

# Vitamin E protects rat testis, eye and erythrocyte from oxidative stress during exposure to radiofrequency wave generated by a BTS antenna model

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## ABSTRACT

**Background:** Radio frequency wave (RFW) generated by mobile phones and wireless communication systems has been reported to cause adverse effects on reproductive function, vision and hematological parameters, possibly through oxidative stress. The aim of this study was to evaluate the effect of RFW generated by base transceiver station BTS on oxidative stress in testis, eye and erythrocyte, and the prophylactic effect of vitamin E by measuring the antioxidant enzymes activity, including: glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), and lipid peroxidation. **Materials and Methods:** Thirty-two adult male Sprague–Dawley rats were randomly divided into four groups and treated daily for 45 days as follows: control, treatment control (vitamin E 200 mg/kg of body weight/day by gavage), sham exposed group (exposed to 900 MHz RFW), and treatment group (received vitamin E and exposed to RFW). Control and treatment control groups were not exposed and were only given the vehicle, i.e., distilled water. On the last day of the study, all the rats were sacrificed and their testes, eyes and erythrocyte were collected and used for measurement of antioxidant enzymes activity and lipid peroxidation. **Results:** Exposure to RFW in the sham exposed group decreased antioxidant enzymes activity and increased lipid peroxidation compared to both control groups ( $p < 0.05$ ). In the treatment group, vitamin E improved antioxidant enzymes activity and reduced lipid peroxidation compared to the sham exposed group ( $p < 0.05$ ). **Conclusion:** RFW causes oxidative stress in eye, testis and erythrocytes and vitamin E improved oxidative stress in these tissues.

**Keywords:** Oxidative stress, radio frequency wave, testis, eye, erythrocyte, vitamin E.

## ► Original article

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## INTRODUCTION

Exposure to radio frequency wave (RFW) emitted from mobile phones and wireless communication systems has caused public concern regarding the possible adverse effects on human health. The health effects of RFWs emitted from these devices have been greatly debated and the exact pathophysiological mechanism(s) of their related health impacts are not entirely known. It was shown previously that non-thermal RFWs exposure could induce several changes in blood-brain barrier permeability<sup>(1)</sup>, altering heat shock proteins<sup>(2)</sup>,

neurotransmitter functions<sup>(3)</sup>, genetic<sup>(4-6)</sup> {Karaca, 2012 #4; McNamee, 2016 #49} and oxidative stress<sup>(7, 8)</sup>, leading to functional changes in the cells. Frequency, duration of exposure and distance from source of RFW are important factors which affect biological responses. Reports of potential adverse effects of RFWs on the heart<sup>(9)</sup>, brain<sup>(10)</sup>, cerebellum and encephalon<sup>(11)</sup>, eye<sup>(7)</sup>, testis<sup>(12)</sup>, liver and kidney<sup>(13)</sup>, epididymal sperm<sup>(14)</sup>, the endocrine system<sup>(15)</sup>, the nervous system<sup>(16)</sup>, hematological parameters and bone marrow<sup>(17)</sup> are available. Effects of exposure to RFWs on the testes, eye and blood cells have been of great

concern, because their continuous exposure to radiation impaired these tissues functions, possibly through oxidative stress.

