

Modulation of human erythrocytes properties post exposed to static magnetic field

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Abstract: The effect of static magnetic field on the biophysical properties of different samples of saline and erythrocytes suspension was investigated. In addition, the effect of magnetized saline on the erythrocytes antioxidant enzymes was evaluated. Biophysical properties including pH, boiling point, conductivity and viscosity showed positive effect on applying magnetic field to the saline samples for different periods. Increasing the time of exposure increases the pH, boiling point, conductivity and viscosity values, which may be due to increasing hydrogen bonding and water clusters. The biophysical properties of erythrocytes were studied for magnetized and unmagnetized suspensions. The results showed that by calculating the NaCl concentration (in the osmotic fragility test) which is able to make 50% hemolysis for the unmagnetized and magnetized erythrocytes suspension sample, it was found to be 0.5% and 0.45% respectively which means that, for the magnetized sample the cell membrane became less permeable to water molecules and higher osmotic pressure is necessary to pump water molecules to the cells, so the erythrocytes cell membrane became stronger to tolerate the osmotic pressure. The oxidative hemolysis of erythrocytes by ascorbic acid was also studied to confirm the result obtained from the osmotic fragility test; it was found that the hemolysis rate for the unmagnetized sample was 0.0083 and that for magnetized sample was 0.0077, which means that the magnetized sample takes a longer time to complete hemolysis or can tolerate the oxidative stress more than the untreated sample. The activity of glucose 6-p dehydrogenase and antioxidant enzymes activities including glutathione peroxidase, glutathione reductase, were measured in erythrocytes before and after exposure to magnetic field. There was a significant increase in the enzymes activities in magnetized as compared with unmagnetized. The observations described in this work are of great interest and importance, in a way that they help in applying magnetic treatment devices technology in various fields such as industry, medicine and agriculture to improve water properties.

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1. Introduction

Human body consists mainly of water and all living process are strongly dependent on water, which is secret of life that leads to investigate the possibility of improving quality of water by passing it through a magnetic field with different devices and strength. This water treatment process has small installation fees, no energy requirements and creates no pollutants (**Gursche et al., 1998**). Different studies have found that various aspects of the liquid water structure, including the size of the water cluster, change when exposed to a magnetic field. The use of a magnetic field to generate large water clusters is of considerable interest in a number of practical applications (**Iwasaka and Ueno; 1998; Nakagawa et al., 1999**). It was found that the number of hydrogen bonds increases slightly as the strength of the magnetic field is increased. This implies that the size of a water cluster can be controlled by the application of an external magnetic field. Also, the structure of the

water is more stable and the ability of the water molecules to form hydrogen bonds is enhanced when a magnetic field is applied. In addition, the behavior of the water molecules changes under the influence of a magnetic field (**Changa and Weng, 2006**). Magnetization is necessary to regulate blood chemistry, flow and keep a pH balance. Since the blood is 90% water, it is obvious that water, which is bio-magnetized, is more effective in maintaining blood quality. But in addition to the influence magnetism has on water and blood, it also is necessary to every aspect of life. The common factor of all aspects of life is dependency on magnetism for survival (**Ohno and Reminick, 2001**).

The effect of magnetized physiological solution (MPS) on isolated, perfused snail heart muscle contractility was studied and found that, The MPS had a depressing effect on ⁴⁵Ca uptake by muscles and intracellular cAMP content and an

elevating effect on intracellular cGMP level. (Ayrapetyan *et al.*, 2005).

Since magnetic field has an impact on biochemical reactions that involve more than one unpaired electron, superoxide dismutase (SOD), one of the enzymes responsible for antioxidant system, was measured under magnetic fields. There has been a significant increase of SOD activity when passed 0, 1, 9 and 15 times at 2.9-4.6 mT magnetic field density for 0, 2.2, 19.8 and 33.0 seconds respectively (Büyüksulu, 2006).

The present work aimed to evaluate the variations in some biophysical markers induced in saline and erythrocytes suspension subjected to magnetic field at different periods. Also to study the variations in some antioxidant enzymes activities in erythrocytes subjected to magnetic field.

2. Materials and Methods

The magnetic treatment device (MTD)

The used MTD in this work is a commercial magnetic double walled cup of stainless steel material from outside and ceramic from inside and of 12 cm height (made in China). The MTD is made up of four super power biomagnets 3000 gauss arranged in an alternating configuration. The cup can hold 220 ml of sample.

