

Glutathione Enhancer Protects Some Biochemical and Haematological Parameters from the Effect of Electromagnetic Field

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THE PRESENT study was designed to study the effect of exposure to electromagnetic field (EMF), emitted from a cellular tower for mobile phone on some biochemical and haematological parameters in male albino rats and to evaluate of the possible protective role of the antioxidant glutathione enhancer on the studied parameters.

Three groups of rats were studied, the control (unexposed), the exposed and the treated exposed groups. Exposed groups were subjected to EMF at frequency of 900MHz, with a peak power of about 60W, power density of $0.05\text{mW}/\text{cm}^2$ at the site of exposure for 24h/ day for 8weeks, at the same time treated group was supplied with oral injection of glutathione enhancer three times weekly.

At the end of experiment, serum levels of thyroid stimulating hormone (TSH), calcium (Ca), uric acid, malondialdehyde (MDA) beside, the activity of lactate dehydrogenase (LDH) were measured also, some haematological parameters were estimated. Impairment of TSH and serum calcium was expressed by a decrease in serum levels of the exposed group. Meanwhile, serum level of MDA, uric acid and the activity of LDH were increased by exposure.

The haematological studies revealed that, exposure to electromagnetic spectrum induced significant reduction in red blood cell counts (RBC's), and haemoglobin concentration (Hb), meanwhile reticulocyte count (Ret) was elevated.

The leucocyte counts (WBC's) and platelets count were not affected by exposure.

The study demonstrated that glutathione enhancer can attenuate the side effects of exposure to electromagnetic field.

Keywords: Electromagnetic radiation, biological parameters, antioxidants.

The ever increasing use of cellular phones and the increasing number of associated base stations are becoming a widespread source of nonionizing electromagnetic radiation (Yurekli *et al.*, 2006). Chronic exposure for electromagnetic frequency waves EMF changes in cellular physiology and biochemistry including biomolecules profile. The most affected biomolecules are protein, hormones, lipid peroxides, reactive oxygen species (ROS), balanced electrolytes and all cellular systems (Akdag *et al.*, 2013 and Dutta *et al.*, 2015).

The cell membrane is considered as the primary site for EMF interaction with cellular systems. The mobilization of cellular calcium ion (Ca^{2+}) by electromagnetic radiation is an important biological response in the regulation of cellular activities (CSIRO, 1994 and Levis *et al.*, 2015). The study of Bergamaschi *et al.* (2004) revealed that EMF induced changes of trans-membrane Ca flux may lead to altered metabolism and/ or secretion of neurohormones including TSH. As a result of TSH perturbation, oxidative stress can be easily achieved especially in case of low TSH level and increased free T3 and T4 (Dutta *et al.*, 2015 and Kesari *et al.*, 2013).

Electrolytic imbalance disturbs the redox potential because Ca level maintain the redox homeostasis by participating in cell biochemical activities to capture free radical formation (Akdag *et al.*, 2013). Accordingly thyroid related hormonal imbalance mediated Ca disturbance were found to increase oxidative stress components such as uric acid, MDA (Schwarz *et al.*, 2012). MDA is one of thiobarbituric acid reactive substances (TBARS), which is considered the main end product of lipid peroxidation, liberated in case of excessive generation of free radicals and their interaction with polyunsaturated fatty acids (PUFAs). Uric acid is the major end product of purine metabolism specifically the catabolism of the purine nucleosides adenosine and guanosine (Alfred, 1994). Uric acid is an effective antioxidant which contributes in the protective mechanisms against oxygen radicals (Ames *et al.*, 1981). The hematopoietic system, like all body systems, is susceptible to damage by radiation, the effects of radiation are dependent on the nature and quantity of radiant energy, the duration of exposure, and the mitotic rate of the exposed cell population. The high mitotic activity of the bone marrow makes it one of the most susceptible organ systems to the toxic effect of radiation (Brain, 1994). One of the constituents of erythrocytes is LDH, its serum elevation may be encountered in variety of disorders including

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haemolytic anaemia (Brain, 1994). The toxicity of free radicals can be neutralized by free radical scavengers and by indirect antioxidants. Molecules which directly scavenge free radicals includes glutathione (Halliwell, 1999), which is an important antioxidant and is used as substrate for glutathione reductase and glutathione peroxidase (Matamoros *et al.*, 2003). Some studies also demonstrated that antioxidants prevent oxidative stress or apoptosis caused by radiofrequency electromagnetic waves (RF-EMW) in animal tissues (Oktem *et al.*, 2005 and Ozguner *et al.*, 2006).

