Iraqi Journal of Veterinary Sciences, Vol. 23, Supplement I, 2009 (105-114)
Proceedings of the 5th Scientific Conference, College of Veterinary Medicine, University of Mosul

Hemato-biochemical and immunotoxicological effects of low electromagnetic field and its interaction with lead acetate in mice

M. A. Hashem*[1] and N. I. El-Sharkawy[2]

[1]Departments of Clinical Pathology, [2]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Zagazig University, Sharkia Province, Egypt. *Corresponding author: **mhashem_vet@yahoo.com**

Abstract

The present study was carried out to investigate the potential effects of extremely low-frequency electromagnetic fields (ELF-EMF) and lead acetate on some hemato-biochemical, immune and pathologic variables in mice. A total 120 female mice were equally divided into eight groups. Gp. 1 kept as control. Gp. 2 exposed to EMF of 2 millitesla intensity and 50 Hz frequency (4h/day) for 30 days. Gps. 3, 4 and 5 were administered lead acetate orally at various doses (1, 5 and 10 mg/kg BW) for 30 days. The last 3 groups (6, 7 and 8) were exposed to EMF- lead combination for the same period. EMF- exposure, for 4h/day during 30 consecutive days, induced a significant increase in the erythrogram, leukogram and platelet counts, compared to control. Anemia, leucopenia, neutropenia, lymphopenia and monocytopenia were recorded with oral administration of lead acetate at various doses. The severity of changes was dose related. The phagocytosis % and phagocytic index were significantly ($P<0.05$) increased in mice exposed to EMF for 30 days (gp.2) but decreased in those given high doses of lead acetate. However, the immune parameters were insignificantly changed in groups 3, 7 and 8. Comparing to unexposed mice, significant variations in biochemical parameters (glucose, enzymes, and protein profiles) were noticed. Lead-EMF combination had synergistic effect on some previous parameters, whereas mice given the highest dose of lead with EMF aggravated hematobiochemical and pathological findings. We concluded that the combined EMF and lead acetate- exposure produced severe changes in the hemato-biochemical and immune parameters which were both real and inconsistent.

***Keywords**:* Electromagnetic fields, Lead, Blood cells, Serum enzymes, Mice.
Available online at http://www.vetmedmosul.org/ijvs

التاثير الدموي–الكيميائي حيوي و السمي المناعي للمجال الكهرومغناطيسي الواطئ وتداخلاته مع خلات الرصاص في الفئران

محمد عبد العزم هاشم[1]و ن. الشرقاوي[2]

[1]فرع التشخيصات المرضية، [2]فرع الطب العدلي والسموم، كلية الطب البيطري، جامعة الزقازيق، محافظة الشرقية، مصر

الخلاصة

اجريت الدراسة الحالية للتحري عن التاثيرات المحتملة للمجالات الكهرومغناطيسة الواطئة جدا وخلات الرصاص على بعض المتغيرات الدموية الكيمياء حيوية والمناعية والمرضية في الفئران. استخدمت في الدراسة ١٢٠ من اناث الفئران والتي قسمت بشكل متساوي الى ثمانية مجاميع. المجموعة الاولى بقيت كمجموعة سيطرة. المجموعة الثانية عرضت للمجال الكهرومغناطيسي بشدة ٢ ملليتيسلا وتردد ٥٠ هرتز وبمعدل ٤ ساعات/يوم ولمدة ٣٠ يوم. المجاميع ٣ و ٤ و ٥ اعطيت جرع عن طريق الفم من خلات الرصاص وبمقدار ١ و ٥ و ١٠ ملغم/كغم من وزن الجسم ولمدة ٣٠ يوم. المجاميع الثلاث الاخيرة ٦ و ٧ و ٨ تم تعريضها للمجال الكهرومغناطيسي مع اعطائها خلات الرصاص وبنفس المقادير والمدة التي استخدمت في المجاميع السابقة. ان التعرض للمجال الكهرومغناطيسي لمدة ٤ ساعات/يوم ولمدة ٣٠ يوم احدث زيادة معنوية في عدد كريات الدم الحمراء والبيضاء وعدد الصفيحات الدموية بالمقارنة مع مجموعة السيطرة. اما اعطاء خلات الرصاص عن طريق الفم فقد احدث فقر دم ونقص في اعداد كريات الدم

البيضاء حيث كانت شدة التغيرات مرتبطة مع مقدار الجرعة. ان النسبة المئوية لعملية البلعمة ودليل البلعمة قد ازدادا وبشكل معنوي ($P<0.05$) في الفئران المعرضة للمجال الكهرومغناطيسي لمدة ٣٠ يوم، ولكنهما قللا في الفئران التي اعطيت جرعات عالية من خلات الرصاص. بينما المعايير المناعية لم تتغير في المحاميع ٣ و ٧ و ٨. كما لوحظ تغيرات معنوية في المعايير الكيمياء حيوية (الكلوكوز، النزيمات، البروتين) في المجاميع المعاملة بالمقارنة مع الفئران غير المعاملة. لقد كان التعريض المزدوج للمجال الكهرومغناطيسي مع خلات الرصاص تاثير تازري في بعض المعايير السابقة، بينما الفئران التي اعطيت الجرعة العالية من خلات الرصاص مع المجال الكهرومغناطيسي اظهرت تغيرات دموية كيمياء حيوية ومرضية عالية. نستنتج من الدراسة ان التعرض المزدوج للمجال الكهرومغناطيسي مع خلات الرصاص يؤدي الى تغييرات حادة في المعايير الدموية الكيمياء حياتية والمناعية والتي كانت حقيقية ومتناقضة.

Introduction

In recent years, several studies have suggested possible bio-effects of magnetic fields on body systems (1). People are exposed to ELF-EMF daily in the home or at work through power lines and the constant use of appliances in every day life such as refrigerators, washing machines and kettles. These household appliances alone may generate magnetic fields of up to 4 μT (2). EMF may interfere with memory performance as there is evidence suggesting impairing effects of stress- induced corticosterone release on object recognition in rats (3), or may certainly increase the risk of both Alzheimer's disease and breast cancer (4). The key events arising from exposure to EMF may include alterations in cell membrane activity and effects on various enzyme systems (5). Exposure of mice to static magnetic field (SMF) increased the blood urea nitrogen, creatinine and glucose concentrations with accelerated glycolysis and glycogenolysis (6).

Lead (Pb) is a multiple-source pollutant, well known for its toxicity, of great risk both for the environment and human health. The main target organs of Pb are the hematopoietic, nervous, and renal systems; there are also reports in support of its impairment effects on the hepatic, reproductive and immune systems (7,8). A significant decreased RBC counts, hemoglobin levels and hematocrit values were reported in male and female mice given dietary Pb (9). Phagocytic cells, such as macrophages, may be used as a biomarker of immunotoxicity in wildlife studies (10).

