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ORIGINAL ARTICLE



Protective properties of *Myrtus communis* extract against oxidative effects of extremely low-frequency magnetic fields on rat plasma and hemoglobin

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ABSTRACT

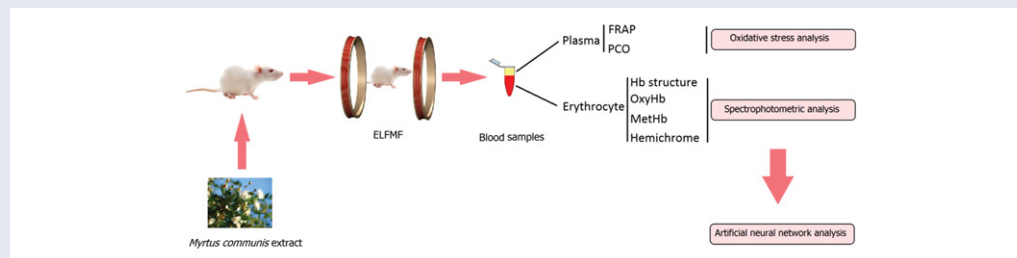
Purpose: This study investigates the protective properties of *Myrtus communis* extract against the oxidative effects of extremely low-frequency magnetic fields (ELFMF). Also, this study is aimed to analyze the conformational changes of hemoglobin, oxidative damages to plasma proteins and antioxidant power of plasma following exposure to ELFMF.

Materials and methods: Adult male rats were divided into 3 groups: (1) control, (2) ELFMF exposure, and (3) ELFMF exposure after *M. communis* extract administration. The magnetic field (0.7 mT, 50 Hz) was produced by a Helmholtz coil for one month, 2 hours a day. The *M. communis* extract was injected intraperitoneally at a dose of 0.5 mg/kg before exposure to ELFMF. The oxidative effects of ELFMF were studied by evaluating the hemoglobin, methemoglobin (metHb) and hemichrome levels, absorption spectrum of hemoglobin (200–700 nm), oxidative damage to plasma proteins by measuring protein carbonyl (PCO) levels and plasma antioxidant power according to the ferric reducing ability of plasma (FRAP). The mean and standard errors of the mean were determined for each group. One-way ANOVA analysis was used to compare the means of groups. The significance level was considered to be $p < .05$. Moreover, artificial neural network (ANN) analysis was used to identify the predictive parameters for estimating the oxyhemoglobin (oxyHb) concentration.

Results: Exposure to ELFMF decreased the FRAP which was in concomitant with a significant increase in plasma PCO, metHb and hemichrome concentrations ($p < .001$). Oxidative modifications of Hb were shown by reduction in optical density at 340 nm (globin-heme interaction) and 420 nm (heme-heme interaction). Administration of *M. communis* extract increased FRAP values and decreased plasma POC, metHb, and hemichrome concentrations. Also, a significant increase in Hb absorbance at 340, 420, 542, and 577 nm showed the protective properties of *M. communis* extract against ELFMF-induced oxidative stress in erythrocytes. ANN analysis showed that optical absorption of hemoglobin at 520, 577, 542, and 630 nm and concentration of metHb and hemichrome were the most important parameters in predicting the oxyHb concentration.

Conclusions: *Myrtus communis* extract enhances the ability of erythrocytes and plasma to deal with oxidative conditions during exposure to ELFMF. Also, ANN analysis can predict the most important parameters in relation to Hb structure during oxidative stress.

GRAPHICAL ABSTRACT



ARTICLE HISTORY

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KEYWORDS

Extremely low-frequency magnetic fields; hemoglobin; *Myrtus communis*; oxidative stress; plasma

Introduction

In recent years exposure to magnetic fields, originating from power lines and electronic devices has been associated with different diseases (Lai and Singh 1997a). In the past 2 decades, concerns have been raised among the public and scientific communities about the potential harms of magnetic fields at frequencies lower than 300 Hz. International Agency for Research on Cancer (IARC) considers ELF-EMF as a carcinogenic factor in biological systems (Güler et al. 2012). There is evidence that changes in energy levels can affect the production and recombination of free radicals (Timmel et al. 1998).

The probability of free radical formation or increased concentration in electromagnetic fields leads to disturbances in the oxidative equilibrium (Adair 1991), which is one of the most important theories addressed in previous studies. For instance, in the presence of a magnetic field, ferric ions react as catalysts with hydrogen peroxide through mitochondrial Fenton reactions and produce hydroxyl free radicals, which cause single-strand and double-strand DNA breaks and psychotoxic damages (Lai and Singh 2004; Phillips et al. 2009).

There are also some investigations, which show that magnetic fields, accompanied by other toxic factors, can exert synergistic effects. For instance, when RAW264 cells are simultaneously exposed to a magnetic field (0.5 mT, 50 Hz) and lipopolysaccharides, the viability of cells is reduced and the number of single-strand DNA breaks is increased synergistically (Nakayama et al. 2016). Moreover, measurement of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in DNA and thio-barbituric acid reactive substances (TBARS) in plasma has shown that a magnetic field (50 Hz, 0.97 mT) can induce cancer as a promoter or co-promoter (Yokus et al. 2005).

