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Evaluation of the Low-Frequency Electromagnetic Fields on Biochemical Parameters in the Absence and Presence of Vitamin C in Mice

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Abstract:
Exposure to low-frequency electromagnetic fields (LF-EMF) has been considered a global concern because of its harmful effects on human health (cancer, neurodegenerative disorders, etc.). According to the International Agency for Research on Cancer, EMF has been classified as a possible cancerous element for human health. Antioxidants such as vitamin C improve the damage caused by EMF by reducing oxidative stress. To evaluate the effects of EMF on the serum total protein, blood sugar, albumin and triglyceride, and the inhibitory role of vitamin C, 40 male BALB/c mice were recruited. Participants were randomly distributed into four groups 1- exposure to LF-EMF, 2- exposure to LF-EMF which received vitamin C (50 mg/kg), 3- exposure to LF-EMF which received vitamin C (100 mg/kg), and 4- control group (no exposure). The experimental groups (1-3) received LF-EMF (50 Hz, 4 mT, 4 hours/day, and 1 month) while both groups 2 and 3 had intraperitoneally injected vitamin C (50 mg/kg, 100 mg/kg) every other day basis respectively. The obtained results demonstrated higher triglyceride and total protein levels and lower albumin and blood sugar levels in the LF-EMF group compared to controls while vitamin C restricts their alterations ($p<0.05$). To sum it up, our data show that intraperitoneal injection of vitamin C restricts the effects of LF-EMF exposure on the biochemical parameters in mice. However, the antioxidant characteristics of vitamin C may be probably involved in the LF-EMF effects of biochemical parameters in mice.

INTRODUCTION

Nowadays, the development of electrical power stations, high-voltage electrical lines, and modern communication devices as well as universe electromagnetic fields precipitously encircled human life. Moreover, man-made electromagnetic fields such as TV, radio, MRI medical instruments, etc. have largely laden our environment. Electromagnetic fields which exist around the electricity supplies, transferring lines and electricity generators are categorized into three main ranges: low-frequency electromagnetic fields (EMF) (50-60 Hz), intermediate frequency (300 Hz-<10 MHz), and radiofrequency range (10 MHz-300 GHz) (1, 2).

The effects of low-frequency electromagnetic fields (LF-EMF) and their biological consequence on human life have attracted researchers globally. Industrialization in developed and developing countries increased the electricity power stations resulting in the widespread concern about the possibility of harmful effects of LF-EMF on human health. Several epidemiologic studies uncovered the deleterious effects of LF-EMF on humans in recent years (3, 4). According to the Wertheimer report, there was a severe correlation between the children's blood cancer and LF-EMF exposure in Denver. He asserted that the higher risk of childhood leukemia at the residential with higher LF-EMF (5, 6). After that, *Savitz et al.* released a publication that supported the Wertheimer report (7). Moreover, researchers published a paper about the association of LF-EMF and various disorders such as suicide (8), cancer (9, 10), and neurodegenerative disorders such as amyotrophic lateral sclerosis and Alzheimer's disease (11).

A solid mass of investigations about the effects of LF-EMF was accomplished on the cellular components including the central nervous system (CNS), genetic material, and proteome and embryogenesis (12, 13). In the short-term exposure, all and altogether do not show significant deleterious effects on physiological and behavioral parameters. Nevertheless, the long-term animal studies need more investigation (14).

Low-frequency electromagnetic field has been described as electromagnetic oscillating waves having below 50-60 Hz frequency with widespread usage in the world. Despite mass investigations conducted on LF-EMF, it seems that further studies are needed to evaluate the effects of LF-EMF exposure on pathophysiological aspects of human health. Based on the *McCann et al.* the exposure to LF-EMFs have not the critical effects on prokaryotes and eukaryotes (15, 16). However, the report of *Sabine et al.* showed the toxicity of LF-EMF (17) and *Feychting et al.* displayed a significant correlation between exposure to LF-EMF and the incidence of childhood cancer (14).