Hence, the current study was performed to shed some light on the health hazards associated with biochemical and physiological changes induced by exposure to electromagnetic waves of Mobile Base Station (MBS). In addition, the study of the possible protective effect of glutathione enhancer is considered against the effect of electromagnetic radiation.

Material and Methods

In this study twenty seven adult male albino rats (*Rattus rattus*), weighing about 130-160g were used. Animals were randomly arranged into three groups (9rats each) as follows: Control (unexposed) group, EMF exposed group, EMF exposed+ glutathione-treated group. Electromagnetic radiation was applied to exposed groups for 8weeks. Rats were obtained from the animal house of the NRC, housed in plastic cages, given standard rodents feed and tap water *ad-libitum* and kept under constant conditions.

Two animal groups were exposed to electromagnetic radiation emitted from a cellular tower (base station) for mobile phone constructed on a roof of a building in Cairo at frequency of 900MHz, with a peak power of about 60W, power density of 0.05mW/ cm² at the site of exposure at a distance of 15meters in front of the antenna, 24h/ day for 8weeks. The field strength emitted by the tower was measured with isotopic probe specified for measuring high frequency and the compartment shaped to standard IEEE C95 (Institute of Electrical and Electronic Engineer), as described by El-Abiad (2002).

The treated group received glutathione enhancer suspension orally using a stomach tube three times/ week for two months during exposure to EMF. Glutathione Enhancer was obtained from International Business Establishment Co. (IBE Pharma) SPI (distributor Nova Pharm.,Egypt). Each tablet (512.5mg) contains 50ng glutathione, 50mg L-Cysteine, 100mg N-acetyl cysteine, 50mg

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L-methionine, 250mg vitamin C and 250mcg selenium. Each two tablets were dissolved in 102.5ml distilled water to obtain a concentration of 10mg/ ml. suspension was given orally at a dose of 1ml/ 100g of body wt.

At the end of experiment (8weeks), animals were fasted overnight then transferred to the laboratory. Blood samples were collected by decapitation for laboratory assessment of the studied parameters. Part of the blood was used for biochemical analysis blood was allowed to clot at 37C° for 30min, centrifuged for 10min at 5000rpm and sera were separated and kept frozen at -20C until analysis. Activity of LDH was estimated by measuring the rate of NADH consumption spectrophotometrically which is proportional to LDH activity (Witt and Trendlenberg, 1982). Total Ca was estimated by using colorimetric method of Tietz (1970) and serum uric acid concentration was measured according to Fossati *et al.* (1980). MDA was estimated colorimetrically by determining TBARs (Draper and Hadley, 1991). Determination of serum TSH was according to the method of Sooes *et al.* (1984) using commercial kits from Immunotech.

Blood for haematological parameters was collected on anticoagulant; disodium ethylenediaminetetra-acetate (EDTA). Haematological measurements erythrocyte counts (RBC's), haemoglobin (Hb), leukocyte counts (WBC's), platelets count (Pt) and reticulocyte count (Ret) were estimated using automatic counter (Guyatt *et al.*, 1992).

Statistical analysis

Data are presented as mean± S. D. and range (lower limit-upper limit), analysed by student's *t*-test and the level of significance was set at $p < 0.05$.

Results

The effect of the exposure of male rats to EMF of MBS, Table 1. showed a significant increase in serum MDA (2.6 ± 0.3) as compared to control group (1.6 ± 0.2) and a significant decrease was noted in the exposed group supplied with glutathione (1.62 ± 0.16) as compared to the exposed one which did not receive glutathione.