Considering the lack of consensus on the biologic effects of static magnetic fields especially in combination with pollutants, this work was aimed to investigate the impact of the combined exposure to EMF and Pb acetate on hemato-biochemical, immunological and pathological findings in mice and to compare these with single treatment.

Material and methods

Animals and experimental design

A total of 120 female healthy Swiss albino mice weighing 20–25 g BW (6-wk-old) were obtained from Laboratory Animal Housing, Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were housed in standard metallic cages, with 15 mice in each cage. The mice were fed a standard pellet diet (El-Nasr Co., Abou-Zaabal, Cairo, Egypt), and water *ad libitum*.

The mice were equally divided into eight groups, control (gp. 1), EMF-exposed (gp. 2), Pb acetate -treated (gps. 3-5) and combined EMF and lead acetate (gps. 6-8). The average daily EMF-exposure was 2 millitesla (2 mT=20 G, Gauss) intensity and the electric field at 50 Hz frequency, 4h/day, for 30 days. The average daily EMF-exposure was 2 millitesla (2 mT=20 G, Gauss) intensity and the electric field at 50 Hz frequency, 4h/day, for 30 days. Lead acetate was dissolved in distilled water and administered orally at doses of 1, 5 and 10 mg/kg BW, for 30 days (11).

The animal experiments have been approved by the Committee of Animal Experimental Ethics of the Faculty of Veterinary Medicine, Zagazig University, Egypt.

Exposure system and magnetic field intensity

Electromagnetic field generator (Fig. 1) was designed and constructed in Biophysics Dept, Faculty of Science, Zagazig Univ., Egypt. The apparatus consists of an open box (width 100 x length 100 x height 50 cm) made of wood, painted mat gray inside.

Magnetic field chamber consisted of a parallel double walled cylindrical cage made from copper plate (2 mm thick) and was 114 cm internal diameter, 140 cm external diameter and 152 cm long (Fig. 1). The two cylinders were sealed at each end with copper to permit water flow between the two layers. A solenoid consisted of coils with 320 turns each from electrically insulated 2.2 mm copper wire were wound around the outer cylinder at equal distance. The four coils were connected in parallel to minimize the total impedance of the wire and allow a homogenous magnetic field within the chamber volume. The cylinder was grounded. A mesh from copper was used to cover both ends of the cylinder. The coils were connected to a Variac fed from the mains (220 V and 50 Hz). The magnetic field inside the chamber was measured at different locations using a hand-held Gauss/ Tesla Meter (Model 4048, F.W. Bell, Division of Bell Technologies,

Orlando, FL). A probe T-4048.001 (USA) of ± 2% accuracy was used to calibrate the magnetic field. The field strength can be varied by means of Variac up to 2 mT inside the homogenous zone without an increase in the chamber temperature (± 0.5 °C). The device was adjusted to induce extremely low frequency of 50 Hz alternating field with a high-intensity vertical magnetic field up to 2 mT (=20 Gauss). The mice cage put in the middle of the coils to get homogenous and magnetic field strength. The cage in the EMF generator contained five mice for each exposure.

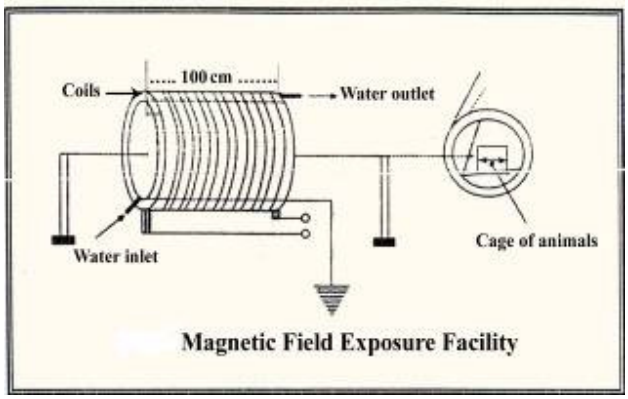


Fig. 1: Electromagnetic field generator designed and constructed in Biophysics Department, Faculty of Science, Zagazig University, Egypt.

Hematological and biochemical studies

Twenty-four hours following the last magnetic exposure, Blood samples were collected from the supra-orbital venous plexus of mice into two tubes. The first tube contained dipotassium salt of EDTA as anticoagulant for RBC, hematocrit, hemoglobin, WBC, neutrophils, eosinophils, lymphocytes, monocytes and platelets analysis, using standard methods (12). Blood sample in the other tube was left for a short time to allow clotting. Clear serum samples were obtained by centrifugation at 3000 r.p.m. for 20 min. and then kept at -20°C prior to biochemical analysis. Serum levels of glucose, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using the enzymatic methods according to manufacturer instructions. The serum creatinine and urea were measured colorimetrically, using commercial diagnostic Kits (Human-Germany).

Immunological studies

The estimation of the humoral immune response was based upon electrophoretic analysis of serum protein fractions by polyacrylamide-gel electrophoresis (Al-Ahram Lab., Tanta, Egypt). Serum total proteins (T.P) were determined by kits supplied from Bio-Analytic, Cairo, Egypt. The phagocytic activity of polymorphnuclear neutrophil was carried out for the evaluation of the cellular immunity (13). Materials used for assessment of phagocytic activity were heparinized tubes for blood collection, Hank′s solution, phosphate buffer saline, Leishman′s stain (14), *Candida albicans* and foetal calf serum (supplied by Animal Health Institute, Dokki, Giza, Egypt). The total number of phagocytic cells, and the phagocytes which ingested yeast cells in individual phagocytes, were determined to calculate the percentage of phagocytosis, and phagocytic index.

Pathological studies

Specimens from the liver, kidneys and spleen were collected and fixed in 10% neutral buffered formalin solution. Five-micron thick paraffin sections were prepared and stained by hematoxylin and eosin (H&E.) (15).

Statistical analysis

Data were computer analyzed by one way ANOVA using SPSS 8.0 for Windows (Statistical Package for the Social Sciences Inc, Chicago, Illinois). The means, showing significant differences in ANOVA, were compared using Duncan's Multiple Range Test. The significant level ($P<0.05$) compared with control.

