AMELIORATIVE EFFECT OF VITAMIN E ON ELECTROMAGNETIC FIELD INDUCED CHANGES IN SOME HEMATOLOGICAL PARAMETERS IN MALE ALBINO RATS

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ABISTRACT

The present work aimed to study the effect of vitamin E on EMF induced changes in some hematological parameters in male albino rats weighting 120 – 150 g. The present results significant increase in total erythrocytes count, hemoglobin content, hematocrit value, (MCH), (MCHC) and total leukocytic count, while total platelets count, (MCV) and (RDW) value recorded low levels in rats exposed to electromagnetic field 2hr/d. for 20 days. Treatment with vitamin E (100 mg/Kg) before exposure to EMF (mobile phone) caused a remarkable recovery in red blood cells , white blood cells and total platelets , while recorded a decrease in Hb content , hematocrit value, MCV, MCH, MCHC and RDW was recorded . Conclusion: We concluded that treatment with vitamin E before exposure to EMF (mobile phone) caused a remarkable recovery in red blood cells, white blood cells and total platelets.

INTRODUCTION

Non-ionizing radiofrequency radiation and microwaves have been implicated in producing effects in the hematopoietic system **(Robert et al., 1997 and Cook et al., 2004).   Juntilainen et al., (2007)** showed that there are no any effects for radiofrequency (RF) electromagnetic fields on micronucleus frequency in polychromatic or normochromatic erythrocytes. The exposure to cell phones radiation causes red blood cells

to leak hemoglobin, the build up of which can cause heart disease and kidney stones **(Nick & James, 2004).**

Ali, (2007) showed that during magnetic resonance imaging (MRI), large number of RBCs affected by the magnetic field (stress) converted its shape from the normal discoid biconcave to spherical shape (spherocytes). The morphological alteration was found and also the morphological index was higher during than post MRI. The presence of iron atoms in hemoglobin makes the red blood cells slightly less diamagnetic than plasma; as a result, RBCs have a tendency to move relative to the plasma toward regions of strong magnetic fields. Electromagnetic field (EMF) induced an increase of RBCs number, Ht and Hb correlated with an increase of MCHC and decrease MCV. The total leucocytes number fluctuated: decrease at 5 and 20 days and increase at 10 days of treatment **(Ion et al., 2005)**. Hemoglobin is sensitive to magnetic field (MF); the morphological examination and the non-linear time course of the sedimentation in plasma indicated that MF increased cell aggregation and there by enhanced ESR **(Lino, 1997)** .

Miryam et al., (2007) recorded that SMF (static magnetic field) caused an increase in MCV and decrease in MCHC indicated the presence of hypochromic RBC in the forms of macrocysts. Also, SMF decreased the red cells distribution width (RDW); this parameter reflects the difference between the smallest and the biggest erythrocytes. Exposure to SMF can carry along agglutination of RBC due to an increase in MCV. The increase in hemoglobin and hematocrit following sub-acute exposure to SMF may be explained by the installation of hypoxid-like status probably resulting from the oxygen binding impairment of hemoglobin or iron metabolism disruption **(Mukewar & Baile, 2003)**. MF increases the risk of various types of cancer, including leukemia, brain and breast tumors and alters the function of reproduction and of the immune system **(Michaelson et al., 1996)**.

Exposure to SMF (1h/day) induced decrease of body weight by the second week of treatment. Moreover, the same treatment caused an increase of hemoglobin concentration, white blood cells, red blood cells and platelets count compared to control rats, where as hematocrit levels remained unchanged **(Pries et al., 1992 and Salem et al., 2006).** Electromagnetic radiation which comes from appliances with frequencies between 1 MHZ and 10 GHZ penetrate the exposed children tissues living in houses with the highest electromagnetic fields [EMFS stronger than 2mG] they were about 24% more likely to develop leukemia than of

children who lived in houses with the lowest EMF [less than 0.65 mg] **(Terry , 1998). Ny et al., (2008)** recorded that, the average time for the neutrophils to respond to the effect of 900-MHz radio frequencies was about 2.5 min.

Intermittent exposures (6min RF on, 2h RF off) at the frequency of 1950Hz on human lymphocytes in different stages of cell cycle do not induce either on increase in micronucleated cells, or change in cycle kinetics; moreover 24h intermittent exposures also fail to affect DNA structure of human leukocytes soon after the exposures, likely indicating that repairable DNA damage was not induced **(Zeni et al., 2008).** Extremely low-frequency magnetic fields have been classified as possibly carcinogenic to humans, mainly based on epidemiological studies consistently showing an association between long-term average exposure to magnetic fields above 0.3/0.4 MT and the risk of childhood leukemia **(Schiiz & Ahlbom, 2008)**.