Serum TSH and Ca exhibited an opposite trend of alteration among the experimental groups, as a function of EMR-exposure and glutathione treatment

in comparison with control group. Where, TSH showed significant decrease in the exposed group (1.11 ± 0.04) as compared to control group (1.15 ± 0.02) and insignificant difference was observed in the exposed treated group (1.12 ± 0.04) when compared to control and exposed groups. The exposed group, also exhibited significant decrease in the level of serum Ca (8.9 ± 0.9) than control (10.13 ± 0.26) and exposed treated groups (9.7 ± 0.63). The exposed treated group showed no significant difference compared to control. In contrast, the level of uric acid expressed a significant rise in exposed group (5.5 ± 0.5) as compared to control group (4.7 ± 0.5), whereas, significant decline was noted in exposed glutathione treated group (4.9 ± 0.4) compared to the exposed one. As regard to enzyme activity, the data in Table 1. revealed that exposure to EMR induced significant elevation in the activity of LDH (637.3 ± 73.3) as compared to control group (444.3 ± 48.8). Treatment with glutathione exerted a significant decline in exposed group (492.1 ± 58.9) in comparison to the exposed group, while no significant difference was recorded compared to control.

TABLE 1. Effect of glutathione on serum MDA, TSH, uric acid, calcium and LDH on rats exposed to electromagnetic radiation of base station for 8weeks.

Parameters	Control	Exposed	Exposed treated
MDA (nmol/ ml) (Range)	1.6 ± 0.2 b (1.3-1.8)	2.6 ± 0.3 a (2.2-3.1)	1.62 ± 0.16 b (1.4-1.9)
TSH (μ u/ ml) (Range)	1.15 ± 0.02 a (1.12-1.16)	1.11 ± 0.04 b (1.1-1.13)	1.12 ± 0.04 a,b (0.13-1.14)
Uric acid (mg/ dl) (Range)	4.7 ± 0.5 b (4.12-5.3)	5.5 ± 0.5 a (4.95-6.36)	4.9 ± 0.4 b (4.29-5.35)
Calcium (mg/ dl) (Range)	10.13 ± 0.26 a (9.33-10.3)	8.9 \pm 0.9b (7.14-9.84)	9.7 ± 0.63 a (8.8-10.96)
LDH (u/l) (Range)	444.3 ± 48.8 b (386-518)	637.3 ± 73.3 a (533-726)	492.1 ± 58.9 b (399-566)

-Data are expressed as means \pm S. D. for 9rats in each group.

-Values in the same raw with different letters (a, b or c) are significantly different.

-Means followed by the same letter (a, b or c) are not significantly different.

-Range is expressed as lower limit and upper limit in each group.

Haematological parameters in rats exposed to electromagnetic radiation in Table 2. The data revealed that there was a significant decrease ($p < 0.05$) in mean values of RBC's count in exposed group (3.9 ± 0.15) as compared to control (4.77 ± 0.3) and the group exposed and treated with glutathione (4.5 ± 0.3) showing insignificant difference from the control group. Also mean value of haemoglobin concentration was significantly lower ($p < 0.05$) in exposed ($11.8 \pm$

0.5) as compared to the control (13.7 ± 0.4) and exposed treated groups (12.8 ± 0.3). Meanwhile, Hb is still significantly decreased in exposed treated group when compared to control group. Contrary, mean value of reticulocyte count was significantly higher ($p < 0.05$) in exposed group (1.3 ± 0.3) as compared to control (0.56 ± 0.15) and exposed treated group (0.86 ± 0.15).

TABLE 2. Effect of glutathione on haematological parameters in rats exposed to electromagnetic radiation of base station for 8weeks.

Parameters	Control	Exposed	Exposed treated
RBC's ($\times 10^6 / \text{mm}^3$) (Range)	$4.77 \pm 0.3\text{a}$ (4.3-5.2)	$3.9 \pm 0.15\text{b}$ (3.7-4.1)	$4.5 \pm 0.3\text{a}$ (4.2-5.0)
Hb (g/ dl) (Range)	$13.7 \pm 0.4\text{a}$ (12.9-14.1)	$11.8 \pm 0.5\text{c}$ (10.8-12.5)	$12.8 \pm 0.3\text{b}$ (12.3-13.3)
WBC's ($10^3 / \text{mm}^3$) (Range)	9.2 ± 0.4 (8.8-10.1)	9.0 ± 0.3 (8.7-9.5)	9.0 ± 0.3 (8.5-9.4)
Ret ($10^3 / \text{mm}^3$) (Range)	$0.56 \pm 0.15\text{c}$ (0.3-0.8)	$1.3 \pm 0.3\text{a}$ (0.9-1.8)	$0.86 \pm 0.15\text{b}$ (0.6-1.1)
PLt (10^3) (Range)	186.8 ± 9.1 (176-200)	182 ± 7.1 (175-195)	187.4 ± 8.2 (176-197)

Legends as in Table 1.