Some reactive compounds such as free radicals, superoxide anions, hydrogen peroxide and hydroxyl radicals are generated during the various metabolic pathways as a by-product. As well as the reactive oxygen species (ROS) are involved in cellular signalling transduction and gene expression. They are usually involved in determining cell growth, anti-inflammatory response, cellular proliferation and differentiation, and oxidative stress response (18). The free-generated radicals and ROS concentration are balanced by the regulation of the rate of production and clearance. The enzymatic and non-enzymatic antioxidants which provide antioxidant tolerance controlled these procedures to maintain the regular redox hemostatic condition (19). Excessive generation of free radicals and ROS causes the imbalance between oxidants and antioxidants in favor of the former which is called oxidative stress (20).

In the represented study, we aimed to show the effects of LF-EMF on biochemical parameters such as blood sugar, triglyceride, total protein and albumin in Mice whereas the role of vitamin C was investigated consequently.

METHODS AND MATERIALS

Animals

Our study was conducted on 40 healthy male BALB/c mice (25-30 gr) which were purchased from the animal room of Razi Institute (Mashhad, Iran). The participants were freely allowed access to fresh tap water and commercial standardized pelleted food. Relative humidity and ambient temperature of the animal room were 65±5% and 25±2^{OC} respectively. They were kept under 12 light-12 dark cycle and all experiments were performed under the animal experimental care approval (21).

Groups

Mice were randomly divided into four groups: 1- LF-EMF that were exposed to low-frequency electromagnetic fields (50 Hz, 4mT, 4 hours/day, 1 month) (n=10), 2- LF-EMF+50 mg/kg/ vitamin C every other day basis (n=10), 3- LF-EMF+100 mg/kg/ vitamin C every-other-day (n=10) and 4- healthy controls (n=10). Animals received vitamin C by Intraperitoneal injection by other basic after exposure to LF-EMF. Our LF-EMF exposure tool generates electromagnetic wave (50 Hz, 4 mT) that consists of a plastic chamber with a copper line coil that the participants were placed in the chamber with the ability to move restriction-free. The controls however were placed in the same condition and similar intervals while there was no exposure to LF-EMF for them.

Measurements of biochemical parameters

At the end of the study period, participants were anaesthetized and 5 ml cardiac blood was collected for determination of biochemical parameters. Samples were centrifuged for 10 minutes at 1800 g and obtained sera were kept at -20^{oC}. The blood serum was processed in a Mindray SAL-6000 Chemistry & Immunoassay Integrated System (Shenzhen Mindray Bio-Medical Electronics Co) for analysis of the following parameters: total protein (TP), triglycerides (22), cholesterol (CHL), and glucose (GLC). The biochemical kits and calibration controls used were acquired from Lab-test Diagnosis (Lagoa Santa, Minas Gerais, Brazil), and were used according to protocols established by the manufacturer.

STATISTICAL ANALYSIS

The mean values and standard deviations were analyzed for obtained data by SPSS software (SPSS 16.0, SPSS Inc., Chicago, IL, USA). The data normality was evaluated by the K-S test; however, the One Way ANOVA statistical test was used to comparison of the obtained results between controls and EMF. The $p<0.05$ was considered as the statistically significant difference.

RESULTS

Total protein

The level of total protein in the LF-EMF exposed group (5.34 ± 0.19 mg/l) was significantly raised in comparison to the controls (4.60 ± 0.08 mg/l) ($p<0.001$). The data demonstrated that the level of total protein in LF-EMF which received vitamin C (50 and 100 mg/kg) were 5.08 ± 0.12 mg/l ($p<0.001$) and 4.44 ± 0.07 ($p<0.001$) that are lower than LF-EMF group respectively (Table 1) (Figure 1).