Vitamin E that maintains a blood concentration of total tocopherols above 0.5mg/dl will also ensure an adequate concentration of the vitamin in all of the tissues. Vitamin E is stored in the liver and it excreted by stools and traces appear in urine **(Harper, 1999).** Vitamin E is present in plasma in 2 forms, α- and γ- tocopherol. It protects unsaturated fatty acids in the cellular membranes from endo-and exogenous free radicals and active oxygen forms, which initiate or propagate damage resulting from non-enzymatic lipid peroxidation **(Combs, 1992 and Pieri et al., 1994).** The deficiency of plasma α -and γ -tocopherol in steelworkers exposed to electromagnetic fields caused an increase susceptibility of tissues to free radicals and increase of lipid peroxidation **(Andrzej et al., 2002). Key et al., (1986)** found that vitamin E is localized primarily in cellular membranes. Its major role is the termination of free-radical chain reactions propagated by the polyunsaturated fatty acids of membrane phospholipids. Erythrocytes of all ages from vitamin E deficient rats behave like old erythrocytes from normal rats, as determined by their susceptibility to phagocytosis, immunoglobulin G [IgG] binding, anion transport ability, and glyceraldehydes-3-phosphate dehydogenase activity. **Ishigaki, (1985)** found that in rabbits with experimental hypercholesterolemia, red cell damage due to oxidation readily occur because of the lack of glutathione and vitamin E.

The aim of this study was to investigate the effect of vitamin E on EMF induced changes in some hematological parameters in male albino rats.

MATERIALS AND METHODS

Male albino rats weighing 120-150 g were used in the present study; the animals were keept under good ventilation.

The animals are divided to four groups as follow.

1-   Control group. It consists of 8 rats and did not receive any treatment.

2-   Vitamins E – treated group. It consists of 8 rats. The animals of this group were injected intraperitoneally (i.p) with Vit E 100mg/kg / day **(Yimaz, 2006)** for consecutive 20 days.

3-   Electromagnetic field (Mobile phone)-exposed group, it consists of 8 rats .The animals of this group were exposed to EMF (1110 MHZ Nokia Mobile phone). The specific absorption rate (SAR) was 2.0 watts / kilogram (w/kg) averaged over 10 grams of tissue, exposed to EMF 2hr/d for 20 days.

4-   Electromagnetic field exposed-vitamin E treated group. It consists of 8 rats. The animals received (i.p) injection with of 100mg vitamin E/kg, before exposed to EMF.

Exposure of rats to EMF (Nokia Mobile- phone)"1110 MHZ", was done by putting rats in transparent plastic cage, and laying two mobile phone on two opposite sides of cage, this until all rats in cage were exposed to EMF 2h/d. for 20 days. Animals of each group were sacrificed; the blood samples were collected in heparinzed tubes, for complete blood count [CBC] by coulter count system.

Results were expressed as mean ± SD. Differences between groups were assessed by T-test **(Lellouch & Lazar 1974)**. P-value of <0.05 was considered as statistically significant.

RESULTS

1-Total erythrocyte count, Hb content, HCT value, MCV value, MCH conten ,MCHC content and RDW%.

Table (1) shows total erythrocytes count (R.B.C$_s$ x 10^6/mm^3), total hemoglobin content (Hb) (g/100ml), hematocrit value (HT)(%), the mean corpuscular value ((MCV)$_{Mm}$^{3}), the mean corpuscular hemoglobin content (MCH)(Pg), the mean corpuscular hemoglobin concentration (MCHC)(g/dl)and red cell distribution width (RDW)(%)in the control and different treated groups .

Table (1): RBCs, Hb , Ht , MCV , MCH , MCHC and RDW in control and different treated groups of male albino rats .