Samples

Saline

Normal saline solution (0.15 M NaCl), was prepared by dissolving 8.766 gm NaCl (Mw 58.44) in distilled water in a clean container to a total volume of 1000 ml. Magnetized saline solution was prepared by dissolving NaCl salt in distilled water then subjected in MTD for 5 hours and stored at room temperature.

Blood

Blood samples were collected from healthy donors on heparinized tubes. Erythrocytes were separated by centrifugation (10 min, 1500 rpm), plasma and buffy coat removed and the cells washed three times with isotonic saline (0.15 M NaCl) at room temperature. By repeated centrifugation (three times), washed erythrocytes were suspended in normal or magnetized saline to reach a concentration of about 3×10^5 cells/ml.

Methods

Biophysical measurements

They include pH value, boiling point, electric conductivity, viscosity, the osmotic fragility test, hemolysis of erythrocytes by ascorbic acid (vitamin C).

pH value

Saline was tested for its pH value before and after keeping the sample in the MTD different periods of time started from one hour up to five hours, using a pH meter model 3200, Genway KFMRC-tcu-008.

Boiling point

In this measurement, the boiling points of the different samples of saline was recorded before and after the samples were magnetized in the cup different periods of time started from one hour up to five hours. This was done by heating each sample and recording the boiling point of it.

Electric conductivity

The conductivity of normal and magnetized saline was measured using portable conductivity meter model FE287/kit – EDT DIRECT ION LTD – UK. The cell constant = 1 cm^{-1} . The apparatus cell contains two platinum electrodes separated by a fixed distance. The conductivity of each sample of saline before and after magnetization in the cup (from one hour up to five hours) was recorded.

Viscosity

The Ostwald viscometer was used for the determination of the relative viscosity of the used samples of saline before and after magnetization in the cup (for different periods of time started from one hour up to five hours) by comparison with a standard liquid (non magnetized saline). The viscometer measures the time interval through which sample takes to pass the capillary tube from one fixed point to another. This interval is compared with the corresponding time interval of the standard liquid and the relative viscosity was calculated for each reading.

The osmotic fragility test

In this test whole blood (0.1ml) was added to varying concentrations of sodium chloride solution (from 0, 0.1 and 0.2 up to 1%) of volume 9.9 ml, and allowed to incubate at room temperature for half an hour. The level of hemolysis of erythrocytes was determined by measuring hemoglobin released from the cells, relative to the total cellular hemoglobin content. The amount of hemoglobin was estimated on the basis of absorbance at 540 nm for the supernatant of saline. The same experiment was repeated by using different concentrations of magnetized saline for a period of five hours of magnetization. Absorbance of the supernatant after complete hemolysis with distilled water (0%) was taken as 100% hemolysis.

Hemolysis of erythrocytes by ascorbic acid (vitamin C)

Erythrocytes suspension was prepared as mentioned above and 0.1 ml of vitamin C (concentration 0.25 mM) was added to 2.9 ml of erythrocytes suspension. To follow the oxidation process, the absorbance of the sample was recorded spectrophotometrically at wavelength of 577 nm each 5 minutes for one hour. The experiment was repeated by using a magnetized saline (for a period of five hours of magnetization).

Biochemical properties

The impact of magnetic field treatment on erythrocytes antioxidants enzymes activities including glutathione reductase, Glutathione Peroxidase and Glucose-6-Phosphate Dehydrogenase were evaluated by kits purchased from RANDOX laboratories Ltd, United Kingdom.

Statistical analysis

The statistical analyses of the data was done by calculating means, standard deviations for biophysical measurements, also P-value, T-test and confidence interval by SPSS program for biochemical properties. The data drawn by excel program.

3. Results and discussion

The effect of static magnetic field on the biophysical properties of saline and erythrocytes suspension was investigated. In addition, the effect of magnetized saline on the erythrocytes antioxidant enzymes was evaluated.

