

Silymarin and Vitamin E modulate 950MHz Electromagnetic Field-induced Oxidative Stress and Hormonal Changes in Male Albino Rats.

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Abstract: The aim of this study was to evaluate the effect of vitamin E, silymarin and their co-administration on oxidative stress and hormonal changes in rats whole body exposed to 950 MHz electromagnetic field (EMF) for 2months (2hrs/day, 3times/week). Vitamin E (1.35mg/Kg BW) and/or silymarin (18mg/Kg BW) were orally administered to rats for 2months before EMF exposure. Exposure to EMF provokes oxidative stress identified by significant increases in serum thiobarbituric acid reactive substances (TBARS), advanced oxidation protein products (AOPP) and protein carbonyl (CO) levels associated to significant decreases in superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities and glutathione (GSH) content. Oxidative stress was accompanied by significant decreases of serum follicle stimulating hormone (FSH), luteinizing hormone (LH), and total and free testosterone levels. Vitamin E as well as silymarin has significantly reduced oxidative stress and ameliorated hormone levels. It is concluded that the co-administration of vitamin E with silymarin would provide a better protection against EMF-induced biological hazards.

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1. Introduction

Over the last decade, due to the development of new technologies, the number and diversity of appliances (TV sets, mobile phones, PC monitors, etc.) with sources of electromagnetic fields (EMFs) significantly increased. Accordingly, everyone is exposed to a complex mix of weak electric and magnetic fields, both at home and at work, from the generation and transmission of electricity, domestic appliances and industrial equipment, to telecommunications and broadcasting. These exposures have been linked to various disorders that may affect different organs, since they penetrate the animal body and act on all organs, altering the cell membrane potential and distribution of ions. These alterations may influence the biochemical processes in the cell. Some studies revealed that direct current electric field pulse induces production of reactive oxygen species and increased lipid peroxide level (Harakawa *et al.*, 2005).

Histological and physiological studies have increased in evaluating the effects of electromagnetic fields on human health. It was reported that extremely low frequency EMF induced tissue damage in different organs of experimental animals (Zare *et al.*, 2007). Studies have shown that exposure to EMF adversely affects spermatogenesis, Sertoli and Leydig cells, alter hormone secretion due to deformation of Leydig and Sertoli cells, which may lead to cell proliferation activated by follicle-stimulating hormones (Roosli *et*

al., 2007), such type of alteration may also decrease the sperm count and may also cause DNA strand break. Wang *et al.* (2003) pointed out that Leydig cells are among the most susceptible cells to electromagnetic waves and injury to these cells may affect spermatogenesis, decrease in sperm count, weight of testicular organs and destruction in Leydig cells due to these radiations are indication of male infertility. Premature aging in genital organs of rats, as a result of devastating effect of EMF on spermatogenic cells and decrease of testosterone secretory cells have been also reported (Khaki *et al.*, 2008).

Vitamin E (DI-alpha-tocopherylacetate) is an essential nutritional element and is a biological antioxidant for a variety of nutrients, metabolites, vitamin A, hormones and enzymes. Absorbed tocopherols are transported unchanged to the tissues and the antioxidant function of the vitamin resulted in its oxidation to alpha-tocopherol quinone through a semi-stable intermediate alpha-tocopherol radical.

Diets rich in naturally occurring polyphenolic flavonoids have been associated with the reduced incidence of certain human cancers. Among these, silymarin, isolated from the fruits of milk thistle, *Silybum marianum* L. Gaertn (Mereish *et al.*, 1991), is being used clinically as an anti-hepatotoxic agent for the treatment of various liver diseases in Europe and Asia and has also been marketed in the USA and Europe as a dietary supplement (Singh *et al.*, 2002).

Silymarin is composed mainly of silibinin with small amounts of other silibinin stereoisomers, namely, isosilybin, dihydrosilybin, silydianin and silychristin (Wagner *et al.*, 1974). Studies with silymarin showed that it could be used against a wide range of liver and gall bladder diseases, including hepatitis and cirrhosis as well as dermatological conditions (Pares *et al.*, 1998). Studies on various animal models using different modes of administration of silymarin showed that it is non-toxic and largely free of adverse side effects in sub chronic and chronic tests even at large doses (Singh *et al.*, 2002). Silymarin is a polyphenolic flavonoid that already has been documented clinically for treatment of hepatic disorders (Abou-Safi, 2005). Another evidence proved that it has a strong antioxidant activity and exhibits anti-carcinogenic, anti-inflammatory, cytoprotective effects and exerts a prophylaxis role against some radiation hazards (El-Gabry *et al.*, 2003).