Groups / Parameters		Normal control	Vitamin E treated group	Irradiated group with electromagnetic wave	Vitamin E + Irradiated treated group
RBCs 10^6/mm^3	Mean	7.47	7.79	8.91	7.37
	S.D.	± 0.75	± 0.79	± 0.87*	± 0.59
	%		+ 4.28	+ 19.28	- 1.34
Hemoglobin content g/dl	Mean	12.88	13.04	15.43	9.10
	S.D.	± 0.87	± 1.15	± 0.86***	± 1.11***
	%		+ 1.24	+ 19.79	- 29.35
Hematocrit value %	Mean	34.95	37.58	41.10	23.90
	S.D.	± 3.11	± 3.53	± 3.21*	± 3.21**
	%		+ 7.53	+ 17.59	- 31.62
MCV µm³	Mean	46.83	48.09	46.23	44.20
	S.D.	± 1.44	± 2.49	± 1.17	± 1.68*
	%		+ 2.69	- 1.28	- 5.62
MCH Pg	Mean	17.30	16.76	17.37	16.86
	S.D.	± 0.74	± 0.27	± 0.47	± 0.44
	%		- 3.12	+ 0.40	- 2.54
MCHC g/dl	Mean	37.00	35.60	37.60	36.16
	S.D.	± 1.53	± 1.99	± 0.28	± 1.09
	%		- 3.78	+ 1.62	- 2.27
RDW %	Mean	17.35	18.58	16.83	15.08
	S.D.	± 1.96	± 2.08	± 0.44	± 0.91*
	%		+ 7.09	- 2.99	- 13.08

Data are expressed as mean ± SD.

Significance as compared with control

* Significant (P<0.05)

** Highly Significant (P<0.01)

*** Very Highly Significant (P<0.001)

The injection of vitamin E only into rats produced a non significant change in RBC$_s$ Count, Hb, Ht, MCV and RDW, MCH and MCHC as compared with control group.

In group treated with vitamin E only, the obtained data showed a non-significant change in RBCs count, Hb, Ht, MCV, RDW, MCH and MCHC as compared with normal control. Rats Irradiated with electromagnetic waves exhibited a significant increase in RBCS count, Ht, MCHC, and a very highly significant increase in Hb, while showed a slightly decrease in both MCV and RDW. Treatment of irradiated rats with vitamin E recorded a remarkable recovery in RBCs count , and showed a decrease in Ht, MCH, RDW, MCV and MCHC as compared with normal control.

2-Total leakocytic count and total platelets count.

Table (2) illustrate total platelets count (10^3/mm^3) and total leukocytic count (WBCs) (10^3/ mm^3) in normal control and different treated groups. Treated rats with vitamin E showed significant decrease in total platelets and increase in total leukocytic count. Exposure rats to electromagnetic wave (mobile phone) caused a significant decrease in total platelets count, while increases in total leukocytic count (WBCs) were recorded as compared with normal control. When irradiated rats treated with vitamin E, a remarkable recovery in WBC$_s$ and in total platelets were observed when compared with normal control group.

Table (2): Total leukocytic count (WBCs 10^3 mm^3) and total platelets count (10^3/mm^3) in control and different treated groups of male rats

Groups / Parameters		Normal control	Vitamin E treated group	Irradiated group with electromagnetic wave	Vitamin E + Irradiated treated group
Total platelets count 10^3 / mm^3	Mean	464.26	416.80	420.40	489.25
	S.D.	**± 28.37**	**± 12.49***	**± 23.16***	**± 15.78**
	%		-10.17	- 9.39	+ 5.44
W.B.Cs 10^3/mm^3	Mean	11.93	13.02	13.55	11.58
	S.D.	**± 1.94**	**± 1.45**	**± 1.39**	**± 1.94**
	%		+ 9.14	+ 13.58	**- 2.93**

Data are expressed as mean ± SD.

Significance   as compared with control

* Significant (P<0.05)

** Highly Significant (P<0.01)

*** Very Highly Significant (P<0.001)

DISCUSSION

The interaction between red blood cells and magnetic resonance imaging apparatus is very complex and involves several stress sources .It causes alteration in blood rheological properties, RBC morphology and membrane properties **(Ali, 2007)**. The present study showed a significant increase in total erythrocytes count, hemoglobin content, hematocrit value, MCH, MCHC and total leukocytic count after exposure to electromagnetic field 2hr/d. for 20 days. Similar results were obtained by **Thomson et al., (1988), Robert et al., (1997) and Salem et al., (2006)** after exposure to SMF.