Biophysical properties of saline including pH, boiling point, conductivity and viscosity showed positive effect on applying magnetic field to the samples for different periods. Increasing the time of exposure increases the pH, boiling point, conductivity and viscosity values as shown in figure.1 (a, b, c, d). The variations or the disturbance of pH balance is due to the effect of the magnetic field in enhancing and decreasing the number of hydrogen bonding and the size of water cluster (Fujimura and Iino; 2009). The increase in boiling point values may be attributed to the stabilization of hydrogen bonds which increases the free energy of water (Fujimura and Iino, 2009). The variations induced in the viscosity values may be due to the compression induced in particles due to the effect of magnetic field which increase the viscosity (Ishii *et al.*, 2005). The increase in conductivity values due to the exposure of the saline samples to magnetic field may be due to the reduction in entropy in saline samples which is a result of strengthening of the hydrogen bonds due

to the application of magnetic field. The decrease in entropy rearrange the molecules in the samples increasing their electric conductivity (Inaba *et al.*, 2004)

After withdrawal of saline samples from magnetic field the measurements indicated that samples kept magnetization effect and tried gradually to return to their original values with time; decreasing the pH, boiling point, conductivity and viscosity values (not included in this work). The effect of magnetic field on the osmotic fragility of erythrocytes was determined to compare between the untreated and treated samples by magnetic field. Figure 2 shows the results of osmotic fragility measurements for treated and untreated erythrocytes, where the percentage of hemolyzed cells is plotted as a function of the concentration percentage of NaCl. From figure 2, it is possible to calculate the concentration of NaCl needed to obtain 50% hemolysis for each treated and untreated samples, which was found to be for the unmagnetized and magnetized erythrocytes suspension samples, 0.5% and 0.45% respectively which means that, the pronounced shift towards lower concentration of NaCl for the magnetized sample indicates that the cell membrane became less permeable to water molecules and higher osmotic pressure is necessary to pump water molecules to the cells. This result indicates that the erythrocytes cell membrane became stronger to tolerate the osmotic pressure (Ibrahim, 2006).

The oxidative hemolysis of erythrocytes by ascorbic acid was also studied to confirm the result obtained from the osmotic fragility test. In this experiment ascorbic acid at concentration of 0.25 mM was used to cause oxidative hemolysis to erythrocytes suspension (Ibrahim *et al.*, 2010). Figure 3 shows the variations in absorbance at wavelength of 577 nm with time due to the oxidative effect of ascorbic acid for the untreated and treated samples with magnetic field. In the figure there is a time interval (in each curve) during which the variations in absorbance with time is nearly linear, the slope of this interval gives the hemolysis rate; the rate at which the number of cells in the suspension decreases. Also it shows that the hemolysis rate for the untreated sample was 0.0083 and that for treated samples with magnetic field was 0.0077, which means that the magnetized sample takes a longer time to complete hemolysis or can tolerate the oxidative stress more than the untreated sample.

Free radical in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology. An over production of these reactive species can occur, due to oxidative stress brought

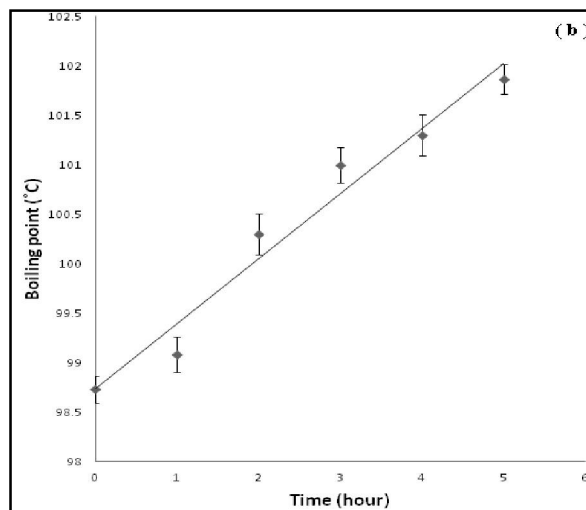
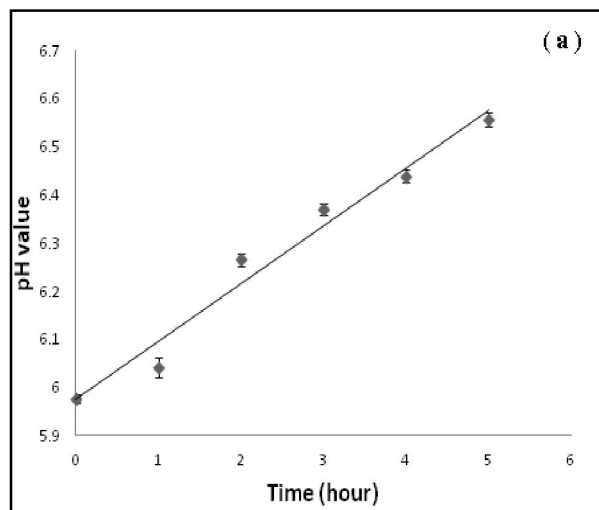
about by the imbalance of bodily antioxidant defense system and free radical formation. These reactive species can react with biomolecules, causing cellular injury and death. This may lead to the development of chronic diseases such as cancers and those that involve the cardio- and cerebrovascular systems (Masella *et al.*, 2005). The consumption of antioxidants has been found to offer protection against these diseases. Antioxidants are often added to foods to prevent the radical chain reactions of oxidation, and they act by inhibiting the initiation and propagation step leading to the termination of the reaction and delay the oxidation process (Valko, *et al.*, 2001).