2. Material and Methods

Animals: Male albino rats Sprague Dawley (10 ± 2 weeks old) weighing 120 ± 20 g were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) and used for the different investigations carried out in the current study. Animals were maintained under standard conditions of light, ventilation, temperature, and humidity and allowed free access to standard pellet diet and tap water. Animals were acclimatized to laboratory conditions before starting the experiment. Biochemical analyses were performed in the morning time at 10 ± 1 a.m. All animal procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the "Guide for the care and use of Laboratory Animals" published by the US National Institutes of Health (NIH publication No. 85-23, 1996).

Electromagnetic wave exposure: Animals were housed collectively in wooden cages (30 x 40 x 40 cm; W x L x H) and exposed to 950 MHz (EMF) at a specific absorption rate of 1W/kg for two hours during a period of 2 months (2hrs/day, 3 times/week). The electromagnetic exposure system was formed of an electromagnetic generator (HP 83712 B) with frequencies range between 0.01 and 20 GHz. HP 8592L spectrum analyzer that covers the range from 9 KHz to 22 GHz. Two horns antennas, one working as a transmitter and the other as a receiver.

Vitamin E treatment: Vitamin E was purchased as a concentrate of dl alpha-tocopheryl acetate with the highest purity, from Pharco-pharmaceutical Company, Cairo Egypt. The concentrate was suspended in distilled water and given orally to rats at a dose of 1.35 mg/Kg body weight (3 times/week) for 2 months (Jaarin *et al.*, 2002).

Silymarin treatment: Silymarin was purchased from South Egypt Drug Industries Company (SEDICO), South Egypt, Egypt. Silymarin was suspended in distilled water and administered to rats via gavages at a dose of 18 mg/Kg body weight (3 times/week) for 2 months (Abou Safi, 2005).

Experimental design: Animals were randomly divided into five groups (n=6) and treated in parallel. 1) Control group: animals neither exposed to radiation nor treated with silymarin; 2) EMF group: Rats exposed to 950 MHz (2hrs/day, 3times/week) for 2 months; 3) Vitamin E + EMF: Rats received Vitamin E (1.35mg/Kg, 3 times/week) for 2 months before exposure to 950 MHz; 4) Silymarin + EMF: Rats received Silymarin (18mg/Kg, 3 times/week) for 2 months before exposure to 950 MHz 5); Vitamin E+ Silymarin + EMF: Rats received Vitamin E parallel to Silymarin (3 times/week) for 2 months before exposure to 950 MHz.

Preparation of samples and biochemical analysis:

All chemicals and reagents used were pure chemical materials from Sigma-Aldrich, St Louis, MO, USA. Animals of all groups were sacrificed at the end of the experiment. Blood samples were collected and serum obtained by centrifugation at 3000 rpm for 10 min. using refrigerated centrifuge (K3 Centurion Scientific Ltd, London, UK). For the assessment of oxidative stress biomarkers, lipid peroxidation was evaluated by measuring thiobarbituric acid reactive substances (TBARS) levels according to the method of Yoshioka *et al.* (1979). The activity of SOD was determined according to the method of Minami and Yoshikawa (1979). Catalase activity was determined following the procedure described by Aebi (1984). Blood glutathione was determined according to Beutler *et al.* (1963). GSH-Px activity was determined by a colorimetric assay according to Lawrence and Burk (1976). Carbonyl group was assayed according to the procedure described by Reznick and Packer (1994). AOPP was assayed according to Witko-Sarsat *et al.* (1996). Serum total and free testosterone, luteinizing hormone and follicle stimulating hormone were determined by the immunometric assay using kits from Diagnostic System Laboratories Inc., Los Angeles, USA (Rajkowski *et al.*, 1977 and Santner *et al.*, 1981) respectively.