Previous studies have shown that the presence of ferric ion in the vicinity of a 5 mT magnetic field can increase lipid peroxidation in rat liver cells, leading to cell death (Jajte et al. 2002). Moreover, a magnetic field (60 Hz, 0.01 mT) can probably increase single-strand and double-strand DNA breaks in brain cells. The probability of this damage is directly related to the exposure time (Lai and Singh 2004). Another study showed that pretreatment of mice with melatonin and N-tert-butyl- α -phenylnitron before and after exposure to a magnetic field (60 Hz, 0.5 mT) could reduce single-strand and double-strand DNA damage in rat brain cells by sweeping free radicals (Lai and Singh 1997b).

Acute exposure to magnetic fields reduces the activity of catalase and superoxide dismutase and impairs the antioxidant status in rat brain. Although these conditions may activate some adaptive responses which controlling the brain oxidative balance (Martínez-Sámano et al. 2012). Other findings suggest that exposure of rats to sinusoidal magnetic fields (50 Hz, 1000 μ T) can increase the effects of gamma-ray induced carcinogenesis at 0.1 Gy (Soffritti et al. 2016).

In recent years, efforts have been made to reduce the potential damage of electromagnetic fields and radiations. In a previous study, administration of garlic extract before exposure to electromagnetic radiation (1.8 GHz, 0.4 W/kg,

1 h/day) could reduce protein oxidation in the rat brain tissues. However, it could not reduce the amount of serum nitric oxides (Avci et al. 2012). Another study showed that the magnetic field (50 Hz, 10 mT) induced mutagenic effects by producing reactive oxygen species (ROS). Moreover, using Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) could reduce the number of micronuclei produced by the magnetic field and bleomycin (Miyakoshi et al. 2012).

Another in vitro study showed that 24-hour exposure to a magnetic field (50 Hz, 100 μ T) could lead to genomic instability and micronucleus formation in SH-SY5Y cells, associated with lipid peroxidation. The results of this study showed that although N-acetylcysteine (NAC) could reduce lipid peroxidation, the magnetic field produced genetic damage, similar to ionizing radiation, which has a dynamic state and is not completely suppressed by antioxidants (Kesari et al. 2015).

A biological system consists of diamagnetic organic compounds, which include paramagnetic molecules such as O₂ and ferromagnetic substructures such as hemoglobin. Hemoglobin contains iron as its main constituent. Nearly half of the iron content in the body is in hemoglobin molecules, which may be influenced by magnetic fields. The presence of compounds with magnetic susceptibility in the external field triggers the total energy, which can affect the biological equilibrium required by the system, including the oxidant/antioxidant equilibrium (Adair 1991).

Erythrocytes are constantly exposed to oxidative damage as a result of high oxygen pressure. Moreover, the presence of high amounts of unsaturated fatty acids and iron ions provides suitable conditions for free radical reactions. Hemoglobin oxidation, methHb formation, iron release (Zbikowska et al. 2014), and formation of ROS through Fenton reactions (Çimen 2008) are the outcomes of oxidative stress in erythrocyte.

Several mechanisms deal with the toxic effects of free radicals. Nonetheless, if high amounts of toxic substances enter the body or the antioxidant system does not have the required efficacy, high amounts of ROS accumulate in biological systems which result in oxidative stress and structural changes in lipids, proteins, and nucleic acids and ultimately lead to the development of various diseases. Erythrocytes are a good model for investigating the oxidative effects of the magnetic field. The flexibility and deformability of heme pocket facilitate the entrance of water and anions. Reactions with these compounds release superoxide anion radicals and produce methHb, which converts to hemichrome and Heinz bodies in erythrocytes (Winterbourn 1985; Signorini et al. 1995).

According to previous studies, magnetic fields can induce oxidative changes in Hb. Exposure to magnetic fields redistributes the electron density in the porphyrin ring of hemoglobin and changes the position of the iron atom. These conformational changes reduce the oxygen affinity of Hb (Shigina 2002). Also, Spectral analysis of Hb has shown a higher absorption at 546 nm and at 581 nm (Motta et al. 1998). In addition to Hb, rat brain proteins undergo oxidative damages and carbonyl formation during exposure to

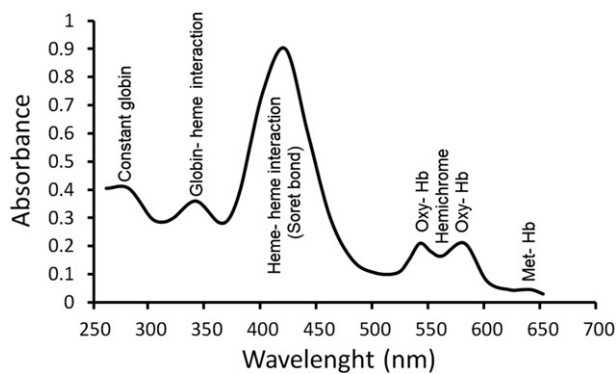


Figure 1. Absorption spectrum of Hb molecule. Absorption band at indicated wavelengths shows constant globin (275 nm), globin-heme interaction (340 nm), heme-heme interaction (420 nm), oxyHb (542 and 577 nm), hemichrome (560 nm) and metHb (630 nm).