Use of mobile phones and Wi-Fi devices was reported to affect some of the reproductive parameters and can cause infertility in human and animal model by decreasing the sperm count, motility, viability and normal morphology or increasing oxidative stress<sup>(18-22)</sup>. As a result of the continuous exposure of the eyes to environmental chemicals, radiation, and atmospheric oxygen, they are important and special organ. It has been proposed that oxidative stress in ocular tissues play a role in pathogenesis of diseases such as cataracts, glaucoma, uveitis, pseudo exfoliation syndrome, and age-related macular degeneration<sup>(23, 24)</sup>. Membrane fluidity of erythrocytes can be affected by oxidative stress<sup>(25)</sup>. Erythrocytes are especially vulnerable to oxidative stress because of 1) the pentose phosphate pathway. 2) active metal protein (hemoglobin), which function as an oxidase and peroxidase, 3) membrane proteins and unsaturated fatty acids (mostly arachidonic acid) which can be oxygenated, and 4) higher tension oxygen than any other cells in the body, with the exception of lung cells<sup>(26)</sup>. Oxidative damage in the erythrocytes can lead to loss of cell function. The results of this phenomenon lead to loss of symmetrical structure of cell membrane lipids, loss of flexibility, impaired water and ions exchange and, ultimately, cell swelling<sup>(27)</sup>. Vitamin E is a major lipophilic chain-breaking antioxidant present in the cell which protects membrane polyunsaturated fatty acids (PUFA)<sup>(20, 28)</sup>. Vitamin E interrupts the lipid peroxidation process and provides a protective effect against several diseases including coronary artery disease and carotid atherosclerosis<sup>(29,30)</sup>. This study was conducted to evaluate the effect of 900MHz (which is used in BTS antenna) RFW s-induced oxidative stress in the rat testis, eye and erythrocytes and the prophylactic effect of vitamin E on these tissues by measuring antioxidant enzymes activity including: glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA).

## MATERIALS AND METHODS

### Animals

Thirty-two adult male Sprague-Dawley rats (220±15gr) were housed (eight rats per cage) in the animal room under controlled lighting (12 h light: 12 h darkness) and temperature (20±2°C) conditions and had free access to a pelleted food and tap water. All of the experimental procedures were conducted between 09:00 and 13:00. This study was accomplished under the approval of the State Committee on Animal Ethics, Shiraz University, Shiraz, Iran. Also, the recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the standards in the protection of animals used for experimental purposes were followed.

### Radio frequency signal generator

The signal generator for producing a 900 MHz signal was made in the Department of Electrical Engineering, Shiraz University, and the output was monitored by a spectrum analyzer (FSH6, from Rohde and Schwarz, Germany) to ensure the correct forward power from the custom-designed mobile base stations on the animals exposed and its usage has already been reported<sup>(11)</sup>.

### Experimental design

The effect of radio frequency wave (900 MHz) (Power density 0.6789mW/cm<sup>2</sup>) on the oxidative stress biomarkers in the testis, eye and erythrocyte of male rat and the protective role of vitamin E were studied by dividing the animals into four groups, each cage included eight animals treated orally as follows:

Group 1: The control group (received vehicle, i.e., distilled water).

Group 2: The treatment control group which received vitamin E (200 mg/kg BW/day) orally by gavage.

Group 3: The sham exposed group was exposed to RFW of 900MHz (received vehicle, i.e. distilled water).

Group 4: Treatment group, received vitamin E orally by gavage (200 mg/kg BW/day) before exposure to RFW.

Group 3 and 4 Animals Cages were placed at

the same distance (5 meters) from signal generator and were exposed to the RFW 4 h/day (between 9:00 and 13:00) during a period of 45 consecutive days. Both control groups were placed in the same conditions in a separate room without applying the RFW. The SAR for whole body was 0.15 W/Kg. No detectable rectal temperature change was observed during exposure to RFW.

### **Hemoglobin preparation**

On the final day of exposure to RFW, all rats were sacrificed, by whole blood collection through heart puncture. The heparinized blood was centrifuged to remove plasma components. The packed red cells were washed three times in saline solution (0.9% NaCl) and red blood cells were osmotically lysed with cold distilled water (2 ml). Hemoglobin (Hb) was measured using cyanmethemoglobin method<sup>(31)</sup>.

### **Sampling and tissue preparation for enzyme assay**

Following sacrificing of rats the left testis and right eye were quickly removed and carefully dissected from the surrounding fat and tissue and immediately rinsed in ice cold saline. The testis was manually homogenized in cold phosphate buffer (pH 7.4, 0.1 M) and centrifuged at 3000g for 10 min. The upper clear supernatants were then recovered and stored at -70°C for evaluation of enzymes activity and protein assays.