Results

Blood haematology

As shown in Table 1, EMF (2 mT intensity and5 0 Hz frequency) exposure, 4h/day, for 30 days (gp. 2) caused a significant ($P<0.001$) increase of RBCs count, Hb concentration and Ht value compared to control mice (gp.1). Mice administered lead acetate at doses of 1, 5 and 10 mg/kg BW alone (gps. 3-5) or in combination with EMF (gps. 6-8) showed a significant ($P<0.01$) decrease in the erythrocyte parameters producing macrocytic hypochromic anemia. EMF-exposed mice revealed significant ($P<0.01$) leukocytosis with neutrophilia, lymphocytosis and monocytosis, in comparison with the control. Total and differential leukocytic counts did not significantly change in gps. 3, 6 and 7, but significantly ($P<0.01$) decreased in groups 4, 5 and 8. The changes were dose- dependent with lead. However, eosinophil count showed insignificant change in all groups. EMF exposure significantly ($P<0.001$) increase the platelet count, but Pb administered mice (gps. 4, 5 and 8) decrease it ($P<0.05$). It showed insignificant change in mice of gps. 3, 6 and 7.

Blood chemistry

As illustrated in Table 2, EMF and lead acetate-treated mice (gps. 2, 4 and 5) showed significant ($P<0.01$) higher levels of serum enzymes (ALT, AST), creatinine and urea than that of control. On contrast, significant ($P<0.01$) hyperglycemia (gp. 2) and hypoglycemia (gps. 4 and 5)

were recorded, when compared with control mice. The changes in lead administered mice were dose-dependent. Insignificant change in these parameters was observed in gp. (3). Compared with the control mice, exposure to EMF-Pb acetate combinations (gps. 6- 8) resulted in the same results for enzymes and renal markers, while serum glucose showed insignificant change.

Table 1: Hematological parameter changes in mice exposed to EMF- and its interaction with lead acetate.

Parameters	Groups							
	Gp. 1	Gp. 2	Gp. 3	Gp. 4	Gp. 5	Gp. 6	Gp. 7	Gp. 8
RBC ($x10^6$/µl)	7.22 ^{B} ±0.42	8.52 ^{A} ±0.16	6.12 ^{C} ±1.22	5.32 ^{D} ±0.66	4.02 ^{E} ±0.80	7.05 ^{B} ±1.02	6.42 ^{C} ±0.86	5.45 ^{CD} ±1.20
Hb (g/dl)	11.50^{B} ±2.18	13.20^{A} ±1.26	9.45 ^{C} ±9.98	9.20 ^{C} ±4.23	8.25 ^{D} ±5.44	10.95 ^{B} ±9.98	10.50 ^{B} ±6.05	9.50 ^{C} ±4.55
Ht (%)	29.80 ^{B} ±2.54	38.00 ^{A} ±1.88	26.50 ^{C} ±3.40	25.00 ^{C} ±4.14	23.90 ^{D} ±1.33	31.80 ^{B} ±1.55	28.55 ^{B} ±3.00	26.44 ^{C} ±3.40
MCV (fl)	41.3^{C} ±1.08	44.60 ^{B} ±1.41	44.00 ^{B} ±0.90	46.99 ^{B} ±1.12	52.7 ^{A} ±1.71	45.11 ^{B} ±1.50	44.47 ^{B} ±1.71	48.51^{A} ±1.50
MCH (pg)	15.80^{B} ± 0.90	15.50^{B} ±0.56	15.44^{B} ± 1.11	17.29^{AB} ± 0.50	20.5 ^{A} ±0.51	15.53^{B} ± 0.43	16.36^{B} ±0.56	17.43^{AB} ± 0.60
MCHC (%)	38.40^{A} ± 3.91	34.74 ^{B} ±2.44	35.20^{B} ± 4.50	36.80 ^{B} ± 8.91	34.50 ^{B} ±3.71	34.43 ^{B} ± 1.43	36.77 ^{B} ±3.71	35.93 ^{B} ± 8.91
WBC ($x10^3$/µl)	10.30^{B} ± 0.19	14.61^{A} ±0.87	10.10 ^{B} ± 1.19	8.32 ^{C} ± 1.11	7.10 ^{D} ±0.87	10.11 ^{B} ± 1.05	10.08 ^{B} ±0.87	8.53 ^{C} ± 1.19
Neutrophil ($x10^3$/µl)	5.20 ^{B} ± 6.07	7.42 ^{A} ± 9.00	5.18 ^{B} ± 7.19	4.28 ^{C} ± 5.17	4.00 ^{C} ± 6.19	5.13 ^{B} ± 7.00	5.00 ^{B} ± 8.45	4.50 ^{C} ± 7.11
Eosinophil ($x10^3$/µl)	0.41 ^{A} ± 0.04	0.42 ^{A} ± 0.10	0.40 ^{A} ± 0.03	0.41 ^{A} ± 0.02	0.43 ^{A} ± 0.11	0.40 ^{A} ± 0.09	0.41 ^{A} ± 0.12	0.40 ^{A} ± 0.19
Lymphocyte ($x10^3$/µl)	3.77 ^{B} ± 3.87	5.39 ^{A} ± 3.70	3.8 ^{B} ± 1.84	2.80 ^{C} ± 1.11	2.15 ^{C} ± 2.88	3.70 ^{B} ± 4.05	3.69 ^{B} ± 5.92	3.37 ^{B} ± 4.09
Monocyte ($x10^3$/µl)	0.93 ^{B} ± 0.05	1.36 ^{A} ± 0.07	0.92 ^{B} ± 0.06	0.60 ^{C} ± 0.02	0.56 ^{C} ± 0.03	0.94 ^{B} ± 0.05	0.92 ^{B} ± 0.04	0.68 ^{C} ± 0.19
Platelet ($x10^3$/µl)	536.00 ^{B} ±86.90	707.20^{A} ±55.17	534.50^{B} ±102.10	350.40 ^{D} ±92.10	300.80^{D} ±88.20	514.66^{B} ±70.10	498.90^{BC} ±100.17	460.25^{C} ±112.10

Mean values within the same row showing different superscript alpha-betical letters are significant different (P<0.01). Values are expressed as means ±SE (15 mice/ group).

Table 2: Serum biochemical parameter changes in mice exposed to EMF- and its interaction with lead acetate.