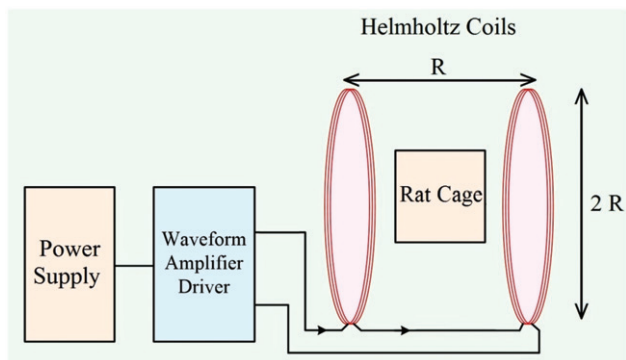


Figure 2. Helmholtz configuration for generating a magnetic field. Helmholtz coils are a pair of circular coils on a common axis with a common radius which produces a region with a nearly uniform magnetic field.

magnetic fields (Megha et al. 2012). Total antioxidant capacity (TAC) of plasma (Sirmatel et al. 2007; Bodera et al. 2013) and FRAP (Goraca et al. 2010) are another parameters for investigating the biological effects of magnetic fields.

The present study aimed to investigate changes in hemoglobin conformation by measuring metHb and hemichrome, oxidative damage to plasma proteins by estimating the carbonyl groups and FRAP following exposure to an ELFMF at 0.7 mT and 50 Hz. Furthermore, the protective role of the aqueous extract of *Myrtus communis* on Hb structure and oxidative condition of erythrocyte and plasma was evaluated after exposure to magnetic fields.

Another purpose of this study was to demonstrate the accuracy of ANN analysis for determining the most important hematological and biochemical parameters during exposure to ELFMF and *M. communis* extract administration. We constructed a multi-layer perceptron network (MLP) with feedforward learning algorithm for demonstrating the Hb oxygenation under oxidative and antioxidative conditions.

Materials and methods

Laboratory animals

The design of this study and experimental groups were according to previous studies (Avci et al. 2012; Martínez-

Sámamo et al. 2012; Jahromy et al. 2016). Twenty-one adult male Wistar rats (3 months old; weight 210 ± 10 g) were kept under standard conditions in terms of temperature, humidity, light, and access to food and water. Same numbers of rats were randomly divided into 3 groups: (1) control group: kept in the same condition but did not receive any treatment. Therefore, the effects of magnetic field on the control group were prevented, (2) magnet group: exposure to ELFMF (0.7 mT, 50 Hz) and 3) magnet and *Myrtus* group: exposure to ELFMF (0.7 mT, 50 Hz) after administration of *M. communis* extract. In this study, 0.5 mg/kg of *M. communis* extract was injected intraperitoneally for one month every day before ELFMF exposure.

The exposure time to the magnetic field was 1 month, every day for 2 hours. One blood sample was obtained from the heart of each rat. All biochemical and hematological experiments were done duplicate. Hemoglobin conformation, Hb derivatives, FRAP assay, and protein carbonyl assay were done independently in each blood sample.

Magnetic field generation

In this study, a Helmholtz coil (60 cm diameter; Figure 1) was used to generate the magnetic field. The required current for the sinusoidal magnetic field (0.7 mT intensity and 50 Hz frequency) was directed on the coils by the power supply and signal generator. To ensure the magnetic field intensity and frequency stability, a Gauss Meter (model GM2, Alphalab, Inc.) and an oscilloscope (MimipaMO-125 OS, 50 MHz, USA) were used, respectively. The inhomogeneity of the magnetic field was about 5% at the cage location.

Structural changes in hemoglobin

The spectrophotometric method was used to study the conformational changes of Hb. Optical absorption of Hb was measured at different wavelengths from 200 to 700 nm (Figure 2). The following equations were used for calculating oxyHb, metHb, and hemichrome concentrations (Ibrahim et al. 2008):

$$\begin{aligned} \text{OxyHb} &= 119A_{577} - 39A_{630} - 89A_{560} \\ \text{MetHb} &= 28A_{577} + 307A_{630} - 55A_{560} \\ \text{Hemichrome} &= -133A_{577} - 114A_{630} + 233A_{560} \end{aligned}$$

Abbreviation A stands for optical absorption at the mentioned wavelengths.

FRAP assay

The FRAP assay introduced by Benzie and Strain (Benzie and Strain 1996) for measuring the total antioxidant power of plasma. Briefly, the antioxidant agents reduce tripyridyl-triazine complex (TPTZ- Fe^{3+}) to ferrous (Fe^{2+}) form which is blue in the acidic medium, with maximum optical absorption at 593 nm.

Table 1. Selected oxidative stress biomarkers as dependent and independent parameters for ANN analysis.

Abbreviation or name of variable	Variable definition	Unit	Type of variable
OxyHb	Oxygenated hemoglobin	uM	Dependent
Cabonyl	Carbonyl content of plasma proteins	nM/mg	Independent
FRAP	Ferric reducing ability of plasma	μM	Independent
MethHb	Methemoglobin	uM	Independent
Hemichrome	Oxidized form of hemoglobin	uM	Independent
A	Absorbance of Hb at indicated wavelengths	Number	Independent

Protein carbonyl assay

Carbonyl assay was performed according to Evans (Evans et al. 1999). In this method, 2,4-dinitrophenylhydrazine (DNPH) reacts with carbonyl groups and produce 2,4-dinitrophenylhydrazone derivatives with maximum optical absorption ($\lambda_{\max}$) at 380 nm and expressed as nmol/ml.