### **Biochemical analysis**

#### **Measurement of superoxide dismutase (SOD) activity**

Total SOD activity was measured in the samples with SOD detection kit (RANSOD kit produced by RANDOX Company, Northern Ireland Antrim, UK) according to the manufacturer's instructions. SOD activity was recorded and expressed as unit per milligram of tissue protein (U/mg protein) and per gram of hemoglobin (U/gHb) for blood.

#### **Measurement of glutathione peroxidase (GPx) activity**

Glutathione Peroxidase Activity was

measured in the samples with GPx detection kit (RANSEL kit; RANDOX Company) according to the manufacturer's instructions. The GPx activity was expressed as unit per mg of tissue protein (U/mg protein) and per gram of hemoglobin (U/gHb) for blood.

#### **Measurement of catalase (CAT) activity**

Activity of tissue CAT was assayed spectrophotometrically by monitoring the decomposition of H<sub>2</sub>O<sub>2</sub> using the procedure of Aebi<sup>(32)</sup>. CAT activity was expressed as the unit that is defined as mmol H<sub>2</sub>O<sub>2</sub> consumed/min per mg tissue protein and amount of hemoglobin gram for blood.

#### **Measurement of lipid peroxidation (MDA)**

Lipid peroxidation (MDA) in testis and erythrocyte was evaluated by a modified HPLC method which is based on the reaction of MDA with thiobarbituric acid (TBA) to form a colored MDA-TBA adduct<sup>(33)</sup>.

#### **Protein content**

The total protein concentration in homogenated tissue was determined according to Lowry *et al.*<sup>(34)</sup>.

#### **Statistical analysis**

The results were statistically analyzed and expressed as mean  $\pm$  standard error of mean ( $\pm$ SEM). The Statistical Package for Social Sciences (SPSS-16.0) was used. The variables between groups were analyzed using one-way analysis of variance (ANOVA). Where a significant difference was found with ANOVA, the source of difference was located followed by Post Hoc multiple comparisons and Tukey test for comparison. Statistical significance was set at  $P < 0.05$ .

## **RESULTS**

The mean ( $\pm$ SEM) activity of GPx, SOD, CAT and MDA (as the biomarker for lipid peroxidation) in the rat testis, eye and erythrocytes is presented in figures 1 - 4, and in

table 1.

Following exposure to RFW, activity of SOD significantly decreased in this group compared to both control groups, while administration of vitamin E could significantly increase the activity of this enzyme compared to the other groups ( $p < 0.05$ ) (figure 1 and table 1). Exposure to RFW also significantly decreased the activity

of GPx and CAT compared to both control groups and administrated vitamin E could prevent this effect (figure 2 and table 1). Exposure of rats to RFW significantly increased lipid peroxidation products (as shown by MDA) compared to other groups, while pretreatment of rats by vitamin E improved MDA level significantly (figure 4 and table 1).

Table 1. Patient characteristics and scan parameters

	SOD (U/gHb)	CAT (U/gHb)	GPx (U/gHb)	MDA (mmol/L)
Control	14.65±391.482 <sup>a</sup>	27.31±1889.5 <sup>a</sup>	13.315±459.419 <sup>a</sup>	1.98±15.304 <sup>a</sup>
Treatment Control	15.94±359.49 <sup>a</sup>	34.98±1794.75 <sup>b</sup>	14.32±466.684 <sup>a</sup>	1.32±10.77 <sup>b</sup>
Sham-expose	11.31±223.702 <sup>b</sup>	27.65±1540.42 <sup>c</sup>	14.35±242.496 <sup>b</sup>	1.65±20.83 <sup>c</sup>
Treatment	19.36±402.684 <sup>a</sup>	38.98±1792.56 <sup>b</sup>	20.341±459.79 <sup>a</sup>	1.89±14.956 <sup>a</sup>