Data showed no significant difference in total leucocytes and platelets count of studied groups as compared to the control group.

Discussion

The widespread use of the mobile phone has initiated many studies on the possible adverse effects of a high frequency EMF, emitted from mobile phones (Hata *et al.*, 2005). As a tax of civilian life, electromagnetic field may induce biochemical and pathological alterations to the cell membrane, various enzymatic systems and electrolytic balanced components. Maganioti *et al.* (2010) mentioned that EMF induce the free radical pair mechanism in the cellular systems. The change includes excessive production of ROS, lipid peroxides and biomolecules such as uric acid a result of DNA breakdown. As the oxidative stress increased, there are expected high level of stress hormones T3 and T4 and disturbance of total electrolytes especially Ca concentration (Maganioti *et al.*, 2010).

The remarkable reduction in serum Ca occurred with exposure can be explained by the effect of EMR which can increase ionized calcium uptake by skeleton, enhanced calcification and oestrogenesis in exposed animals. Results

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are consistent with those of Bortkiewicz (2001) who attributed the effect of the electromagnetic field on the flow of ions across cell membranes. Also data are consistent with those of Persson *et al.* (1989) and Brovkovich *et al.* (1991) who reported that parasympathetic over activity during long term exposure to electromagnetic field released gastrocalcin that promoted the uptake of blood calcium into bone causing hypocalcaemia. Another data suggested that EMF induced opening of the calcium channel in the membrane rather than increase of the calcium mobilization from endoplasmic reticulum (Goodman *et al.*, 1995). In general calcium profile disturbance mediated EMF can produce change in thyroid related hormones and oxidative stress.

TSH is secreted from the anterior pituitary gland and is controlled by thyroid hormones and thyrotropin-releasing hormones. In the current study, the significant decrease in serum TSH in rats exposed to EMR as compared to that of control group can be attributed to the effect of EMF on the hypothalamic centres responsible for thyrotropin-releasing hormones which become less sensitive to feedback inhibition by thyroid hormones. Several studies revealed that the activity of thyroid hormones increased by the effect of EMR (Adey, 1981, Dumanskiĭ *et al.*, 1976 and Marzook, 2006). Accordingly, it can cause a decrease in TSH by feedback inhibition. Results are in accordance with those of Koyu *et al.* (2005) who found that 900MHz EMF emitted by cellular telephones decreases serum TSH levels. Meanwhile, results disagree with those of Djeridane *et al.* (2008) who revealed that TSH was not disturbed by RF-EMFs emitted by mobile phone Ahmedpoor *et al.* (2016).

Electromagnetic radiation or RF field of cellular mobile phones may affect biological systems by increasing free radicals, which appear mainly to enhance lipid peroxidation, by changing antioxidant defines systems of human tissues (Ozguner *et al.*, 2005) and blood (Moustafa *et al.*, 2001) leading to oxidative stress. Thus the significant increase in serum MDA in exposed group as compared to control one could be attributed to the overproduction of ROS due chronic exposure to EMR. Oxidative stress occurred as a consequence of imbalance between ROS and body antioxidant capacity (Ahmedpoor *et al.*, 2009 and Yakymenko *et al.*, 2014). This imbalance could happen as a result of increased ROS generation, impaired antioxidant defines system or their combination. It was found that electromagnetic radiation causes biological effect by increasing free radicals, which enhance lipid peroxidation, and by changing the antioxidant

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defence system of human tissues, leading to oxidative stress (Ozguner *et al.*, 2005 and Johanson, 2009). Results agree with those reported by Stopczyk *et al.* (2002) and Ilhan *et al.* (2004). A significant rise in MDA level in rats exposed to EMF indicated that those animals suffered from oxidative stress which was neutralized by the use of antioxidant glutathione. Results are also consistent with those of Ilhan *et al.* (2004) who found increased MDA level in brain tissue of rats exposed to 900MHz of MBS for seven days that was prevented by treatment with Ginkgo biloba and the improvement reported by Ozguner *et al.* (2005) after treatment caffeic acid phenyl ester.