Protein concentration was calculated as mg/ml according to a comparison with standard curve and related absorbance at 280 nm. Carbonyl concentration of plasma proteins was assayed according to the following equation:

$$\text{Carbonyl conc. (nmol/mg of protein)} = \text{carbonyl conc. (nmol/ml)} / \text{protein conc. (mg/ml)}$$

Statistical analysis

In this study, IBM SPSS statistics for windows, version 20 (IBM Corp., Armond, N.Y.) was used for regression analysis and statistical analysis and Microsoft Excel 2010 (Redmond, WA) for classifying the data. The mean and standard error of mean (SEM) were determined for each group. ANOVA was used to compare the means of different groups. The significance level was considered to be $p < .05$.

ANN analysis

To investigate the predictive parameters for oxyHb concentration, the ANN analysis was performed using a four-layer perceptron (1,5,5,11). The input layer included independent variables, which were considered as the predictive variables (Table 1). Hyperbolic tangent, identity, and sigmoid functions were tested in the hidden layer in output layer, oxyHb was considered as the dependent variable. During ANN analysis, 70% and 30% of data were randomly used in training and testing phases, respectively. To improve the training process of ANN analysis, the standardization method was used for rescaling the training data, using the following formula:

$$\text{Standardization indicator} = (\times \text{mean}) / \text{SD}$$

This study was carried out after obtaining approval from the Vice Chancellor for Research and Technology (Grant No. 2109) and Ethics Committee of Arak University of Medical Sciences (Ethics Code 95-176-5). Guidelines in the Declaration of Helsinki were considered in all stages of this

study. Also, all mandatory laboratory health and safety procedures have been complied with in the course of conducting our study.

Results

In this research, for studying the protective effects of *Myrtus communis*, adult male Wistar rats were divided into 3 groups. In the magnet group, animals were exposed to ELFMF (0.7 mT, 50 Hz). In magnet and *Myrtus* group, animals were exposed to ELFMF (0.7 mT, 50 Hz) after administration of *M. communis* extract. In the control group, animals were kept in the same condition but did not receive any treatment. Therefore, the effects of magnetic field and *M. communis* extract on the control group were prevented.

Figure 3(A) shows the plasma antioxidant power based on the FRAP. In the group with magnetic field exposure, the FRAP level was reduced from 701 to 601 μM, which indicates a 14.3% decrease in the plasma antioxidant power in comparison to control group ($p < .001$). The use of *M. communis* extract before magnetic field exposure caused an increase in the FRAP level from 601 to 630 μM, which shows a 4.8% increase in the plasma antioxidant power; nonetheless, it was still lower than that of the control group ($p < .001$).

As presented in Figure 3(B), the amount of PCO in control group was 2.58 nmol/mg of protein. After exposing the rats to magnetic radiation, the level of carbonyl groups increased to 3.73 (nmol/mg, $p < .001$). These findings show that magnetic field radiation leads to the oxidation of plasma proteins. Nevertheless, administration of *M. communis* extract under magnetic field exposure caused a significant decrease in the carbonyl groups from 3.73 to 3.27 (nmol/mg), showing a 12.33% reduction in the oxidative effects of magnetic field on plasma proteins ($p < .001$, Figure 3(B)).

Figure 4 shows the mean concentration of Hb derivatives in each group. In the group with magnetic field exposure, a significant reduction in oxyHb was observed from 33.38 to 19.54 μmol, indicating a 41.46% decrease in the erythrocyte capacity to carry oxygen ($p < .001$, Figure 4(A)). Similarly, reduction of Hb optical absorption at wavelengths of 542 and 577 nm indicated a decline in the concentration of oxyHb (Figure 5).

Administration of the extract in rats which exposed to magnetic fields increased the concentration of oxyHb to 26.72 μmol, indicating a 36.8% increase in the erythrocyte capacity to carry oxygen (Figure 4(A)). These results showed that administration of *M. communis* extract can protect erythrocytes against the oxidative effects of magnetic field