Albumin

The mean level of albumin in the serum of controls was 3.10 ± 0.06 mg/l but it decreased in the LF-EMF group to 2.48 ± 0.04 which showed a statistically significant

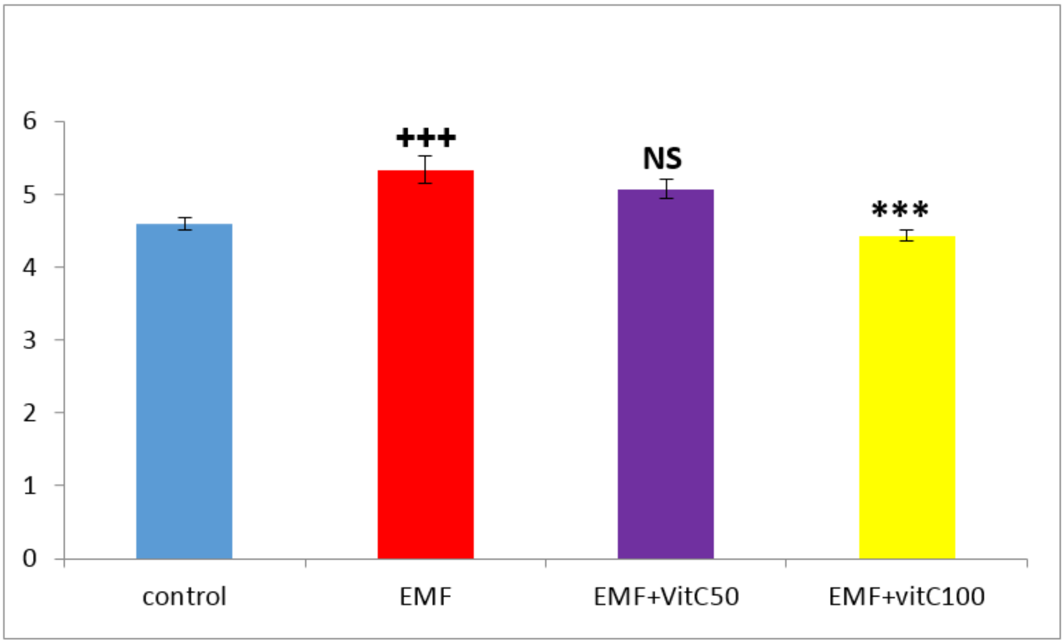

Fig 1. Figure1: Evaluation of total protein level in participants
+++: significant difference between controls and EMF group (p<0.001)
***: significant difference between EMF and EMF+vitamin C (100mg/kg/any other day) group (p<0.001)
NS: Non significant correlation

Table 1. Serum biochemical parameters in control and different exposed groups after 1 month exposure to low-frequency electromagnetic fields

Biochemical parameters	Control	LF-EMF	LF-EMF+50 mg/kg vitamin C	LF-EMF+100 mg/kg vitamin C
Total protein mg/l	4.60 ± 0.08	5.34 ± 0.19	5.08 ± 0.12	4.44 ± 0.07
Albumin mg/l	3.10 ± 0.06	2.48 ± 0.04	2.80 ± 0.08	2.84 ± 0.04
Blood sugar (Glucose) mg/100 cc	250.4 ± 12.6	178 ± 5.3	223 ± 6.5	234 ± 23.6

difference between the two groups (p<0.001). Moreover, the level of albumin in LF-EMF exposed group received 50 and 100 mg/kg vitamin C (2.80 ± 0.08) and (2.89 ± 0.03) were significantly higher than LF-EMF exposed group (p<0.01) (Table 1) (Figure 2).

Triglyceride
The level of triglyceride in controls was 46.6 ± 2.9 mg/dl while it raised to 79.6 ± 9.3 in LF-EMF exposed group (p<0.001). The levels of triglyceride in the LF-EMF exposed group received 50 and 100 mg/kg vitamin C were 53.8 ± 3.3 and 42.6 ± 4.1 which significantly decreased in comparison to the LF-EMF exposed group (p<0.01 and p<0.001) (Table 2) (Figure 3).