Parameters	Groups							
	Gp. 1	Gp. 2	Gp. 3	Gp. 4	Gp. 5	Gp. 6	Gp. 7	Gp. 8
Glucose (mg/dl)	102.00 ^{B} ± 3.19	122.00 ^{A} ±2.55	97.00 ^{B} ± 2.19	75.00 ^{C} ± 2.33	60.89 ^{D} ± 2.12	110.80^{AB} ±3.11	94.90 ^{B} ±1.28	96.30 ^{B} ±2.10
ALT (U/L)	18.35 ^{E} ±1.05	59.66 ^{C} ±2.11	19.30 ^{E} ±1.02	44.62 ^{D} ±2.04	78.11 ^{B} ±3.15	68.33 ^{C} ±2.9	84.35 ^{B} ±3.22	100.00 ^{A} ±4.00
AST (U/L)	23.83 ^{E} ±3.14	60.01 ^{C} ±4.52	25.45 ^{E} ±2.09	49.85 ^{D} ±3.22	64.55 ^{C} ±4.6	73.00 ^{B} ±4.13	85.83 ^{A} ±4.35	90.13 ^{A} ±5.44
Creatinine (mg/dl)	1.07 ^{D} ±0.08	1.99 ^{C} ±0.15	1.05 ^{D} ±0.06	1.97 ^{C} ±0.13	2.21 ^{B} ±0.11	2.11 ^{BC} ±0.14	2.27 ^{B} ±0.12	2.55 ^{A} ±0.16
Urea (mg/dl)	35.57 ^{D} ±1.93	79.44 ^{B} ±4.14	35.99 ^{D} ±1.66	66.37 ^{C} ±3.12	84.50 ^{B} ±3.05	85.57 ^{B} ±4.11	105.34 ^{A} ±4.14	119.00 ^{A} ±5.50

Mean values within the same row showing different superscript alpha-betical letters are significant different (P<0.01). Values are expressed as means ±SE (15 mice/ group).

Immunological results

As shown in Table 3, EMF- exposure of mice during 30 consecutive days significantly increased the serum total protein levels (P<0.05), gamma (P<0.01), and total globulins (P<0.01) compared to control group. However, serum albumin and other globulin fractions showed no statistical change. Administration of 10 mg lead significantly (P<0.05) decreased the serum total proteins, albumin, and globulins (α, β, γ) compared to the control values. Contrarily, low doses of Pb acetate (1 and 5 mg/kg) or combined EMF- Pb acetate treatments (gps. 6 and 7) produced insignificant change. Mice of gp. 8 showed a significant (P<0.05) decrease in serum proteins, albumin, α and β- globulins, with insignificant change in gamma and total globulins.

As indicated in Table (4) and Figures (2-7), the percentage of phagocytosis and phagocytic index were significantly (P<0.001) increased in EMF- exposed mice for 30 days (gp.2), while administration of lead exerted a significant (P<0.01) dose-dependent decrease (gps. 4 and 5), in comparison with gp. (1). These tests were insignificantly changed in mice of groups (3, 7 and 8), but increased significantly (P<0.05) in mice of gp. (6).

Table 3: Proteinogram changes in mice exposed to EMF- and its interaction with lead acetate.

Parameters	Groups							
	Gp. 1	Gp. 2	Gp. 3	Gp. 4	Gp. 5	Gp. 6	Gp. 7	Gp. 8
Total protein (g/dl)	6.10^{B} ±1.42	6.90^{A} ±1.50	6.32^{B} ±1.22	6.20^{B} ±1.20	4.12^{C} ±1.03	5.99^{B} ±1.05	5.90^{B} ±1.12	4.86^{C} ±1.14
Albumin (g/dl)	3.10^{A} ± 0.22	3.04^{A} ± 0.19	3.00^{A} ± 0.14	2.80^{A} ± 0.17	1.95^{B} ± 0.06	2.60^{A} ± 0.12	2.60^{A} ± 0.10	2.00^{B} ±0.15
α_1-Globulins (g/dl)	0.42^{A} ± 0.19	0.44^{A} ± 0.12	0.45^{A} ± 0.11	0.42^{A} ± 0.09	0.35^{B} ± 0.19	0.45^{A} ± 0.15	0.44^{A} ± 0.07	0.36^{B} ± 0.12
α_2-Globulins (g/dl)	0.61^{A} ±0.11	0.62^{A} ±0.13	0.61^{A} ±0.05	0.61^{A} ±0.04	0.34^{C} ±0.11	0.60^{A} ±0.13	0.55^{AB} ±0.09	0.40^{C} ±0.01
β-Globulins (g/dl)	1.14^{A} ±0.50	1.09^{A} ±0.71	1.12^{A} ±0.80	1.13^{A} ±0.71	0.85^{B} ±0.02	0.99^{AB} ±0.06	1.06^{A} ±0.25	0.90^{B} ±0.40
γ-Globulins (g/dl)	1.19^{B} ±0.50	1.85^{A} ±0.65	1.21^{B} ±0.23	1.17^{B} ±0.57	0.64^{C} ±0.45	1.23^{B} ±0.43	1.11^{B} ±0.36	1.24^{B} ±0.91
Total globulins (g/dl)	3.00^{B} ±1.00	3.80^{A} ±1.10	3.40^{B} ±1.03	3.40^{B} ±1.05	2.47^{C} ±0.50	3.29^{B} ±1.14	3.30^{B} ±1.16	2.94^{BC} ±1.12

Mean values within the same row showing different superscript alpha-betical letters are significant different (P< 0.05). Values are expressed as means ±SE (15 mice/ group).

Table 4: Percentage and index of phagocytosis in mice exposed to EMF- and its interaction with lead acetate.

Parameters	Groups							
	Gp. 1	Gp. 2	Gp. 3	Gp. 4	Gp. 5	Gp. 6	Gp. 7	Gp. 8
Phagocytic %	43.10^{C} ±5.30	56.90^{A} ±4.11	43.31^{C} ±5.60	36.20^{D} ±3.82	24.41^{E} ±3.50	50.20^{B} ±6.12	43.90^{C} ±4.72	40.80^{C} ±5.22
Phagocytic index	0.71^{B} ± 0.22	0.95^{A} ± 0.19	0.70^{B} ± 0.14	0.65^{C} ± 0.17	0.60^{C} ± 0.33	0.85^{A} ± 0.12	0.75^{B} ± 0.13	0.73^{B} ±0.15

Mean values within the same row showing different superscript alpha-betical letters are significant different (P<0.05). Values are expressed as means ±SE (15 mice/ group).

Pathological findings

Group 1: Liver, kidney and spleen sections from a control animal showing normal parenchyma respectively (Figs. 8, 9 and 10).