Statistical analysis: Experimental data were analyzed using one way analysis of variance (ANOVA) using SPSS (statistical package for social sciences, 1999; ver.10.0), followed by Duncan test as post hoc ANOVA test. The significance among samples was compared at $P \leq 0.05$. Results were represented as mean \pm Standard error (n =6).

3. Results

The current study results revealed that exposure to 950 MHz (2 hr/day for 2 months, 3times/week) induced a significant increase ($P<0.05$) in serum TBARS, AOPP and CO levels, compared to their respective control levels. Administration of vitamin E as well as silymarin during 2 months before exposure to 950 MHz reduced the level of oxidants, compared to their respective levels in the EMF group. However, the antioxidant effect of vitamin E was superior to silymarin. Their co-administration was more efficient compared to each treatment given alone (Table 1).

Exposure to 950 MHz induced significant decreases ($P<0.05$) in serum SOD, CAT, GSH-Px activities and GSH content, compared to their respective control levels. Silymarin treatment before

EMF exposure enhanced SOD and CAT activities better than vitamin E treatment, while vitamin E increased GSH-Px activity and GSH content better than silymarin treatment. Their co-administration showed the same trend recorded for silymarin and vitamin E treatment, respectively (Table 2).

Exposure to 950 MHz induced a significant decrease ($P<0.05$) in FSH, LH, total and free testosterone contents, compared to their respective control levels. Administration of vitamin E or silymarin before EMF exposure induced a nearly similar significant increase ($P<0.05$) in their contents regarding their respective values in the serum of EMF exposed rats. Their co-administration was more efficient for FSH and LH while had no effect on total and free testosterone (Table 3).

Table (1): Effect of vitamin E and silymarin on serum lipid peroxidation (TBARS), advanced oxidation protein products (AOPP) and protein carbonyl (CO) in rats exposed to electromagnetic field, 950 MHz (EMF).

Animal groups	TBARS (nmol/ml)	AOPP (μ mol/l)	CO (nmol/ml)
Control	14.6 \pm 0.04	44.3 \pm 0.62	12.9 \pm 0.30
EMF	45.7 \pm 1.02 ^a	110.7 \pm 4.00	34.9 \pm 0.92 ^a
% of changes vs control	213%	150%	170%
Vitamin E + EMF	27.3 \pm 0.66 ^{ab}	78.8 \pm 2.00 ^{ab}	24.5 \pm 0.48 ^{ab}
% of changes vs control	87%	78%	90 %
Silymarin + EMF	35.6 \pm 0.88 ^{abc}	89.6 \pm 1.53 ^{abc}	32.3 \pm 0.50 ^{abc}
% of changes vs control	144%	102%	150%
Vitamin E + Silymarin + EMF	37.9 \pm 0.56 ^{abcd}	69.2 \pm 0.57 ^{abd}	20.0 \pm 0.69 ^{abcd}
% of changes vs control	59%	56 %	55%

Each value represents the mean \pm SE (n=6). a: significant vs control, b: significant vs EMF, c: Significant vs vitamin E+EMF, d: Significant vs silymarin + EMF.

Table (2): Effect of vitamin E (E), silymarin and their co-administration on serum superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities and glutathione (GSH) content in rats exposed to Electromagnetic Field, 950 MHz (EMF)

Animal groups	SOD (U/ml)	CAT (U/ml)	GSH-P _x (U/ml)	GSH mg/dl
Control	4.82 \pm 0.53	55 \pm 1.0	5.22 \pm 0.48	32.8 \pm 1.9
EMF	2.41 \pm 0.10	36 \pm 0.9 ^a	1.38 \pm 0.20 ^a	9.5 \pm 0.6 ^a
% of changes vs control	-50%	- 34%	-74%	- 71%
E + EMF	3.00 \pm 0.12 ^{ab}	45 \pm 0.6 ^{ab}	3.19 \pm 0.31 ^{abd}	28.3 \pm 2.3 ^{abd}
% of changes vs control	- 38 %	-18%	-39%	- 14%
Silymarin + EMF	4.08 \pm 0.05 ^{abc}	52 \pm 2.1 ^{bc}	2.06 \pm 0.11 ^{ab}	15.6 \pm 0.8 ^{ab}
% of changes vs control	- 15%	-5%	- 61%	- 52%
E + Silymarin + EMF	3.95 \pm 0.11 ^{abc}	53 \pm 2.8 ^{bc}	3.24 \pm 0.21 ^{abd}	28.9 \pm 0.6 ^{abd}
% of changes vs control	- 18%	-4%	-38%	- 12%

Each value represents the mean \pm SE (n=6). a: significant vs control, b: significant vs EMF, c: Significant vs vitamin E+EMF, d: Significant vs silymarin + EMF.