Cholesterol
The level of Cholesterol in controls, LF-EMF, LF-EMF received 50 and 100 mg/kg vitamin C were 119.4 ± 5.8, 119.8 ± 4.8, 115.2 ± 9.3, and 114.4 ± 4.6 mg/dl respectively which demonstrated no statistically significant different among them (Table 2) (Figure 4).

Blood sugar (Glucose)
Our obtained results uncovered that the level of glucose in controls was 250.4 ± 12.6 mg/dl but it reduced significantly in the LF-EMF exposed group to 178 ± 5.3 mg/dl (p<0.01). The mean of serum glucose in LF-EMF received 50 mg/kg vitamin C was 223 ± 6.5 mg/dl showing the no significant difference, while it was raised to 234.4 ± 23.6 in LF-EMF received 100 mg/kg vitamin C showed a statistically significant difference with LF-EMF exposed group (p<0.05) (Table 1) (Figure 5).

Liver weight
The mean range of lever weight in controls, LF-EMF, LF-EMF received 50 and 100 mg/kg vitamin C were 0.049 ± 0.004, 0.055 ± 0.002, 0.052 ± 0.003, and 0.037 ± 0.008 gr respectively. According to our data, the weight of the liver in the LF-EMF received 100 mg/kg vitamin C was significantly lower than that of the LF-EMF exposed group (p<0.05) (Table 3) (Figure 6).

DISCUSSION
Vitamin C is known as an important antioxidant that plays a role as a cofactor in many enzymatic reactions during infections and inflammation and also protects cells from various oxidative damages.

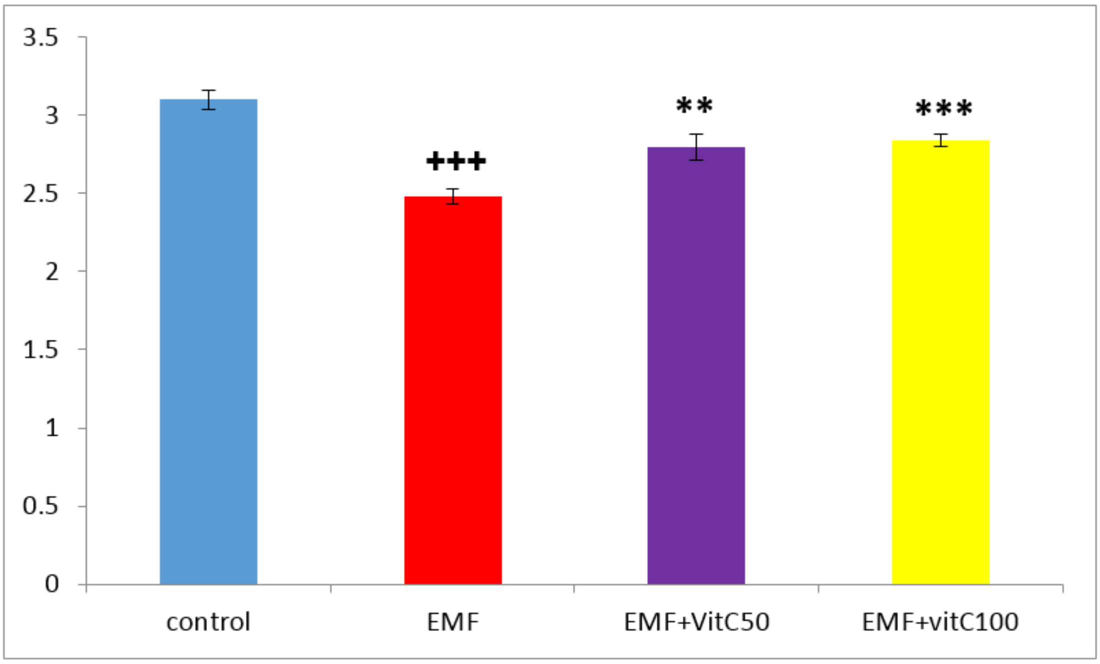

Fig2. Figure 2: Evaluation of the level of Albumin in participants
+++: significant difference between controls and EMF group (p<0.001)
***: significant difference between EMF and EMF+ vitamin C (100mg/kg/any other day) group (p<0.001)
**: significant difference between EMF and EMF+ vitamin C (50mg/kg/any other day) group (p<0.01)