Group 2: The liver from exposed mice to EMF showed focal centrolobular necrosis of the hepatic cells surrounded by severe hydropic degeneration involving the majority of hepatic parenchyma (Fig. 11). Congestion of hepatic blood vessels and sinusoids, mild lymphocytic infiltration with mild fibroblast, proliferative biliary epithelium and round cell infiltration were encountered. The kidneys of EMF exposed mice showed congestion of renal blood vessels, contracted glomerular tufts of some glomeruli and focal leukocytic aggregation (Fig. 12). A few leukocytes infiltration mainly lymphocytes were encountered in the interstitial tissues of renal cortex. Some renal tubules has

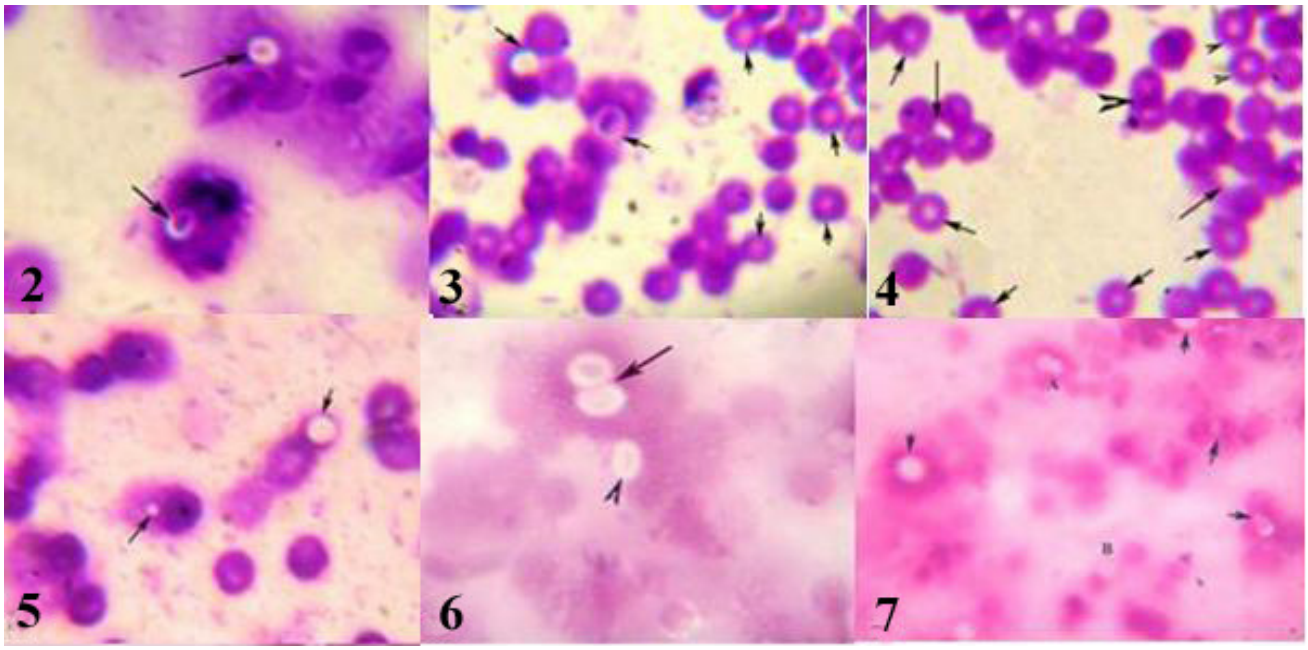
edematous vascular wall with congestion. An area of coagulative necrosis infiltrated with few lymphocytes and plasma cells in the renal tubules was seen. Some splenic white pulps of spleen suffered from lymphoid depletion and the others became hyperplastic with proliferation of megakaryocytes, beside hemosiderosis in red and white pulps (Fig. 13).

Group 3: mice given 1mg/kg BW of Pb acetate showed mild lesions in liver, kidney and spleen, when compared with mice given other doses of lead.

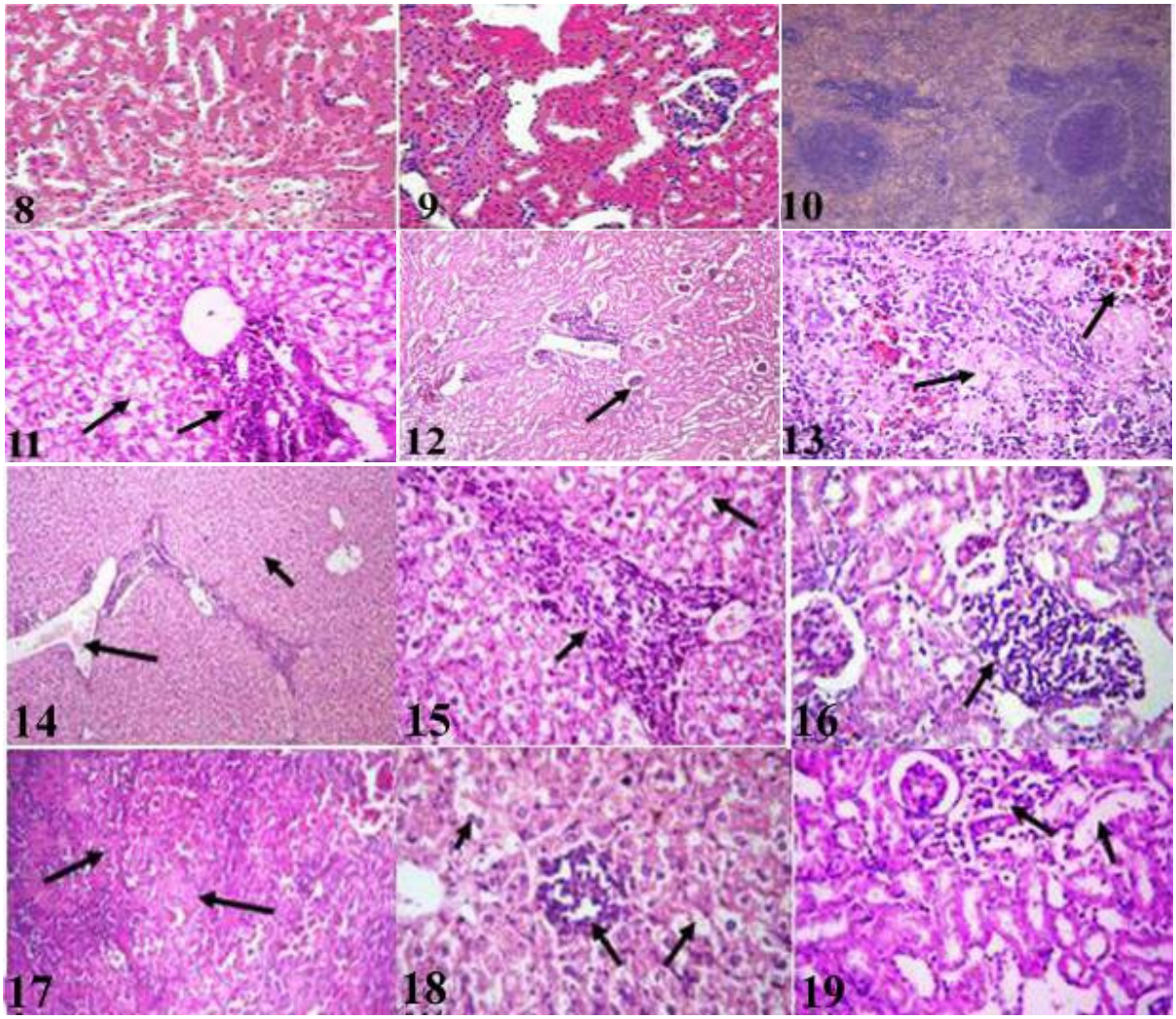
Group 4: The liver sections from mice given 5mg/kg BW of Pb acetate showed congestion of blood vessels, portal leukocytic infiltration and hydropic degeneration (Figs. 14 and 15), beside focal necrotic area invaded with lymphocytes in the hepatic parenchyma. Kidneys revealed large area of necrosis in the renal cortex and focal replacement of some renal tubules by lymphocytes and erythrocytes. Spleen showed the same lesion of gp. 2. In addition, few hemosiderin granules were scattered in the white and red pulps. Thickening of splenic capsule and depletion of subcapsular sinuses were observed in some cases.