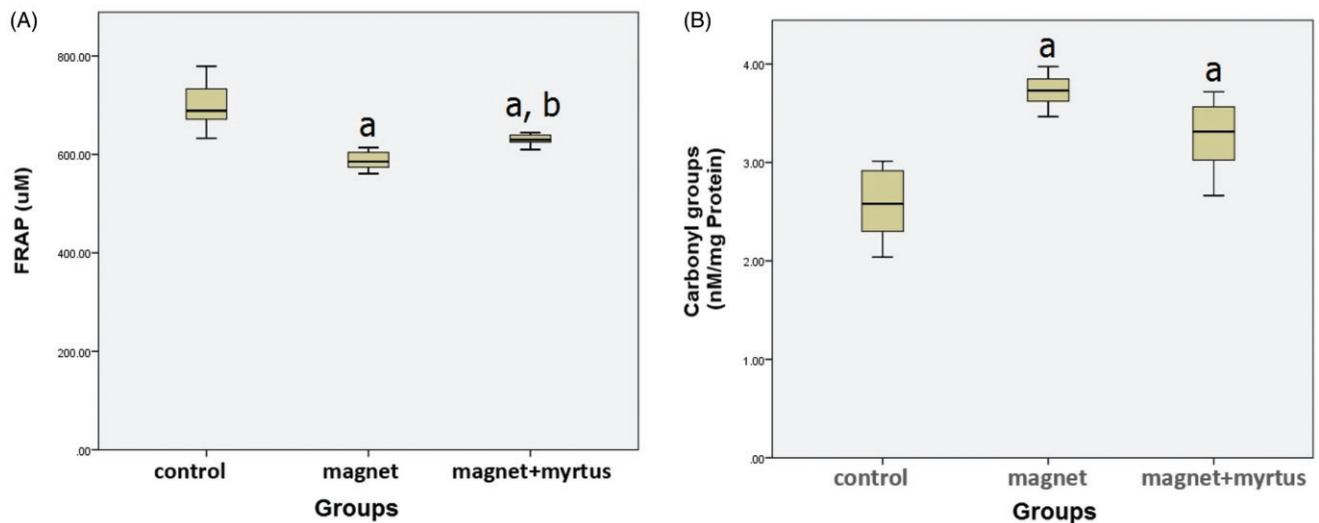


Figure 3. Ferric reducing ability of plasma (A) and carbonyl content of plasma proteins (B). Blood samples were obtained from adult male Wistar rats. Control: without any treatment; Magnet: after one month of daily exposure to ELFEMF (0.7 mT, 50 Hz), Magnet + Myrtus: after one month of daily administration of *Myrtus communis* extract and exposure to ELFEMF (0.7 mT, 50 Hz). Lowercase letters (a and b) indicate significant differences ($p < .001$) from control and magnet groups, respectively.

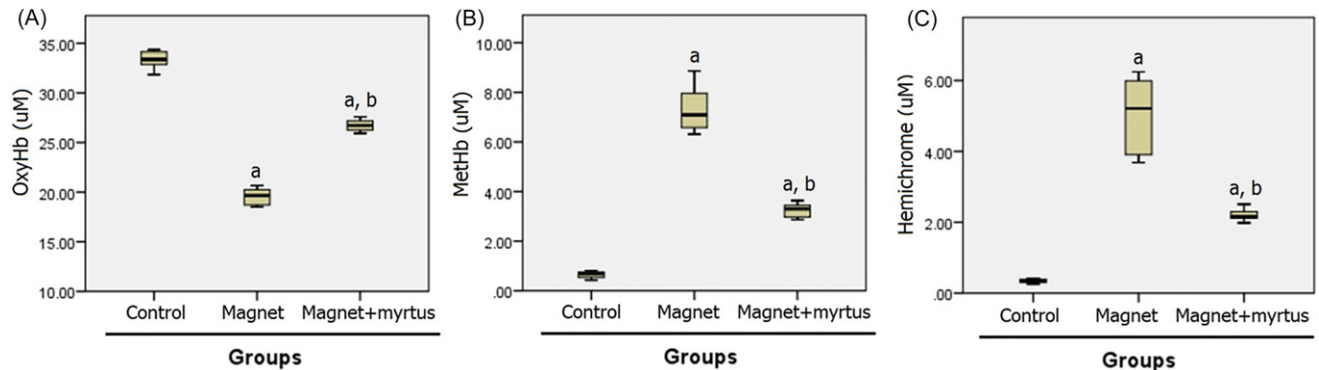


Figure 4. The mean values of oxyHb, metHb and hemichrome as Hb derivatives. Hb concentration was 4 $\mu\text{mol/L}$ in hypotonic phosphate buffer pH 7.4. Data are in terms of $\mu\text{mol/L}$. Blood samples were obtained from adult male Wistar rats. Control: without any treatment; Magnet: after one month of daily exposure to ELFEMF (0.7 mT, 50 Hz); Magnet + Myrtus: after one month of daily administration of *Myrtus communis* extract and exposure to ELFEMF (0.7 mT, 50 Hz). Lowercase letters (a and b) indicate significant differences ($p < .001$) from control and magnet groups, respectively.

radiation. These findings are in agreement with the significant increase in the optical absorption of hemoglobin at 577 and 542 nm in the group received the extract (Figure 5).

According to the results of the present study, changes in oxyHb concentration are relative to changes in metHb and hemichrome concentrations. The metHb level was 0.64 μmol in the control group, which increased to 7.34 μmol after exposure to ELFEMF that indicates the 11.5-fold increase in Hb oxidation (Figure 4(B)). On the other hand, administration of the extract reduced the metHb level to 3.23 μmol (a 56% reduction), which shows a significant protective effect of *M. communis* against Hb oxidation.

Hemichrome concentration was 0.34 μM in control group, which increased to 4.99 μmol after magnetic field exposure, indicating a 14.7-fold increase in Hb oxidation (Figure 4(C)). After administration of the *M. communis* extract, the hemichrome concentration was reduced to 2.21 μM ($p < .001$), which was in association with the increase in Hb absorption at 560 nm (Figure 5).