Table (3): Effect of Vitamin E (E), Silymarin and their co-administration on serum follicle stimulating hormone (FSH), luteinizing hormone (LH), total testosterone and free testosterone levels in rats exposed to Electromagnetic Field, 950 MHz (EMF).

Animal groups	FSH Pg/dl	LH mIU/dl	Total testosterone ng/dl	Free testosterone Pg/dl
Control	4.70±0.19	4.20±0.11	3.90±0.07	13.60±1.06
EMF	0.55±0.05 ^a	0.31±0.03 ^a	2.30±0.09 ^a	8.10±0.87 ^a
% of changes vs control	-88%	-92%	-41%	-40%
E + EMF	1.48±0.08 ^{ab}	1.40±0.08 ^{ab}	3.30±0.18 ^b	12.50±0.76 ^b
% of changes vs control	-69%	-66%	-15%	-8%
Silymarin + EMF	1.10±0.06 ^{ab}	1.30±0.06 ^{ab}	3.50±0.27 ^b	14.40±0.35 ^b
% of changes vs control	-76%	-69%	-10%	6%
E + Silymarin + EMF	2.52±0.10 ^{abcd}	2.40±0.09 ^{abcd}	3.70±0.10 ^b	12.8±0.72 ^b
% of changes vs control	-46%	-42%	-6%	-6%

Each value represents the mean ± SE (n=6). a: significant vs control, b: significant vs EMF, c: Significant vs vitamin E + EMF, d: Significant vs silymarin + EMF

4. Discussion

The biological effects and health hazards of non-ionizing radiation have been debated for over fifty years. The results of extensive studies showed that acute exposure to non-ionizing radiation can lead to increased internal temperature, speeds up chemical reactions rate which result in biochemical and physiological effects. Some studies showed that MF and EMF may change the hormonal balance of sex hormones and affects the reproductive activity (Al-Akhras *et al.*, 2006).

Kartashev (1992) suggested that the chronic applications of alternating electric field disturb proliferation and differentiation in mammals. Cucullo *et al.* (2005) found that electric field may affect cellular function by changing the structure of ion channel and the integrity of the cell membrane. Lai and Singh (1996) reported damage in rat brain cells exposed to 245 GHz which support that EMF radiation may lead to oxidative stress due to overproduction of ROS. Moreover, Soeradi and Tadjudin (1986) reported that the exposure of male rats to electrostatic fields leads to reduction in their fertility. They also suggested that the primary interaction between testis and electrostatic fields takes place at the molecular level. Lokhmatov and Pastukhova (1993) also demonstrated that desquamation of spermatogenic epithelial layers in the seminiferous tubes was increased along with reduction of the number of testosterone producing cells and concomitant with microcirculatory changes.

On the other hand, Margonato *et al.* (1993) suggested that 50 Hz electric field exposure did not cause harmful effects on tissue with high cellular turnover rates and did not impair the reproductive function of rats.

The outcome of oxidative damage induced by electromagnetic fields depend on various factors, including the oxidative status of the cell, capability of

endogenous antioxidant enzymes and processes to counteract free radical build up, availability of exogenous antioxidants, the parameters of exposure (e.g., generated power, duration of exposure and wave shape), and whether the oxidative damage is cumulative.

The results obtained in the present study revealed that exposure to 950MHz for 2 months (2hrs/day, 3times/week) provoked oxidative stress demonstrated by a significant increase in the level of TBARS, AOPP and CO (Table 1) associated with a significant decrease in SOD, CAT, GSH-Px activities and GSH content (Table 2), compared to their respective levels in the control group. Oxidative stress was accompanied by hormonal disturbances recorded by significant decreases in FSH, LH, and total and free testosterone contents (Table 3).