Stanis et al., (2001) pointed to the existence of an increase of hematological parameters on lactating, was exposed to a pulsed electromagnetic field. The increase of hemoglobin, red blood cells, MCH, MCHC and white blood cells levels could be explained by the hypoxia- like status induced by electromagnetic field , these results were

recorded by **Pries et al., (1992), Stashkov and Gorokhov (1988), Salem et al., (2006) and Chater et al., (2006**) after exposure to static magnetic field. Hb is the blood transporter of oxygen within the mammalian body. Oxygen is bound to the iron atoms of the Hb **(Fadel et al., 2003).** The stimulation of hematopieosis can be associated to the change in the conformation of Hb under SMF action **(Atef et al., 1995)** this effect may be explained by the setting up of an hypoxia − like status as reported by **Stashkov and Gorokhov (1998) and Amara et al., (2006).**

Mainiu and Neacsu (2004) recorded that the effects of EMF action on rat positive parameters depends on parameters type and treatment duration. Thus, EMF had a different influence on hematological indices and leucocytes dynamic number. This effect can be determined by a selective action of EMF on hematopoietic processes and the cell maturation rhythm and on sanguine reservoir. From the obtained data hemoglobin recorded an increased value according to increasing of MCH and MCHC values, indicating a better level of respiratory performances of hematological indices as a result of EMF action. These effects assume hemoglobin synthesis stimulation by EMF. MCV and RDW recorded low values, possibly owing to an effect of EMF on membrane permeability with a loss of the cellular water and a cellular volume reduction. This effect is accompanied by the MCHC increase with preserve of cellular respiratory capacity **(Ion et al., 2005 and Miryam et al., 2007).**

In the present study exposed to electromagnetic field caused an increase in white blood cells count because EMF alter the immune system and cause leukemia **(Savitz and Loomis, 1995., Michaelson et al., 1996., Salem et al., 2006 and Miryam et al , 2007).**

Exposure to EMF from mobile phone 2hr/d. for 20 days caused a decrease in total platelets count (table,2) , this because free radical which produced from EMF action caused a chain reaction of oxidation of polyunsaturated fatty acids being included in cytoplasmic membranes phospholipids, was adecissive effect on blood platelets function causing activation of the mentioned blood morphotic elements (adhesion, and aggregation) , which lead to decrease platelets counts , **(Brocklehurst & Mclauchlan, 1996 and Buczynski et al., 2005).**

The present results showed that treatment with vitamin E (100mg/kg) before the exposure to EMF (mobile phone) caused a remarkable protection of red blood cells, white blood cells and total platelets. This, because vitamin E protects unsaturated fatty acids in the

cellular membranes from endogenous and exogenous free radicals and active oxygen forms , which initiate or propagate damage resulting from non- enzymatic lipid peroxidation **(Pieri et al ., 1994 and Andrzej et al., 2002).**

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تأثير فيتامين هـ علي التغيرات الضارة للمجال الكهرومغناطيسي علي بعض قياسات الدم في ذكور الجرذان البيضاء

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اجريت هذه الدراسة علي ذكور الجرذان البيضاء التي تزن (١٢٠ – ١٥٠ جرام). وعوملت الجرذان معاملة جيدة من حيث التهوية والتغذية . وقسمت الجرذان الي اربعة مجموعات . المجموعة الآولي قياسية غير معاملة . المجموعة الثانية معاملة بفيتامين هـ (100mg/kg) بالحقن داخل الغشاء البريتوني لمدة ٢٠ يوم . المجموعة الثالثة معرضة للمجال الكهرومغناطيسي   (تليفون محمول نوع نوكيا 1110MHz) ، لمدة ساعتين في اليوم لمدة ٢٠يوم . المجموعة الرابعة المعاملة بفيتامين هـ بالحقن داخل الغشاء البريتوني (100mg/kg) قبل التعرض للمجال الكهرومغناطيسي لمدة ٢٠يوم . تعرض الفئران للمجال الكهرومغناطيسي بوضعها في قفص بلاستيك ووضع تليفونين علي جانبين متعاكسين من القفص لكي تتعرض جميع الجرذان للمجال الكهرومغناطيسي لمدة ساعتين في اليوم لمدة ٢٠ يوم . في هذه الدراسة شوهد زيادة في العدد الكلي لكريات الدم الحمراء و محتوي الهيموجلوبين و MCH وMCHC والعدد الكلي لكرات الدم البيضاء ، بينما سجل نقص في العدد الكلي للصفائح الدموية و MCV و RDW بعد التعرض للمجال الكهرومغناطيسي . وعند المعاملة بفيتامين هـ ظهر تحسن واضح في العدد الكلي لكريات الدم الحمراء و كرات الدم البيضاء والصفائح الدموية .