The effect of magnetic field on Glucose-6-Phosphate dehydrogenase and antioxidant activities were evaluated in this study. Results obtained as shown in figure 4 showed that, the activity of Glucose-6-Phosphate dehydrogenase was statistically significant increased after exposed to magnetic field and comparing with none magnetized ($P < 0.001$). In addition, the activity was shown to be significantly decreased by time (from one to three minutes). However, the magnetic field enhances the activity but doesn't reach to normal values. The enhancement of antioxidant activity by magnetic field was attributed to stimulation the conversion of inactive zymogens to active enzyme form. A major defense mechanism against free

radical involves the antioxidant enzymes, including SOD, catalase and glutathione peroxidase (GPx), which convert active oxygen molecules into non-toxic compounds.

The lipid peroxidation is accelerated when free radicals are formed as the results of losing a hydrogen atom from the double bond in the structure of unsaturated fatty acids. The activities of Glutathione peroxidase and Glutathione Reductase as shown in figures 5&6 respectively were statistically significant increased after exposed to magnetic field comparing with non magnetized ($P < 0.001$). In addition, the activity was shown to be significantly decreased by time. However, the magnetic field enhances the activity but doesn't reach to normal values.

The results obtained revealed that, magnetic field enhances the antioxidant activities. The antioxidant properties are attributable to the ability of magnetic field to induce the activation properties of these enzymes. Also, this study suggests that magnetic field has a potent antioxidant activity. These observations were documented by biochemical results that supporting the potential clinical use of magnetic field in the treatment of some diseases. Further studies will be carried out to evaluate the *in vivo* effects on experimental animals.



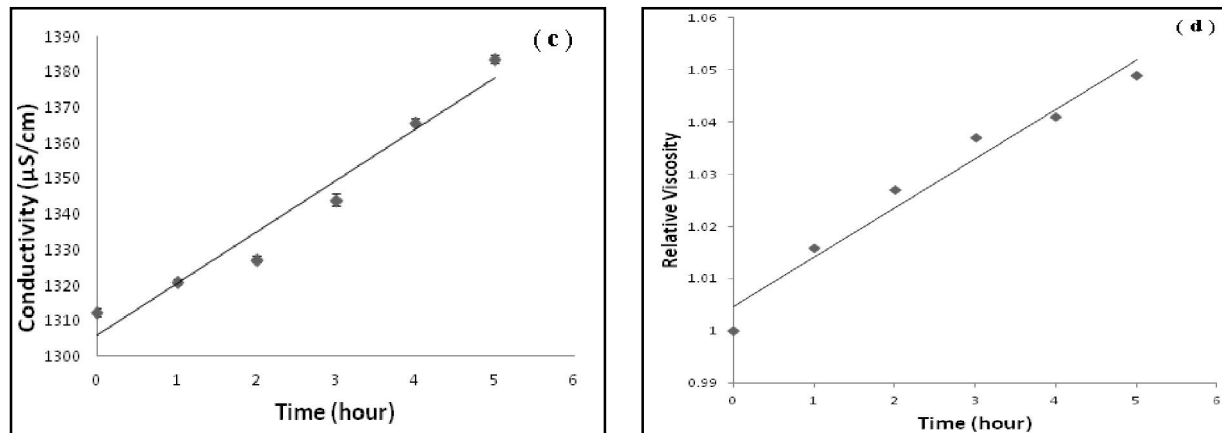


Fig. 1: The change in biophysical parameters [pH (a), boiling point (b), conductivity (c) and relative viscosity (d)] of saline with the time of exposure to magnetic field.

