioxidant activity of the extract increased when the extract concentration increased (Baydar et al. 2007). Accordingly our results were in agreement with previous data, the ameliorated and the radiprotective effect of GSE could be explained by its

ability to trap harmful free radicals and consequently decrease their cytotoxic effect.

The adverse effects of of 2.45 GHz radiation (0.25 mW /cm^2) on the erythrocyte membrane were confirmed by measuring the Hb osmofragility (expressed as the degree of hemolysis) after exposure to radiation and after treatment with GSE protector. Our results illustrated change in the RBCs osmotic fragility of exposed animals which reflects changes in the properties of RBCs membranes. It was reported by (Savopol et al. 1996) that power levels, 2.45 GHz irradiation induced significant Hb loss due to transient permeabilization of irradiated erythrocytes. The degree of hemolysis increased with increasing microwave power density.

Free radicals formed during microwave irradiation can cause a variety of membrane changes including lipid peroxidation, amino acid residue damage in membrane proteins and lipid-protein crosslinks (Schon et al. 1994). Kuchel et al. (1997) reported that modification in the physical condition of the proteins on the cell membrane leads to change in the permeability of the RBCs membrane. Some proteins on the cell membrane (ion channels) act as pores through which the liquid (water) bound to ions carried inside the cell. The applied radiation may cause variation on the electrical charge distributed on the RBCs membrane. The redistribution of the electrical charges may affect the ion channels proteins as a cause of unbalance in the ionic concentration, which may leads to Changes in membrane structures and affect the cytoskeleton. The combined effects of free radicals on the red blood cell membrane and cytoskeleton may contribute to the leak of hemoglobin out of the cells. The hemolysis of the red blood cells reflects the loss of integrity of the cells which can leads to the liberation of intracellular hemoglobin (Schon et al. 1994, Ali et al. 2003). The width at half maximum of the differential plot ($W_{h\ max}$) as shown in (Fig. 3) (Table 2), represents the elastic limit of RBCs' membrane. The increase in $W_{h\ max}$ represents the increase in cellular membrane elasticity, so the elasticity of RBCs membrane decreased as a result of MW exposure.

Treatment by GSE returned the erythrocyte membrane elasticity to its normal trend. Wang et al. (1999) reported that proanthocyanidins could reduce the aggregation of platelets, increase the red blood cell, decrease blood viscosity and increase the fibrinolytic activity. In addition, proanthocyanidins improve the oxygen carrying capacity of blood, and increase the strength and elasticity of blood vessel wall by binding with collagen. Flavonoids in general and proanthocyanidins in particular are free of side effects, since they are water soluble, any excess proanthocyanidins are excreted via water or urine. GSE is effective in preventing the oxidative stress associated loss of membrane surface charge which maintains the erythrocyte membrane integrity and function (Balu et al. 2005, Devi et al. 2006). According to the results obtained it could be concluded that grape seed extract is acting in concert science it has potent antioxidant activity.

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