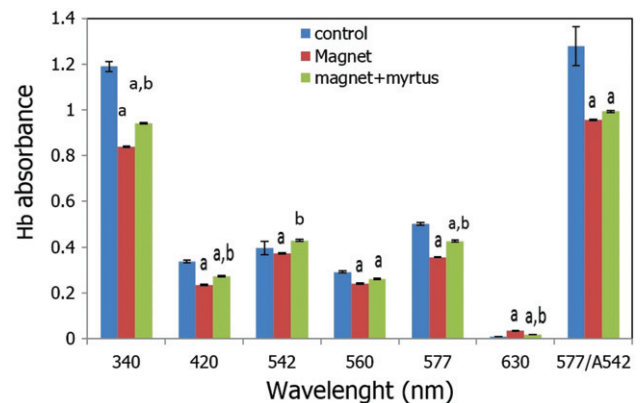


Figure 5. Comparison of Hb absorbance at different wavelengths. Blood samples were obtained from adult male Wistar rats, washed 3 times with isotonic phosphate buffer (pH 7.6) and lysed with hypotonic phosphate buffer (pH 7.6). Control: without any treatment; Magnet: after one month of daily exposure to ELFEMF (0.7 mT, 50 Hz); Magnet + Myrtus: after one month of daily administration of *Myrtus communis* extract and exposure to ELFEMF (0.7 mT, 50 Hz). Error bars show SEM, and lowercase letters (a and b) indicate significant differences ($p < .05$) from control and magnet groups, respectively.

Table 2. Model summary of our proposed ANN model.

Training	Sum of squares error	.100
	Relative error	.017
	Stopping rule used	1 consecutive step(s) with no decrease in error ^a
	Training time	0:00:00.00
Testing	Sum of squares error	.041
	Relative error	.011

This table displays a summary of the neural network results by partition and overall, including sum-of-squares error, relative errors, and the training time.

^aError computations were based on the testing sample, Dependent variable: OxyHb.

The Soret band (420 nm) indicates the heme-heme interactions in Hb structure. In the experimental group which exposed to ELFMF, a significant decrease of Soret band was observed from 0.34 to 0.24 ($p < .05$; Figure 5). After the administration of the *M. communis* extract, optical absorption in the Soret band increased to 0.27 which indicates an enhancement of heme ring interactions and hemoglobin protection against the oxidative effects of magnetic fields ($p < .05$; Figure 5). Magnetic field exposure decreased optical absorption of Hb from 1.19 to 0.84 at 340 nm, which indicates a 29.4% decline in heme-globin interactions ($p < .05$; Figure 5). Interestingly our results showed a significant increase in optical absorption of Hb to 0.94 following the administration of *M. communis* extract in rats that exposed to magnetic fields ($p < .05$; Figure 5).

The decrease in A577/A542 ratio indicates the conversion of oxyHb to metHb. In the group under magnetic field exposure, the corresponding ratio decreased from 1.28 to 0.96, indicating a 25% increase in the conversion of oxyHb to metHb. These results are in association with a significant increase in optical absorption at 630 nm, which indicates the formation of metHb. Administrations of *M. communis* extract to rats that exposed to magnetic fields increase the A577/A542 ratio to 0.99, indicating its protective effects on the conversion of oxyHb to metHb. Simultaneously, Hb optical absorption decreased at the wavelength of 630 nm, which indicates a reduction in metHb concentration in erythrocyte and confirms the protective effects of *M. communis* extract (Figure 5).

ANN analysis was performed to determine predictive parameters for oxyHb concentration. The four-layer perceptron method (11,5,5,1) was used to optimize the analysis. Hyperbolic tangent and identity functions were applied as activation functions in the hidden layer and output layer, respectively. An appropriate ANN model was selected based on R^2 , sum of squared errors, and relative errors in training and testing steps. It is generally accepted that in an appropriate model, the error level should be less than 20%. As presented in Table 2, in our proposed model, the sum of squared errors in the training and testing steps were 0.100 and 0.041, respectively, and the relative errors were 0.017 and 0.011, respectively.

Figure 6(A) shows a linear relationship between the predicted and observed values of oxyHb ($y = 0.93 + 0.96x$ and $R^2 = 0.989$). Figure 6(B) shows the relationship between the predicted and residual values. The random distribution of data represents the high accuracy of the proposed model. The A577/A542 ratio represents the mutual conversion of oxyHb and metHb and is the most important parameter for

predicting oxyHb concentration. Other predictive parameters for oxyHb concentration with a normalized value more than 50% was as follows: A560, metHb concentration, A630, A542, hemichrome concentration, A577, and A420 (Table 3).

Discussion

The results of the present study showed that ELF-MF induces a significant decrease in the antioxidant power of plasma (Figure 3). Considerably, the *M. communis* extract retained the plasma antioxidant power and protected against the oxidative effects of the magnetic field which is in accordance with previous studies about antioxidant effects of *M. communis* extract (Yadegarinia et al. 2006; Mimica-Dukić et al. 2010; Wannes et al. 2010).

Oxidative stress induces conformational damages in proteins. The degrees of damages depend on the type and concentration of oxidants, efficiency of the antioxidant systems and source of free radicals. Many studies examined the effects of electromagnetic waves and electromagnetic fields on protein carbonylation (Goto and Radák; Alamdari et al. 2005; Dalvi et al. 2013; Wadley et al. 2016) which indicates the oxidative modification of proteins. According to our results, ELF-MF increased the carbonyl groups in plasma proteins which are associated with the reduced antioxidant power of plasma (Figure 3).

Consales showed that ELF-MF exposure and subsequent oxidative stress induces α -synuclein expression and increase the risk of neurodegenerative diseases (Consales et al. 2018).