Table 2. The level of triglyceride and cholesterol in serum of control and different low-frequency electromagnetic fields exposed groups after 1 month exposure

Lipid parameters	Control	LF-EMF	LF-EMF+50 mg/kg vitamin C	LF-EMF+100 mg/kg vitamin C
Triglyceride mg/100 cc	46.6 ± 2.9	79.6 ± 9.3	53.8 ± 3.3	42.6 ± 4.1
Cholesterol mg/100 cc	119.4 ± 5.8	119.8 ± 4.8	115.2 ± 9.3	107.6 ± 9.1

Vitamin C plays an important role in reducing the risk of diseases such as cancer, Alzheimer's, Parkinson's and other degenerative diseases (22). The various effects of electromagnetic fields on living cells are a complicated phenomenon involved in the different metabolic pathways (23). These effects have been initiated by physicochemical reactions such as the polarization of electrolytes and structural biomolecules leading to the generation of ROS and free radicals, weakening covalent bonds, hydration alteration, and change of dipoles' spin which consequently affects the biochemical parameters (24-26). In the present study, exposure to low-frequency electromagnetic fields (LF-EMFs) altered the biochemical parameters in mice serum in the presence and absence of vitamin C. Accordingly, the level of total protein, albumin, triglyceride, and blood glucose along with the liver weight were significantly affected among participants.

In the current study, the level of total protein was raised significantly in the LF-EMF exposed group whereas the level of albumin was reduced which may demonstrate the higher level of immunoglobulin generation in participants exposed to LF-EMF. In other words, it can be inferred that exposure to LF-EMF increased the level of antibodies, inflammatory and pro-inflammatory proteins, homeostatic and fibrinolytic polypeptides and proteolytic modulators (27). In other words, exposure to electromagnetic fields not only increased the generation of ROS and free radicals in the liver and other tissues, but it also led to a significant alteration in the level of antioxidants in plasma that showed oxidative stress (28). Based on our investigations, LF-EMF may be involved in oxidative stress-related biomolecules and/or tissue impairments which activated the immune responses and raised the level of total protein in our study. Determination of albumin level demonstrated a significant reduction in the LF-EMF exposed group, demonstrating the severe hepatic problem. The liver is the unique source of albumin production. Clinical association of liver damage is consequently mirrored in albumin concentration which is associated with severity of impairment (29).

Our results showed that the intraperitoneal administration of vitamin C increased the albumin level in participants reduced by LF-EMF exposure. Thereby, LF-EMF may cause serious hepatic damage that deceased albumin production whereas vitamin