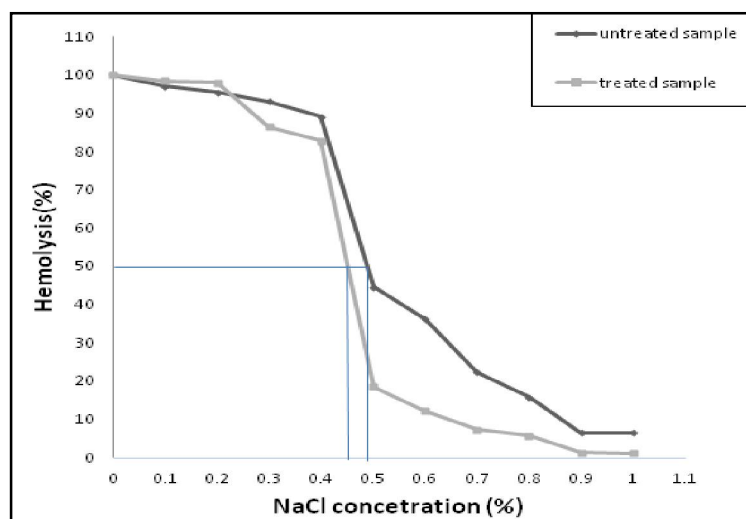


Fig. 2: The variation in the percent hemolysis of erythrocytes as a function of NaCl concentration for untreated and treated samples with magnetic field.

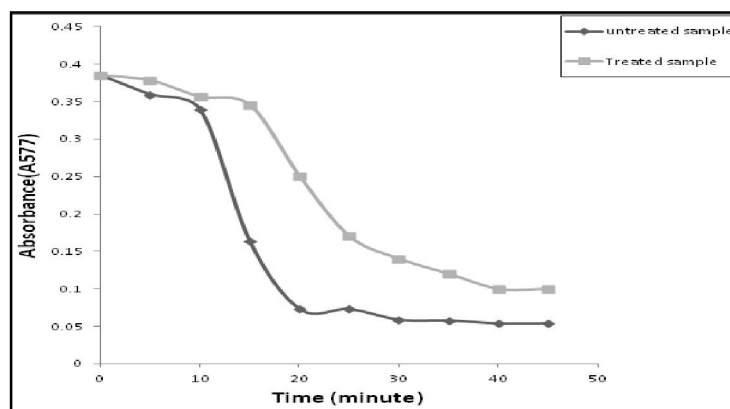


Fig. 3: The change in absorbance at wavelength 577 nm with time for untreated and treated erythrocytes with magnetic field.

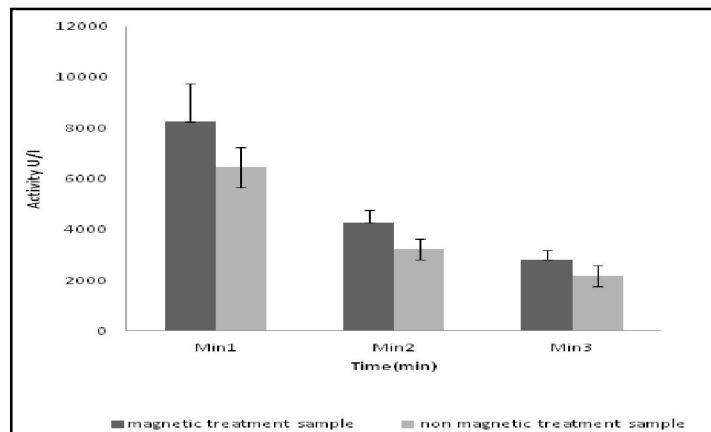


Fig. 4: Comparison between magnetic treatment blood samples with non magnetic treatment blood sample on the activity of erythrocytes Glucose-6-Phosphate dehydrogenase at different time

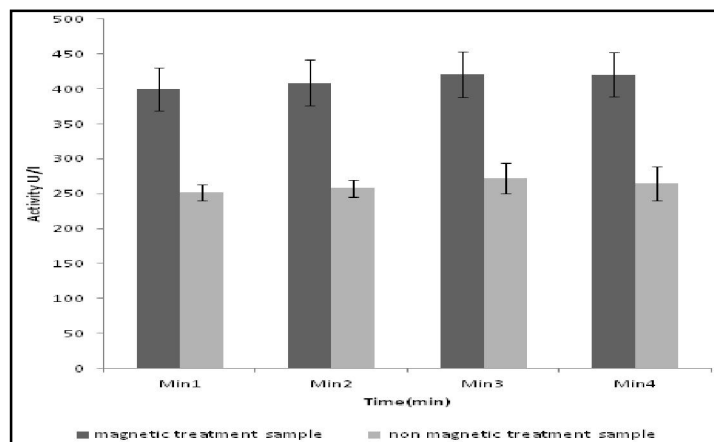


Fig. 5: Comparison between magnetic treatment blood samples with non magnetic treatment blood sample on the activity of erythrocytes Glutathione Peroxidase (U/I) at different time.