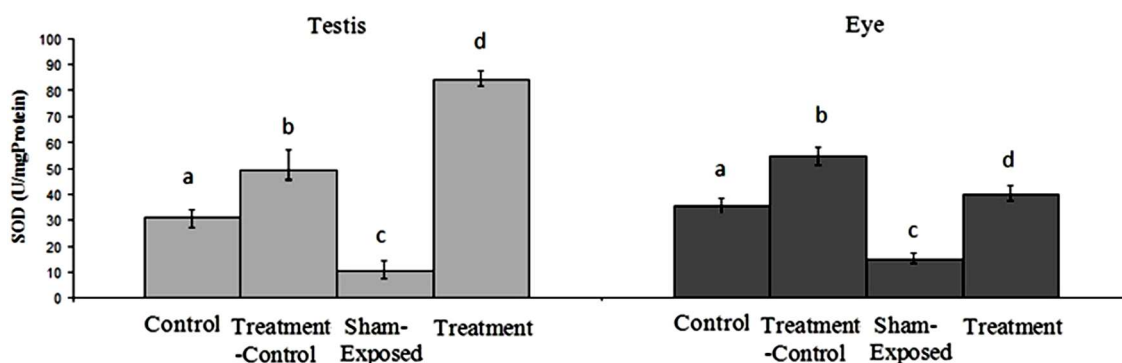


Figure 1. SOD activity in testes and eye significantly increased following treatment with vitamin E in RFW exposed group (n= 8). Values represent mean± SEM of enzyme activity (U/mg protein of tissue). Different alphabets show significant difference between groups in each tissue ( $p < 0.05$ ).

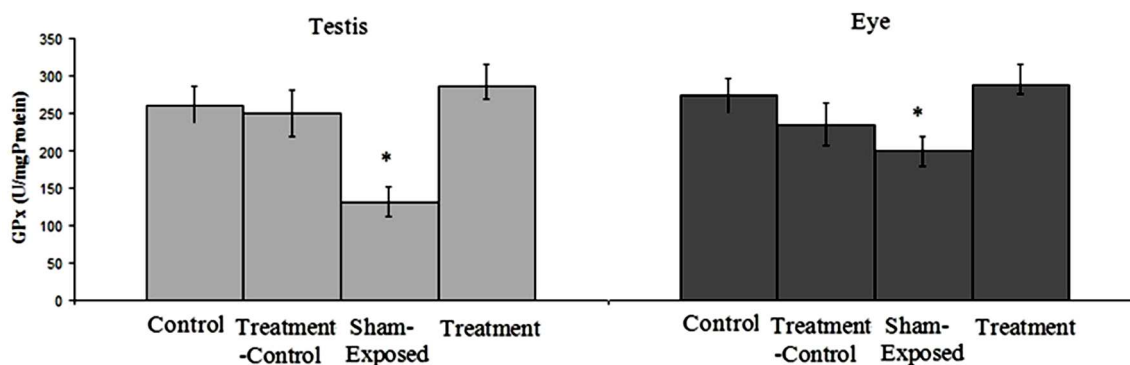


Figure 2. Comparison of mean (± SEM) GPx activity (U/mg protein of tissue) in testes and eye among different groups (n= 8). \*indicates statistical difference compared to other groups in each tissue ( $p < 0.05$ ).