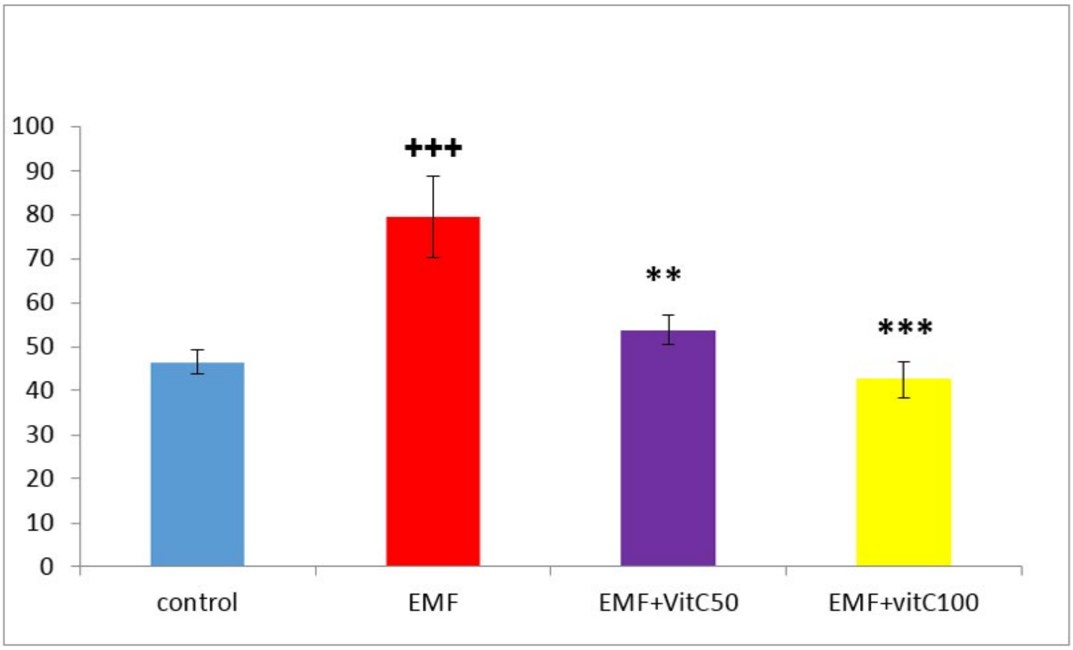

Fig3. Figure 3: Evaluation the level of triglyceride among participants
+++: significant difference between controls and EMF group (p<0.001)
***: significant difference between EMF and EMF+ vitamin C (100mg/kg/any other day) group (p<0.001)
**: significant difference between EMF and EMF+ vitamin C (50mg/kg/any other day) group (p<0.01)

Table 3. The weight of liver in participants after 1 month exposure

Tissue weight	Control	LF-EMF	LF-EMF+50 mg/kg vitamin C	LF-EMF+100 mg/kg vitamin C
Liver (mg)	49 ± 4	55 ± 2	52 ± 3	37 ± 8