The increased EMF induced oxidative stress may lead to increased T3 and T4 which in turn induces overproduction of uric acid (Hashem and El-Sharkawy, 2009). Hyperuricaemia may be caused by either increased production of uric acid or decreased renal excretion. Increased production is seen with increased nucleic acid turnover which leads to increased catabolism of purines which can be seen in rapid proliferation of cells as in lymphoproliferative disorder, malignancy or in haemolytic anaemia, primary gout and many other factors including radiation (Alfred, 1994). Thus, the significant alteration of serum uric level between control animals and EMR exposed group noted herein may be attributed to increased catabolism of purines or due to increased haemolysis caused by effect of electromagnetic radiation exposure in this study. Also increased uric acid may be a compensatory mechanism trying to counteract oxidative stress since uric acid is considered as antioxidant which contributes in the mechanism against oxygen radicals (Ames *et al.*, 1981 and Hamed *et al.*, 2004). Result was consistent with those described by Dasdag *et al.* (1999) who investigate the effects of RF and microwave (MW), non-ionizing radiation, and observed an increase serum uric acid. Improvement after treatment of exposed animals with glutathione could be attributed to increased TSH level accordingly decreased T3 and T4 as well as oxidative stress.

The blood integrity is a picture of normal blood picture and normal enzymatic profile like Lactate dehydrogenase. LDH is present in all body cells but its higher concentrations are found in liver, heart, kidney, skeletal muscle and erythrocytes. Total LDH-concentration in serum or plasma is increased in patients with liver disease, renal disease, myocardial infarction, many malignant diseases, progressive muscular dystrophy and almost any cause of haemolysis

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(Friedman and Young, 2001). The results obtained revealed that the activity of LDH raised significantly over control in exposed group and improvement was noticed in glutathione treated group. Thus, the increased level of LDH in the current study may be associated with the increased tissue damage due to exposure (Ozgur *et al.*, 2014). Improvement which occurred in the treated group can be attributed to the protective effect of glutathione enhancer on LDH activity which can remove free radicals developed by EMF (Reiter, 1994). Results are in agreement with those of Vojisavljevic *et al.* (2007) who studied the effect of electromagnetic radiation (550-850nm) on l-lactate dehydrogenase kinetic and he found increased activity of the enzyme.

EMF appeared to decrease significantly the RBC's in exposed group in comparison with the control and treated groups. This fact can be explained by the effect of EMR on the haematopoietic system which is susceptible to be damaged by radiation and that the high mitotic activity of the bone marrow make it the most vulnerable organ system to the effect of radiation (Marzook, 2006 and Fathi *et al.*, 2014). Improvement noticed in treated group could be attributed to protective effect of antioxidants against oxidative damage of cells by free radicals and by preventing the oxidation of polyunsaturated fatty acids in the cell membrane. Results are in agreement with those of Heikinen *et al.* (2001), Nageswari (2003), Mukewar and Baile (2003) and El Abiad *et al.* (2007). Also, mean value of Hb concentration was significantly decreased in all exposed groups as compared to the control group which are consistent with those of Nageswari (2003), Mukewar and Baile (2003) and Ali *et al.* (2003). The antioxidant activity of glutathion and its protective effect on blood parameters was evident in this study and agreed with the study of El-Abiad *et al.* (2007) and El-Shafey *et al.* (2009). Results are in disagreement with Heikinen *et al.* (2001) who found increased hemoglobiin concentration in groups of rats exposed to EMR.

Also data showed that mean values of reticulocytes count was significantly increased in exposed group as compared to control and treated groups. The present results suggest that increased reticulocytes count may be due to physically induced haemolytic anaemia by the effect of chronic exposure to the EMF. Haemolytic disorders may be caused by many factors including physical agents (John, 1994). The haemolytic anaemia results primarily from increased

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red cell destruction (Brain, 1994) which stimulates erythropoiesis. The evaluation of suspected haemolytic states should include measures of increased red cell destruction and parameters of accelerated erythropoiesis which is measured by the reticulocyte count. Results are consistent with those of Germann (2004) and El Abiad *et al.* (2007). These results could be explained on the base of EMR exposure may be responsible for the changes of blood and glutathione enhancer can provide protection against the effect of EMR of MBS.