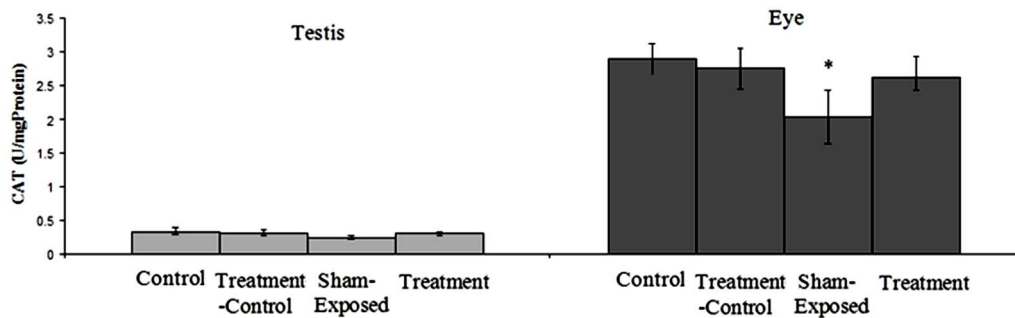


Figure 3. Comparison of mean ( $\pm$  SEM) of CAT activity (U/mg protein of tissue) in testes and eye among different groups (n= 8). Asterisk (\*) indicates statistical difference compared to other groups in each tissue (p<0.05).

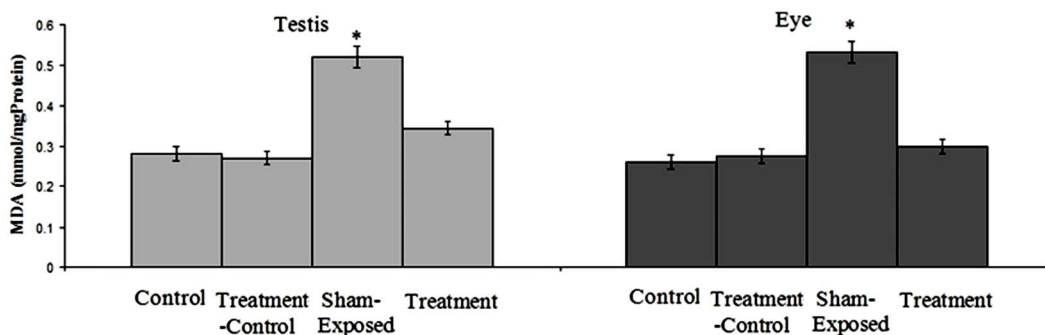


Figure 4. Comparison of mean ( $\pm$  SEM) MDA level (mmol/mg protein of tissue) in testes and eye among different groups (n= 8). Asterisk (\*) indicates statistical difference compared to other groups in each tissue (p<0.05).

## DISCUSSION

Our results showed significant increases in indices of oxidative stress in testis, eye and erythrocyte following exposure of animals to RFW, which is in agreement with previous reports (7, 8, 11-13, 18, 19, 35). Administration of vitamin E to exposed group could significantly reduce oxidative stress. Vitamin E was reported to improve antioxidant enzymes activity, lipid peroxidation and inflammation in asthenozoospermic patients (35) and cardiovascular disease (29,30,36). It also improves the erythrocyte deformability in sodium nitroprusside-induced oxidative stress by its antioxidant effects on the lipid peroxidation and antioxidant enzymes activity (37).

Non-thermal RFWs are able to increase free radicals and lipid peroxidation, and decrease antioxidant content and thereby induce oxidative stress. ROS production in plant and animal cells stimulate signaling pathways of cell's activities, which is due to changes of inner and outer physiological conditions. Oxidative stress results in series of events, deregulates the

cellular functions leading to various pathological conditions including: premature aging, cancer, neurodegenerative diseases, cardiovascular dysfunction, glaucoma, metabolic dysfunction of almost all vital organs and infertility (16, 19, 23, 24, 38). Non-enzymatic antioxidants system consists of vitamin E, A, C and proteins such as albumin, glutathione, etc. MDA is the by-product of the major chain reactions leading to the oxidation of polyunsaturated fatty acids and, thus, serve as a biomarker of oxidative stress-mediated lipid peroxidation (39). Deleterious effects of the oxidant/antioxidant imbalance in the testis, eye and other tissues exposed to electromagnetic waves emitted by mobile phone has been shown in experimental studies (7, 11, 12, 19, 38, 40-42). Exposure to electromagnetic fields (128 mT; 1 h/day, for 30 days) was reported to decrease the antioxidant enzymes activity (SOD, CAT and GPx), increased lipid peroxidation level and production of free radicals, particularly superoxide anion in rat testis (40). Agarwal *et al.* (2011) showed that chronic exposure to electromagnetic radiation reduced the enzymes activity of SOD, GPx and CAT and increased lipid