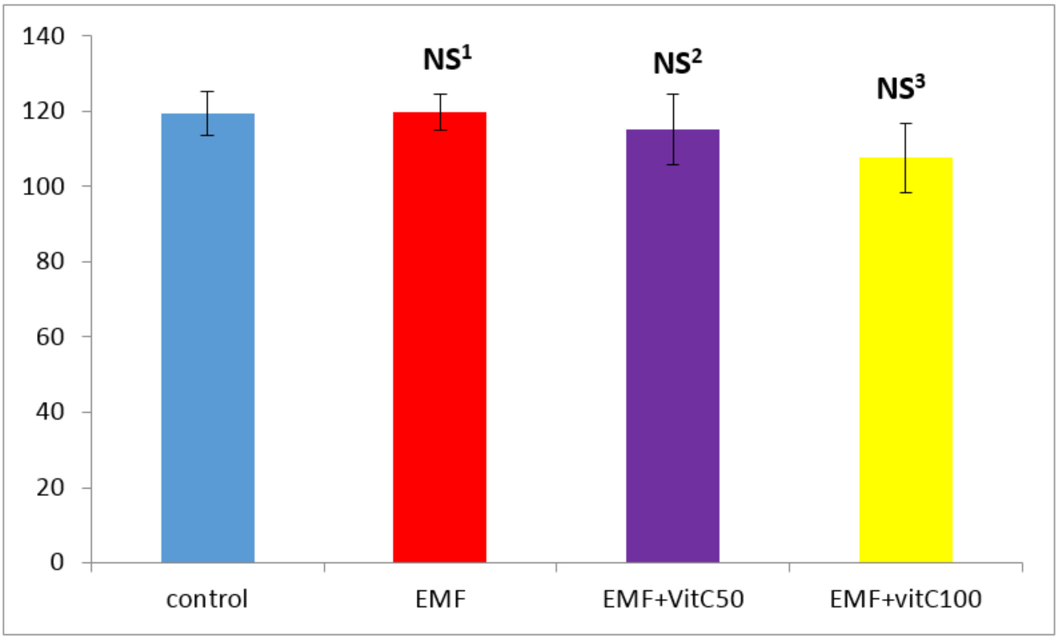

Fig4. Figure 4: Evaluation the level of cholesterol among participants
NS1: No significant difference among control and EMF groups
NS2: No significant difference among EMF and EMF+ vitamin C (50mg/kg/any other day) groups
NS3: No significant difference among EMF and EMF+ vitamin C (100mg/kg/any other day) groups

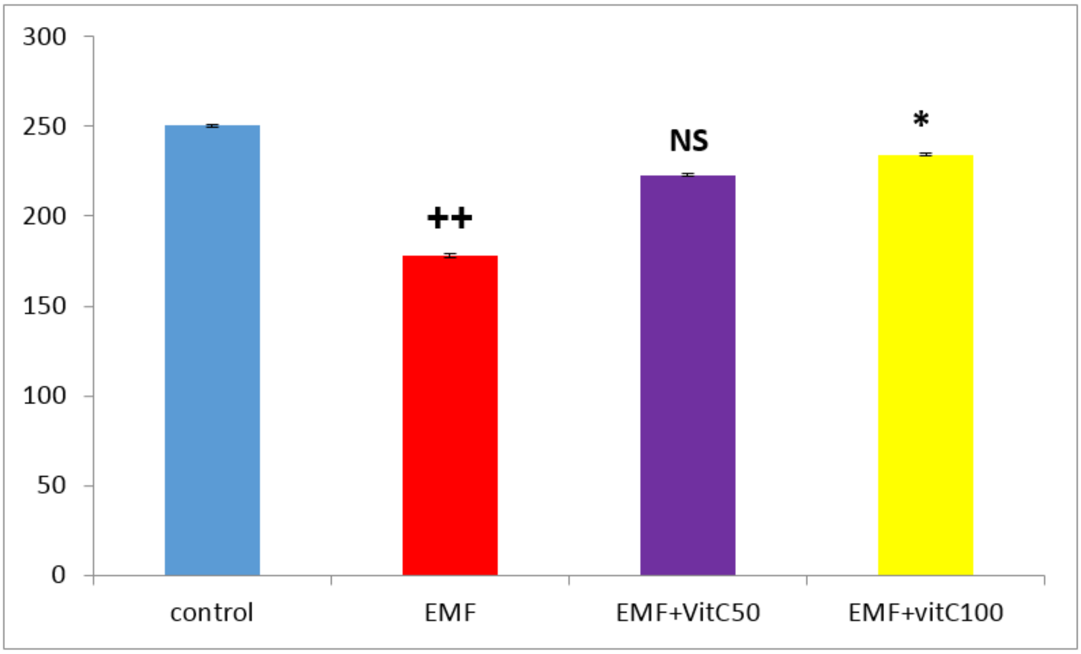

Fig5. Evaluation the level of glucose among participants
++: significant difference between controls and EMF group ($p<0.01$)
*: significant difference between EMF and EMF+ vitamin C (100mg/kg/any other day) group ($p<0.05$)
NS: significant difference between EMF and EMF+ vitamin C (50mg/kg/any other day) group