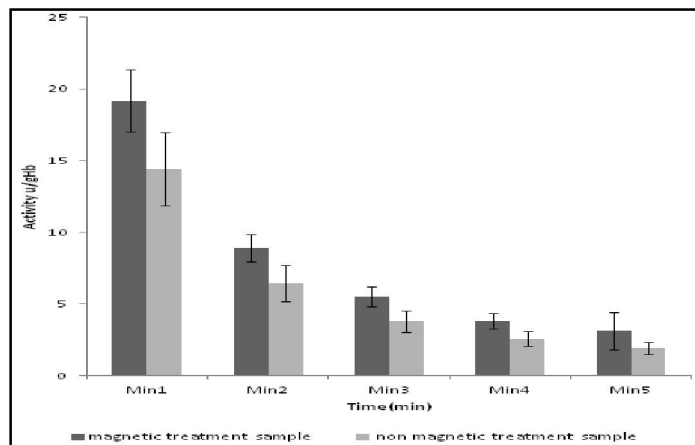


Fig. 6: Comparison between magnetic treatment blood samples with non magnetic treatment blood sample on the activity of erythrocytes Glutathione Reductase (u/gHb) at different time.

Conclusion:

It was concluded from this study that the magnetic field can affect the biophysical properties of saline and erythrocytes; also it can increase the activity of antioxidant enzymes in erythrocytes. The obtained results in this work are of great interest and importance, in a way that they help in applying magnetic treatment devices technology in various fields such as industry, medicine and agriculture to improve water properties.

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References

- 1-Ayrapetyan G., Hayrapetyan H., Ayrapetyan S. (2005). Metabolic pathway of magnetized fluid-induced relaxation effects on heart muscle. *Journal of Bioelectromagnetics*. 26:624-630.
- 2-Büyüksulu N. (2006). The effect of magnetic field on the activity of superoxide dismutase. *Journal of Cell and Molecular Biology*. 5: 57-62.
- 3-Changa K. Weng C. (2006). The effect of an external magnetic field on the structure of liquid water using molecular dynamics simulation. *Journal of applied physics*. 100:7-11
- 4-Fujimura Y., Iino, M. (2009). Magnetic field increases the surface tension of water. 3rd International Workshop on Materials Analysis and Processing in Magnetic Fields *J. Phys.Conf.* 156 (1).
- 5-Gursche S., Rona Z.P., Alive Research Group (1998). *Encyclopedia of Natural Healing: The Authoritative Reference to Alternative Health & Healing: a Practical Self Help Guide*, first edition, Vancouver, BC, Alive Publishing and Inc.112-119.
- 6-Ibrahim IH, Ahmed YM, Al-Malki AL, Abulnaja KO, Al-Othmani MT (2010). Effect of some water soluble vitamins on human erythrocytes. *Journal of Biophysical and Biomedical Sciences*.3 (1):254-257.
- 7-Inaba H., Tozaki K., Hayashi H. (2004). Effect of the magnetic field on the melting transition of H₂O and D₂O measured by a high resolution and supersensitive differential scanning calorimeter. *Journal of Applied Physics*. 96:(6127).
- 8-Ishii K., Yamamoto M., Nakayama H. (2005). Relative change of viscosity of water under a transverse magnetic field of 10 T is smaller than 10⁻⁴. *Chem. Lett.* 34: 874.
- 8-Iwasaka M., Ueno S. (1998). Structure of water molecules under 14 T magnetic fields. *Journal of Applied Physics*. 83:11.
- 9-Masella R., Di Benedetto R., Vari R., Filesi C., Giovannini C. (2005). Novel mechanisms of natural antioxidant compounds in biological systems: Involvement of glutathione and glutathione related enzymes, *J. Nutr. Biochem.* 16:577-586.
- 10-Nakagawa J., Hirota N., Kitazawa K., Shoda A. (1999). Magnetic field enhancement of water vaporization. *Journal of Applied Physics*. 86:2923-2925.
- 11-Ohno U., Reminick H. (2001). The naturally magnetized water difference in blood composition and circulation. *Explore! for the Professional*. 10:5
- 12-Valko M., Morris H., Mazur M., Raptap P., Bilton R.F. (2001). Oxygen free radical generating mechanisms in the colon: Do the semiquinones of Vitamin K play a role in the aetiology of colon cancer? *Biochim. Biophys. Acta.* 1527:161-166.

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