Group 5: The liver section from mice given 10 mg/kg BW of Pb acetate showed more severe lesions than those of gp. (4). Kidneys revealed focal replacement of renal parenchyma by lymphocytes (Fig. 16). The spleen showed severe lymphoid depletion, rudimentary white pulps, and hemosiderosis in red and white pulps, beside thickened splenic trabeculae (Fig. 17).

Mice exposed to a combination of lead and EMF (gps. 6 and 7) showed more severe lesions than previous groups. Liver (gp. 8) showed portal and interstitial lymphocyte aggregations, hyperplastic Kuffer cells, hydropic degeneration, hyperchromatic nuclei and disorganized hepatic cords, (Fig. 18). Kidneys were suffered from multiple lymphocyte aggregations in renal cortex, hemorrhages, albuminous (granular) and hyaline casts in the tubular lumen. Periglomerular lymphocytic infiltrations with individual coagulative necrosis were noticed in the renal tubules (Fig. 19).



Figs. 2-7: Phagocytosis of *C. albicans* by neutrophils in mice exposed to EMF- and its interaction with lead acetate. 2- Phagocytosis of *C. albicans* by neutrophils of control mice (gp.1). One cell of *C. albicans* was engulfed by neutrophils (arrow), Leishman′s stain, X100. 3 and 4- Phagocytosis of *C. albicans* by neutrophils of EMF exposed mice (gp.2). One and two C. *albicans* engulfed by neutrophils were represented by arrow (Fig. 2) and arrow-head (Fig. 3) respectively, Leishman′s stain, X100. 5- Phagocytosis of *C. albicans* by neutrophils of mice administered lead acetate (gp.4). One *C. albicans* was engulfed by neutrophils (arrow), Leishman′s stain, X100. 6- Phagocytosis of *C. albicans* by neutrophils of mice administered lead acetate (gp.5) showing two cells of *C. albicans* were engulfed by neutrophils (arrow) and one cell attached to the surface of neutrophil (arrow-head), Leishman′s stain, X100. 7- Phagocytosis of *C. albicans* by neutrophils of mice administered lead acetate (gp.6) showing two cells of *C. albicans* were engulfed by neutrophils (A) and one cell attached to the surface of neutrophil (B) and/ or engulfed (arrow), Leishman′s stain, X100.



Figs. 8- 19:- Histopathological findings in mice exposed to EMF- and its interaction with lead acetate. 8- Liver section from control mice (gp. 1) showing normal structure, H&E., x1200. 9- Kidney section from control mice (gp. 1) showing normal structure, H&E., x1200. 10- The liver section from mice of gp. (2) showed focal centrolobular necrosis of the hepatic cells surrounded by severe hydropic degeneration involving the majority of hepatic parenchyma and round cells infiltration, H&E., x1200. 11- The kidney section (gp. 2) suffered from congestion of renal blood vessels, contracted glomerular tufts of some glomeruli and focal leukocytic aggregation, H&E., x300. 12- Spleen section from control mice (gp. 1) showing normal structure, H&E., x1200. 13- Spleen section (gp.2) showed lymphoid depletion in splenic white pulps and hemosiderosis in red and white pulps, H&E., x1200. 14- Liver section from mice given 5mg/kg BW (gp. 4) of lead acetate showed congestion of blood vessels, portal leukocytic infiltration and hydropic degeneration, H&E., x300. 15- High power of Fig. (14) showed leukocytic infiltration in the portal area and hydropic degeneration, H&E., x1200. 16- Kidney section from mice given 10mg/kg BW of lead acetate (gp. 5) revealed focal replacement of renal parenchyma by lymphocytes, H&E., x1200. 17- Spleen section from gp. (5) showed severe lymphoid depletion, hemosiderosis in red and white pulps beside thickened splenic trabeculae, H&E., x1200. 18- Liver section of mice exposed to a combination of lead and EMF (gp. 8) showed focal replacement of hepatic parenchyma by lymphocytes, hyperplastic Kuffer cells, hydropic degeneration, hyperchromatic nuclei and disorganized hepatic cords, H&E., x1200. 19- Kidney section from mice of gp. (8) showed periglomerular lymphocytic infiltrations with individual coagulative necrosis, H&E., x1200.

Discussion

One of the most contentious issues in the scientific communities today is that of the biological effects of EMFs, and whether or not they are adversely affecting our health. The choice of 2 mT intensity/50 Hz magnetic fields which they are below ICNIRP standard (16) and since they are to a certain degree realistic in terms of human and animal exposure. Repeated exposure to EMF of 2 mT intensity (4h /day) for 30 days induced an increase in the count of RBCs, platelets, Hb content, Ht values and leukocytosis with neutrophilia, lymphocytosis and monocytosis, compared to control unexposed mice. Indeed, previous data induced by EMF suggesting the hypoxia-like status resulting probably from the oxygen-binding impairment of Hb (17,18). Other studies also showed that erythrocytes orient with the applied MF (17). These data are probably associated with the change in the conformation of Hb under MF action. In this context, an increase in Hb concentration, RBC, WBC and platelet numbers in rats following exposure to EMF for 1h/day during 30 consecutive days have demonstrated (19,20). Contrarily, insignificant change was in that parameters, except for increased hematocrit value in male welders exposed to ELF-EMF of 10 μT– 2 T/ 5 kHz (21). These hematological results were confirmed by pathological findings in spleen which showed lymphoid depletion in some splenic white pulps and the others became hyperplastic with proliferation of megakaryocytes, precursor cells of blood platelets.

Lead acetate administration alone or in combination with EMF produced macrocytic hypochromic anemia, associated with increased MCV and decreased MCHC. It was reported that anemia following lead poisoning is in part the result of various inhibitory effects of Pb on heme biosynthesis (22). Besides, excessive Pb exposure inhibits the body's ability to make hemoglobin by interfering with several enzymatic steps in the heme pathway, through inhibiting aminolevulinic acid dehydratase and ferrochelatase activity, leading to anemia and erythrocytes degeneration or destruction (23,24). This data is in coincidence with previous reports (8,9). Dose dependent changes in total and differential WBCs, as well as platelet counts were detected in Pb alone or with EMF-treatment groups. On the other hand, mice given Pb acetate orally at doses 1 and 10 mg/kg BW for one month produced a significant leukocytosis (11,25).