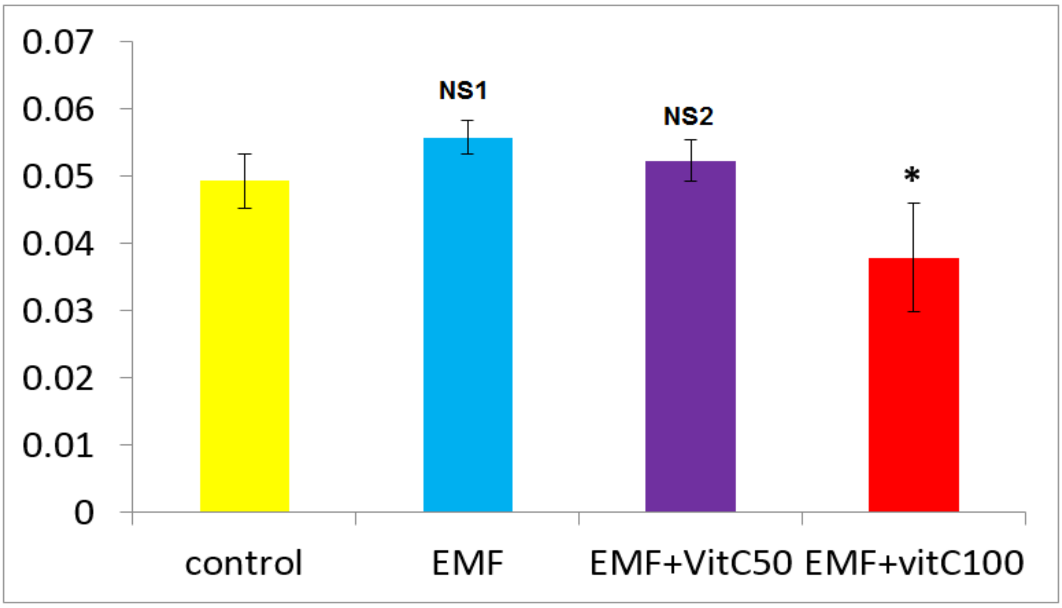

Fig6. Figure 6: Evaluation the weight of liver among participants
*: significant difference between EMF and EMF+ vitamin C (100mg/kg/any other day) group ($p<0.05$)
NS1: no significant difference between EMF and control group
NS2: no significant difference between EMF and EMF+ vitamin C (50mg/kg/any other day) group

C administration improved albumin generation consequently. These changes are considering the serious pathological responses correlated to oxidative stress represented due to LF-EMF exposure. The liver weight was increased significantly demonstrating a higher amount of inflammation and consequently higher blood circulation in the liver in comparison to the LF-EMF exposed group received 100 mg/kg vitamin C.

Obtained data demonstrate that the level of triglyceride and Cholesterol were increased while the level of glucose decreased in the LF-EMF exposed group. It may show that one month of exposure to LF-EMF (50 Hz, 4 mT) caused gradual metabolic changes with reduced levels of total protein and glucose in the participants when compared to controls (30). However, the pathological effects of oxidative stress were preceded more logically. Intraperitoneal administration of vitamin C in exposed mice improved the level of biochemical parameters.

Exposure to an electromagnetic field (50 Hz) can cause oxidative stress and stimulate the secretion of mineralocorticoids (such as cortisol) which increases the glucose level in exposed animals (31). Exposure to EMF affects cellular and/or tissue membranes by changing the ion disturbance, protein, and membrane charges and making dysfunction in bilayer lipid membrane lead to excessive permeability of glucose to cell/tissue (32). Administration of vitamin C with the extremely low standard potential of reduction (280 mV) improves the intracellular/extracellular antioxidant capacity, thereby decreasing lipid peroxidation, free radical generation, and neutralizing ROS (33-35).

CONCLUSION

Striking effects of LF-EMF on biochemical parameters were observed in the exposed mice. We can conclude that the electromagnetic fields can affect the biochemical parameters. Exposure to LF-EMF (50 Hz, 4 mT, 4 hours/day, 1 month) increased the total protein and decreased the albumin level whereas vitamin C regulated the alternation of both parameters. The pathological effects of LF-EMF seem associated with oxidative damage caused by generated free radicals and ROS The antioxidant capacity of vitamin C restricts the LF-EMF damage to the liver, and also regulates the level of biochemical parameters in participants. To investigate the precise effects of LF-EMF exposure on liver damage and the role of pathologic oxidative stress on biochemical parameters further studies are needed.

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Conflict of interest

The authors reported no conflict of interest in the present study.

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