Falone reported different results in young rats which showed that ELF-MF enhances neurotrophic signaling and antioxidant enzymes and protect them from oxidative stress-induced neuronal pathologies (Falone et al. 2008).

Also, studies in neuroblastoma cells showed that ELF-MF activates cytoprotective and antioxidative pathways and induces a survival and proliferative advantage during aggressive behavior (Falone et al. 2017).

In agreement with our results, some studies have shown that an electric field of 50 Hz and 12 kV/m significantly increases the protein carbonylation (Güler et al. 2009). Although in contradiction with the present results, in another study (Ferreira et al. 2006), exposure of pregnant rats to electromagnetic waves (peak, 600 mW; 834 MHz; 26.8–40 V/m; vertical polarization) did not change the carbonyl levels in the plasma or liver of newborn mice. In this regard, Ali et al (Ali et al. 2003), applied a similar frequency (50 Hz), but lower flux density compared to our study

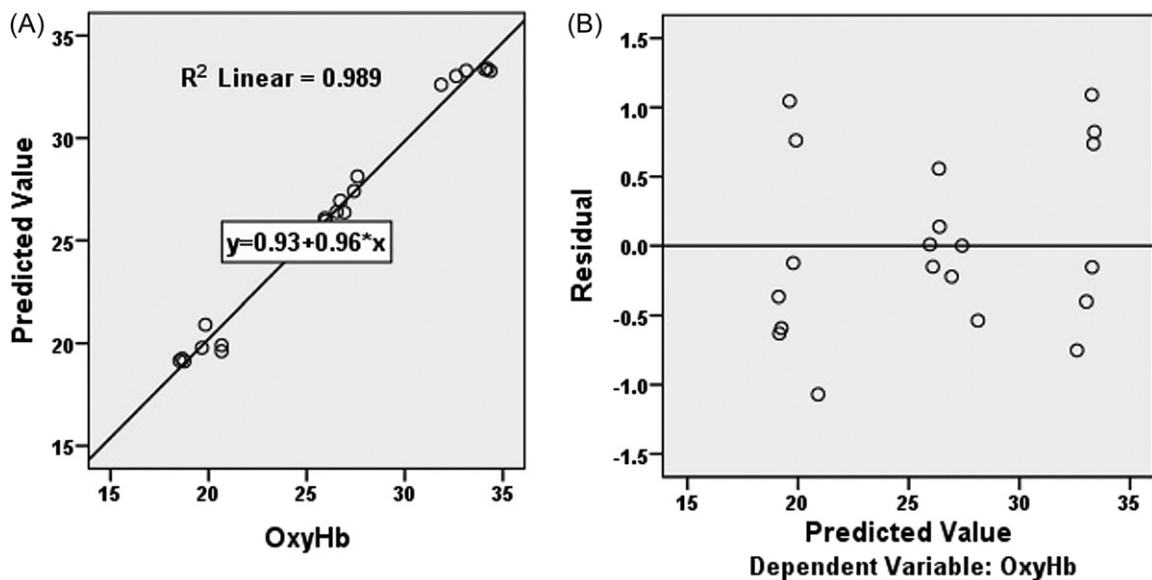


Figure 6. Predicted by observed (A) and residual by predicted (B) charts for the proposed ANN model. Predicted by observed chart displays a predicted-by-observed-value chart for oxyHb as a dependent variable. Residual by predicted chart displays a residual-by-predicted-value chart for oxyHb as a scale-dependent variable.

Table 3. Independent variable importance analysis for our proposed ANN model.

	A ₅₇₇ /A ₅₄₂	A ₅₆₀	MetHb	A ₆₃₀	A ₅₄₂	Hemichrome	A ₅₇₇	A ₄₂₀	FRAP value	A ₃₄₀	PCO
Importance	0.148	0.147	0.115	0.101	0.093	0.085	0.083	0.081	0.072	0.052	0.021
Normalized importance (%)	100.0	99.1	77.5	67.8	63.0	57.0	56.3	54.8	48.6	35.2	14.4

This table performs a sensitivity analysis, which computes the importance of each predictor in this study. The analysis is based on the combined training and testing samples.

A: Absorbance of Hb at indicated wavelengths; FRAP: Ferric Reducing Ability of Plasma; MetHb: Methemoglobin; PCO: Carbonyl content of plasma proteins.

(0.2 mT vs. 0.7 mT) and showed structural changes in hemoglobin and reduction of elasticity, and permeability of the erythrocyte membrane. On the other hand, a study by Lin et al., showed that by decreasing the permeability of erythrocytes in static magnetic fields (0.2 T and 0.4 T), results a decrease in biophysical stability of cell membrane. Moreover, the survival of frozen erythrocytes was increased (Lin et al. 2013), without changing erythrocyte morphology. Studies showed that the oxidative effects of electromagnetic fields depend on the characteristics, source, intensity, frequency, and exposure time. Elferchichi confirmed the effects of magnetic fields on erythrocyte, hemolysis, and finally hypoxia (Hassan and Abdelkawi 2010).

Magnetic fields can induce oxidative changes in Hb, redistribute the electron density in the porphyrin ring of hemoglobin and changes the position of the iron atom. These conformational changes reduce the oxygen affinity of Hb (Shigina 2002).