The present results comes in coincidence with the results of Ozguner *et al.* (2005) that MDA levels increased while SOD and CAT activities decreased in some tissues of EMF RF exposed animals. In the same context Yurekli *et al.* (2006) reported that MDA levels increased and GSH decreased significantly at 900 MHz RF radiation. Moreover, Oktem *et al.* (2005) indicated that acute exposure to radiofrequency fields of commercially available cellular phones enhances lipid peroxidation and reduce SOD activity. In contrast to our results Irmak *et al.* (2002) illustrated that serum SOD activity increased in EMR-exposed rabbits compared to control while MDA levels did not show significant changes. Ferreire *et al.* (2006) found that acute ultra-high frequency electromagnetic field (800-1800MHz) exposure is not able to produce detectable oxidative stress in rats from any age tested. While Balci *et al.* (2007) found that MDA level and CAT activity increased significantly in rats after exposure to 900 MHz RF radiation.

In the current experiment, the significant decrease of serum hormones recorded after exposure to 950MHz may be explained by the fact that microwaves (especially waves from electromagnetic field of cellular phones) produces temperature elevation and energy distribution in live tissues. The temperature is an important factor in regulating the endocrine hormones.

Previous studies have demonstrated that long-term (9 months) exposure to electrical fields (17 KHz, 100 KV/m) increased destructive processes in both spermatogenous epithelium cells and in steroid producing cells (Lokhmatova, 1993). It is well documented that the function of the testis is controlled by the hypothalamic-pituitary mechanism. Exposure to EMF (non-ionizing radiation) increases the process of lipid peroxidation in the brain and causes damage to both the pituitary gland and hypothalamus resulting in hypopituitarism (Brodsky *et al.*, 1996). However, McGivern *et al.* (1990) indicated that electromagnetic field exposure did not interfere with regulation of the hypothalamic-pituitary-gonadal axis, and testosterone level did not differ in exposed compared with sham-exposed rats. Al-Akhras *et al.* (2006) reported that exposure of rats to electromagnetic field (long term) provokes a decrease in testosterone level while Shahryar *et al.* (2008) reported that long term exposure to EMF can increase testosterone levels in serum.

The decreased FSH, LH, and testosterone levels may be attributed to hypothalamic and pituitary gland dysfunction which interferes with hormone production. The decreased levels of male sex hormones may also resulted by the direct effect of non-ionizing radiation on testis and the produced free radicals attack the testicular parenchyma causing degeneration of the seminiferous tubules and Leydig cells (Constine *et al.*, 1993).

The results obtained in the current study revealed that administration of vitamin E for 2 months before EMF exposure afford rats with a significantly higher protection than silymarin against the increase of oxidants and the decrease of GSH-Px activity and GSH content, while administration of silymarin was more efficient against the decrease of SOD and CAT activities. Their co-administration exhibit a higher efficacy compared to each treatment alone. Regarding hormone levels administration of vitamin E as well as silymarin before EMF exposure induced significant increases and their co-administration was better compared to each treatment alone.

The protective role of silymarin may be attributed to its distribution in several important organs of the body (Singh *et al.*, 2002) where it acts as a free-radical scavenger and membrane stabilizer (El-Gabry *et al.*, 2003). The antioxidant and free radical scavenging activities are attributed to the presence of active

flavonolignons, silibin, silydianin and silychristine, collectively known as silymarin.

Because the lipid component in the membrane is particularly susceptible to radiation damage, it could be reasoned that vitamin E might play an important protective role against such damage. Large doses of vitamins increase the antioxidant potential of the rat tissues. In fact, it has been reported that vitamin E prevents peroxidation of polyunsaturated fatty acids in vitro and erythrocyte damage in vivo. Furthermore, it has been shown that systemic and topical applications of vitamin E reduced ultraviolet-induced lipid peroxidation and skin damage (Record *et al.*, 1991) and prevented the biological effects of radiation on mice liver. Moreover, vitamin E is the most important lipid soluble vitamin, which acts in adipose tissue, plasma lipoproteins, membranes mitochondria and cell membranes including those of erythrocytes (Bjorneboe *et al.*, 1990). It is a lipid soluble free radical scavenger and ubiquitous component present among the lipid constituents of cell membranes and lipoproteins.

According to the results obtained in the current study, it is concluded that the co-administration of vitamin E with silymarin before EMF exposure afford a better protection, compared to each treatment alone, against oxidative stress and hormonal changes.

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