Exposure to EMF increased significantly serum glucose level and transaminases activities. The increase in the glucose level agrees well with previous findings with EMF with different strengths (17,26). Serum transaminases have been widely utilized as biomarkers for hepato-cellular injury (19). Magnetic field induced structural changes in hepatocytes, primarily in mitochondria, and in turn significant increase in ALT activity, which indicates citotoxic effect (20,27,28). EMF exposure increases serum creatinine and urea levels. Similarly, Tsuji at al. (29) stated that mice exposed to SMFs (5 T) for 48 h increased blood urea nitrogen and creatinine levels. This may be due to the renal dysfunction associated with contracted glomeral tufts of some glomeruli and focal leukocytic aggregation by pathologic examination. Contrarily, SMF exposure had no effect on serum creatinine and urea levels in rats (20).

The hyperproteinemia due to EMF exposure suggesting the change in protein metabolism of stressed mice or the increase in the globulin component. These results agree with similar reports (19,30). On contrary, a significant decrease in the levels of total protein, albumin and /or globulins were observed in steelworkers exposed to EMF (1.3 mT intensity and 50 Hz frequency, mean 6.8h /day) for 5days (6), and in rats exposed to EMF, 4-8hr daily, for 2months (31). This discrepancy could be due to the difference of the intensity of the EMF and the exposure scenario and duration. The increased phagocytosis % and phagocytic index in mice with EMF exposure indicated that the role of electromagnetic field is prevalent in the formation of effects of the intensity and completeness of phagocytosis (32). There is no generally accepted mechanism to explain how extremely low frequency fields might initiate bioeffects, if any, on immune system (33). The present results supported by the pathologic lesions in the liver (Fig. 9) and kidney (Fig. 10). Similarly, Krstic et al. (34) reported that experimental exposition of mice by mobile telephones showed a slightly increased number of micronuclei and discrete perivenular fatty changes in liver. Zare et al. (35) showed highly cytoplasmic vacuolation of liver and swelling of epithelial cells of kidney tubules with subsequently cell necrosis in two groups of guinea pigs exposed to EMFs of 0.013 μT or 0.207 μT with 50Hz frequency, 2 or 4hours daily for 5 days.

Chronic lead administration (5 and 10 mg Pb acetate/kg, daily for 30 days) resulted in a dose- dependent increase in serum parameters; however, serum glucose was significantly decreased. Lead can cause adverse effects to hepatic cells owing to its storage in the liver after Pb acetate exposure. An elevation in the levels of AST and ALT was previously (36,37). The observed elevation in creatinine concentrations may indicate impairment in kidney function, which is in agreement with Ghorbe et al. (38). Enhanced protein catabolism together with accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpret the elevated levels of urea or due to the previously reported destruction of RBCs (39). The combination of EMF with Pb acetate showed synergic effects on some hepato-renal parameters.

Interestingly, the decreased in serum proteins may be attributed to hepatotoxicity or renal toxicity resulting from the highest dose of lead. Experiments conducted in male rats exposed to 0.2 g/l dietary Pb for 10 weeks showed normal serum total proteins and albumin (40). Blood lead >

or = 25 microg/dL can cause a significant decrease in immunoglobulins with dysfunctions in different organ systems of the body, such as the immune system (41). Observations of suppression of phagocytic activity neutrophil in relationship to Pb acetate exposure were in parallel with others (42-44). It causes a primary impairment of the chemotactic and phagocytic activities of neutrophil leucocytes. The combined effects of EMF and of Pb acetate were found to potentiate the toxic effects. Similar interpretations were given by Chernykh (32). The most common and constant findings was a portal leukocytic infiltration, hydropic degeneration and loss of normal architexture in the liver. Light microscopy of kidney revealed focal replacement of renal parenchyma by lymphocytes and coagulative necrosis. Similar histopathological lesions have been reported in experimental Pb acetate toxicity with different species (8, 45-47).

In conclusion, several experiments are still necessary with the purpose of explaining which frequency, intensity, exposure time and other parameters involved with EMF, especially concurrent with environmental pollutants to protect ourselves from that harms.

Acknowledgments

The author thanks Dr. Abd El-Moneim Aly, Prof. of Pathology, Faculty of Veterinary Medicine and Dr. Magda Hanafy, Prof. of Physics, Faculty of Science, Zagazig University, Egypt for their assistance in the histopathology and supplying electromagnetic field generator respectively.