The Hb oxidation resulted in the formation of metHb. This study showed an increase in metHb and a decrease in oxyHb concentrations after exposure to magnetic fields. Also, our results showed a significant reduction of A₅₇₇/A₅₄₂ ratio which represents the conversion of oxyHb to metHb.

Methemoglobin reductase plays an important role in the conversion of metHb to oxyHb. However, if oxidative conditions are severe or metHb reductase cannot reduce the

metHb molecules, it converts to hemichrome that precipitate in erythrocytes and convert to Heinz bodies. On the other hand, metHb plays a central role in oxidative stress which can lead to hemolysis (Mohammed and Ahmad 2015; Ballard et al. 2016). The results of the present study are in accordance with Abdul Moiz (Elferchichi et al. 2016), which concluded that although metHb has diamagnetic effects, it can be influenced by the magnetic fields, based on the mass susceptibility, molar susceptibility, Curie constant, and magnetic moment.

Interestingly, our results showed that administration of *M. communis* extract protected Hb against the oxidative effects of the magnetic field by preventing metHb formation.

According to this study, the magnetic field caused a significant reduction in Hb optical absorption at 340 nm, indicating a disturbance in noncovalent bonds of His-H7 with the heme iron. Moreover, a significant increase in the Hb optical absorption at 630 nm is indicative of the weakening of iron-nitrogen bond in the porphyrin ring and interaction between the globin and heme ring. These changes reduce the amount of Hb affinity to oxygen and ultimately lead to hypoxia.

Although the characteristics of magnetic fields were different in our study and Hassan (Hassan and Abdelkawi 2010), but the results of both studies indicate an increase in optical absorption at 630 nm and a decrease in the A₅₇₇/A₅₄₂ ratio after exposure to ELFMF. This could indicate

destabilization of hemoglobin molecules during exposure to a magnetic field. Based on our results a significant reduction in the Hb optical absorption at 420 nm (Soret band) reflects the reduction in heme-heme interactions due to oxidative stress during exposure to magnetic fields. Our results also showed a reduction in optical absorption at 577 nm which indicates the heme-heme interaction. With the administration of *M. communis* extract, the increase in optical absorption at 577 nm indicates protection against conformational changes of globin moieties.

One of the limitations of this study, was that the control group did not receive any treatments such as normal saline or vehicle. Although according to previous studies, normal saline or vehicle administration in control or sham groups did not change the oxidative parameters such as MDA, glutathione (Assimakopoulos et al. 2005; Haleagrahara et al. 2011; Arab et al. 2018), carbonyl content of proteins, lipid hydroperoxides (Assimakopoulos et al. 2005; Haleagrahara et al. 2011), protein thiols, protein disulfides, and apoptosis (Assimakopoulos et al. 2005).

In this study, a multi-layer perceptron network with feed-forward learning algorithm was constructed for indicating the most important parameters on Hb oxygenation. Dai applied artificial neural network for monitoring hemoglobin oxygenation during invasive brain surgery (Dai et al. 2008). Also, Ding showed the effectiveness of spectrophotometric analysis and back propagation artificial neural network as a non-invasive detection of Hb concentration (Ding et al. 2014). Our designed ANN showed the importance of Hb absorbance at 420–577 nm and metHb and hemichrome concentrations (Table 3).

Conclusion

In our previous study, we exposed erythrocyte to magnetic fields and lead ions and found that the oxidative effects of metal-catalyzed systems are intensified in magnetic fields and lead erythrocyte towards the oxidative challenge. Similarly, in the present study, we concluded that changes in Hb conformation could be associated with the formation of oxygen free radicals due to exposure to the magnetic fields.

Since the reduced forms of heme iron and globin chains are necessary for their biological activity, oxidative damage to Hb, and proteins can reduce erythrocyte lifespan and cause pathological changes and anemia. Interestingly administration of *M. communis* extract induces a significant protection against these oxidative damages.

Innovations

In this research, a quantitative and sensitive algorithm of biochemical and hematologic methods was proposed to investigate the oxidative effects of magnetic fields and the protective effects of *M. communis* extract, changes in plasma antioxidant power, carbonyl groups in plasma proteins and changes in Hb conformation and heme structure.

Furthermore, the predictive factors for the level of oxyHb were studied by ANN analysis.

In the present study, the spectrophotometric analysis showed that the *M. communis* extract protects the erythrocytes against the oxidative effects of magnetic fields by retaining the Hb conformation and reduction of carbonyl groups. These results are in association with a significant increase in antioxidant power of plasma.

In addition, an ANN model was proposed to study the most effective parameters on oxyHb concentration. This model showed that optical absorption of Hb at 577, 560, and 542 nm were the most important parameters for predicting the oxyHb concentrations. Also considering our results, the ANN analysis is an accurate and reliable approach for determining the Hb oxygenation during healthy and pathologic situations.

Limitations

This study aimed to examine Hb and plasma protein oxidation. For a comprehensive study of the effects of magnetic fields and the protective role of *M. communis* extract, other oxidation parameters, such as antioxidant enzymes, lipid peroxidation, and oxidation of carbohydrates and nucleic acids can be considered in future studies. Also, the ANNs could not explain the relationship between dependent parameters.

Disclosure statement

No potential conflict of interest was reported by the authors.

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