The present study revealed negligible changes in total leucocytes due to exposure. These results are in accordance with the finding of Tuschl *et al.* (1999) and Germann (2004) who studied the effect of high frequency radiation on persons exposed to GSM mobile phones. Also data are consistent with those of Marzook (2008) who found insignificant difference in leucocyte counts in a group of rats exposed to EMR radiation of bas station of frequency of 900-930MHz Data disagree with those of Goldoni (1990) who found decreased leucocytes and platelets counts in group of men occupationally exposed to pulsed MW. Meanwhile, study of Svedenstal and Holmberg (1993) found that total leucocytes count was elevated in pulsed electromagnetic field exposed animals (CBA mice) compared to non-exposed control. This variation can be attributed to the differences in the frequency of EMF used and/ or duration and time of exposure, the mitotic rate of the exposed cell population and the differences in animal species used (Vinodha and Raghavan, 2015).

In conclusion, the current study indicates that glutathione enhancer as a potent antioxidant provide a great defines mechanism in EMR exposed animals.

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الجلوتاثيون المحفز يقى بعض المعايير الكيموحيوية و الدموية من تأثير المجال الكهرومغناطيسي

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تلقي هذه الدراسة نظرة شاملة على الأضرار الناتجة عن الثورة الهائلة في الاتصالات اللاسلكية و خاصة محطات المحمول ، بالإضافة إلى دراسة الدور الوقائي لمحفز الجلوتاثيون كمضاد للأكسدة ضد تأثير المجال الكهرومغناطيسي و الناتج عن التعرض لمحطات المحمول في ذكور الجرذان.

لذلك تمت دراسة تأثير الموجات الكهرومغناطيسية على كلا من إنزيم لاكتيكت ديهيدروجينيز ، و عنصر الكالسيوم و حمض اليوريك و كذلك ناتج أكسدة الدهون (المالونالدهايد) ، و هرمون المحفز للغدة الدرقية و صورة الدم و الخلايا الشبكية.

تم تقييم تأثير التعرض على الجرذان باستخدام عدد 27 من ذكور الجرذان قسموا إلى ثلاث مجموعات متساوية كالاتي : المجموعة الأولى الضابطة التي لم تتعرض للمجال الكهرومغناطيسي ، و مجموعة معرضة و غير معالجة ، و مجموعة معرضة و معالجة بمحفز الجلوتاثيون ثلاث مرات اسبوعيا المجموعات المعرضة للجرعة من المجال المغناطيسي بتردد حوالي 900 - 930 ميغا هرتز و قدرة كثافية 0.5 و مللي وات لكل سم 2 عند موقع التعرض و ذلك طوال اليوم لمدة ثمانية اسابيع.

و قد اوضحت النتائج ان التعرض للمجال الكهرومغناطيسي قد سبب زيادة معنوية في إنزيم لاكتيكت ديهيدروجينيز في المجموعة المعرضة مقارنة بالمجموعة الضابطة ثم تراجع في المجموعة المعرضة المعالجة بمحفز الجلوتاثيون. و لوحظ ايضا ان ناتج أكسدة الدهون (المالونالدهايد) و حمض اليوريك قد إزدادوا بشكل ملحوظ في المجموعة المعرضة مقارنة بالمجموعة الضابطة و المجموعة المعرضة المعالجة بمحفز الجلوتاثيون. و على عكس ذلك حدث نقص معنوي في الكالسيوم بشكل ملحوظ في المجموعة المعرضة مقارنة بالمجموعة الضابطة و المجموعة المعرضة المعالجة بمحفز الجلوتاثيون. كما وجد نقصا معنويا في كرات الدم الحمراء و كذلك نقصا معنويا في نسبة هيموجلوبين الدم و زيادة معنوية في نسبة الخلايا الشبكية و ذلك في المجموعة المعرضة مقارنة بالمجموعة الضابطة و لم يلاحظ أى تأثير للتعرض للمجال المغناطيسي على خلايا الدم البيضاء و الصفائح الدموية و ذلك في المجموعة المعرضة و حدث تحسن معنوي بعد المعالجة بالجلوتاثيون.

نستخلص من ذلك ان محفز الجلوتاثيون يمكن ان يستخدم كمضاد للاكسدة لتقليل الاثار الضارة الناتجة عن التعرض للموجات الكهرومغناطيسية.