peroxidation<sup>(38)</sup>. Balci *et al.* (2007 and 2009) and Agarwal *et al.* (2011) reported that electromagnetic field emitted by mobile phone leads to oxidative stress due to increased MDA levels in the cornea and lens and other tissues<sup>(38, 41, 42)</sup>. It was reported that exposure to RFWs emitted from BTS (4 h/day, for 45 days) decreased the antioxidant enzymes activity and increased lipid peroxidation in the eye and testis<sup>(7, 12)</sup>.

Vitamin E, an important antioxidant in biological membrane, can neutralize free radicals. It appears that this effect is dose-dependent<sup>(43)</sup>. It scavenges all three important types of ROS, namely superoxide anion, H<sub>2</sub>O<sub>2</sub>, and hydroxyl radicals<sup>(20, 44)</sup>. A major antioxidant function of vitamin E is inhibition of lipid peroxidation<sup>(35, 45)</sup>. Devaraj *et al.* (2007) showed that high-dose of alpha-tocopherol supplementation reduces biomarkers of oxidative stress, lipid peroxidation, inflammation and carotid atherosclerosis in patients with coronary artery disease<sup>(29)</sup>. Suleiman *et al.* (1996) showed that vitamin E supplement can lead to decreased lipid peroxidation and increase sperm motility in asthenozoospermic patients<sup>(35)</sup>.

Arachidonic acid (a polyunsaturated fatty acid exists abundant in cytoplasm and in membranes of erythrocytes) is highly susceptible to oxidation both as a free fatty acid and as a component of phospholipid membranes. Superoxide radicals caused by auto-oxidation of hemoglobin level can alter unsaturated lipids in erythrocyte membranes, resulting in a loss of membrane fluidity and subsequent cell lysis<sup>(28)</sup>. The susceptibility of RBC to oxidation is strongly correlated with RBC vitamin E content<sup>(46, 47)</sup>. Sun *et al.* (2012) showed that vitamin E supplementation apparently decreases oxidative stress in healthy middle-aged to elderly people, at least in part by protecting erythrocyte membrane fluidity and reducing erythrocyte hemolysis, presumably because vitamin E dwells primarily in cell membranes where it protects polyunsaturated fatty acids from oxidative damage<sup>(48)</sup>.

Several lines of evidence indicate that among different forms of vitamin E, alpha-tocopherol

has potential protective effects with regard to cardiovascular disease<sup>(29, 30, 36)</sup> and reproductive damage caused by gossypol<sup>(49)</sup>.

Alpha-tocopherol supplementation in human subjects and animal models has been shown to decrease lipid peroxidation, superoxide production by impairing the assembly of nicotinamide adenine dinucleotide phosphate (reduced form) oxidase as well as by decreasing the expression of scavenger receptors (SR-A and CD36), particularly important in the formation of foam cells. It was shown that alpha-tocopherol therapy, especially at high doses, decreases the release of pro-inflammatory cytokines, the chemokine IL-8 and the plasminogen activator inhibitor-1 (PAI-1) levels as well as decreasing adhesion of monocytes to endothelium<sup>(36, 50)</sup>.

## CONCLUSION

Our results suggest that RFW leads to oxidative stress in eye, testis and erythrocyte, and vitamin E improved antioxidant enzymes and decreased lipid peroxidation and supports the hypothesis that dietary supplementation of vitamin E can effectively increase eye, testis and erythrocyte resistance to oxidative stress. Further study with different frequencies and exposure periods may be needed in order to discover the effects of radio frequency signal-induced oxidative stress in these tissues.

**Conflicts of interest:** Declared none.

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