References

1. Jolanta J, Janina G, Marek Z, Elzibieta R, Mariola S, Marek K. Influence of a 7mT static magnetic field and irons ions on apoptosis and necrosis in rat blood lymphocytes. J Accup Health 2001;43:379-381.
2. Fernie KJ, Reynolds SJ. The effects of electromagnetic fields from power lines on avian reproductive biology and physiology:a review. J Toxicol Environ Health B Crit Rev. 2005;8:127–140.
3. Mostafa RM, Mostafa YM, Ennaceur A. Effects of exposure to extremely low-frequency magnetic field of 2 G intensity on memory and corticosterone level in rats. Physiol Behav. 2002;76:589-595.
4. Davanipour Z, Sobel E. Long-term exposure to magnetic fields and the risks of Alzheimer's disease and breast cancer:Further biological research. Pathophys, 2009;Mar 9. 01.005 doi:10.1016/j.
5. Upfal M. Liver enzymes among microelectronics equipment maintenance technicians. J Occup Med. 1992;34:384-390.
6. Boguslaw K, Andrzej S, Rozalia G, Danuta P. Effect of electromagnetic field on serum biochemical parameters in steelworkers. J Occup Health 1999;41:177–180.
7. Teijón C, Olmo R, Blanco D, Romero A, Teijón JM. Low doses of lead:effects on reproduction and development in rats. Biol Trace Elem Res. 2006;111:51-165.
8. Durgut R, Koc A, Gonenci R, Bal R, Celik S, Guzel M, Altug M, Atesoglu E. Effects of high dose lead toxication on liver, kidneys, heart, brain and blood in rabbits:an experimental study. J Appl Biol Sci. 2008;2:11-18.
9. Iavicoli I, Carelli G, Stanek EJ, Castellino N, Calabrese EJ. Effects of low doses of dietary lead on red blood cell production in male and female mice. Toxicol Lett, 2003;137:193-199.
10. Fournier M, Cyr D, Blakley B, Boermans H, Brousseau P. Phagocytosis as a biomarker of immunotoxicity in wildlife species exposed to environmental xenobiotics. Integr. Comp. Biol. 2000;40:412-420.
11. Schlick E, Friedberg KD. Bone marrow cells of mice under the influence of low lead doses. Arch. Toxicol. 1982;49:227-236.
12. Feldman BF, Zinkl JG, Jain N.C. Schalm's Veterinary Hematology. 5th Ed., Lippincott Williams and Wilkins. A Wolters Company. Philadelphia, Baltimore, New York, London, 2000.
13. Antley PP, Hazen KC. Role of yeast cell growth temperature on *Candida albicans* virulence in mice. Infect Immun. 1988;56:2884-2890.
14. Cruickshank R, Duguid JP, Marinion BP, Swain RH. Textbook of Medical Microbiology, 12th Ed. E.S. Living Stone Limited Edinburgh and London, 1975.
15. Bancroft GD, Stevens A. Theory and Practice of Histopathological Technique. 4th Ed., Churchill Livingstone Edinburgh, London, Melbourne and New York, 1996.
16. ICNIRP. Guidelines for limiting exposure to time-varying electric, magnetic and electromagnetic fields (up to 300GHz). Health physics 1998;74:494-522.
17. Chater S, Abdelmelek H, Sakly M, Pequinot JM, Ben RK. Effects of sub-acute exposure to magnetic field on blood hematological and biochemical parameters in female rats. Turk J Hematol. 2006;23:182-187.
18. Stashkov AM, Gorokhov IE. Hypoxic and antioxidant biological effect of multi-day application of a weak variable super-low frequency magnetic field. Biofizika 1998;43:807-810.
19. Amara S, Abdelmelek H, Ben Salem M, Abidi R, Sakly M. Effects of static magnetic field exposure on hematological and biochemical parameters in rats. Brazilian Archives of Biology and Technology 2006;49:889-895.
20. Salem A, Hafedh A, Rached A, Mohsen S, Khémais BR. Zinc prevents hematological and biochemical alterations induced by static magnetic field in rats. Pharmacol Rep. 2005;57:616-622.
21. Dasdag S, Sert C, Akdag Z, Batun S. Effects of extremely low frequency electromagnetic fields on hematologic and immunologic parameters in welders. Arch Med Res. 2002;33:29-32.
22. Kim Y, Yoo CI, Lee CR, Lee JH, Lee H, Kim SR, Chang SH, Lee WJ, Hwang CH, Lee YH. Evaluation of activity of erythrocyte pyrimidine 5-nucleotidase (P5N) in lead exposure workers, with focus on the effect on hemoglobin. Ind Health 2002;40:23-27.
23. Patocka J, Cerný K. Inorganic lead toxicology. Acta Medica. 2003;46:65-72.
24. Gurer-Orhan H, Sabir H, Ozgüneş H. Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. Toxicology 2004;195:147-154.
25. Hogan GR, Adams DP. Lead-induced leukocytosis in female mice. Archives of Toxicology 2004;41:295-300.
26. Armario A, Marti J, Gil M. The serum glucose response to acute stress is sensitive to the intensity of the stressor and to habituation. Psychoneuroendocrinology 1990; 15:341-347.
27. Parafiniuk M, Gorczynska E, Gutsch A, Parafiniuk W. Effect of constant magnetic field on the liver of guinea pig. Electron microscopic studies. Folia Histochem Cytobiol. 1992;30:119-123.
28. Djindjić B, Sokolović D, Radić S, Najman S, Krstić D, Marković V. Effect of long- term non-ionizing radiation on activity of hepatic enzymes in serum, Clinical Chemistry Laboratory Medicine. Barcelona, Spain:Monduzzi Editore S.p.A.-Medimond Inc., 2003, p. 1063-1066.
29. Tsuji Y, Nakagawa M, Suzuki Y. Five-tesla static magnetic fields suppress food and water consumption and weight gain in mice. Industrial health 1996;34:347-357.

30. Kumosani TA, Qari MH. The effect of magnetic field on the biochemical parameters of mice blood. Pak J Med Sci. 2003; 19: 36 – 40.
31. El-Abiad NM, Marzook EA. Effect of environmental microwave radiation exposure emitted from cellular phone base station on some biochemical parameters in rats. Sci. Med. J. ESCME. 2005; 17: 1-19.
32. Chernykh AM. Hygienic evaluation of the combined effect of electromagnetic fields and pesticides in experimental conditions. Gig Sanit. 2003; 2: 56-58.
33. Balcavage WX, Alvager T, Swez T, Goff CW, Fox MT, Abdullyava S, King MW. A mechanism for action of extremely low frequency electromagnetic fields on biological systems. Biochem Biophys Res Commun. 1996; 222: 374–378.
34. Krstic DD, Dinctic BJ, Sokolovic DT, Markovic VV, Petkovic DM, Radic SB. The results of experimental exposition of mice by mobile telephones. Mikrotalasna revija 2005; 11: 34-37.
35. Zare S, Alivandi S, Ebadi AG. Histological studies of the low frequency electromagnetic fields effect on liver, testes and kidney in guinea pigs. World Applied Sciences J. 2007; 2: 509-511.
36. Sivaprasad R, Nagaraj M, Varalakshmi P. Combined efficacies of lipoic acid and meso-2 3-dimercaptosuccinic acid on lead-induced erythrocyte membrane lipid peroxidation and antioxidant status in rats. Hum Exp Toxicol. 2003; 22: 183–192.
37. Shalan MG, Mostafa MS, Hassouna MM, El-Nabi SE, El- Refaie A. Amelioration of lead toxicity on rat liver with vitamin C and silymarin supplements. Toxicology 2005; 206: 1–15.
38. Ghorbe F, Boujelbene M, Makni-Ayadi F, Guermazi, F, Kammoun A, Murat J, Croute F, Soleilhavoup JP, El-Feki A. Effect of chronic lead exposure on kidney function in male and female rats, determination of lead exposure biomarker. Arch Physiol Biochem. 2001; 109: 457-463.
39. Ashour AA, Yassin MM, Abu Assia NM, Ali RM. Blood, serum glucose and renal parameters in lead-loaded albino rats and treatment with some chelating agents and natural oils. Turk J Biol. 2007; 31: 25-34.
40. Mahaffey KR, Carpar SG, Gladen BC, Fowler BA. Concurrent exposure to lead, cadmium and arsenic effects on toxicity and tissue metal concentrations in the rat. J Lab Clin Med. 1981; 98: 463–481.
41. Ayatollahi M. Study of the impact of blood lead level on humoral immunity in humans. Toxicol Ind Health 2002; 18: 39-44.
42. Di Lorenzo L, Silvestroni A, Martino MG, Gagliardi T, Corfiati M, Soleo L. Evaluation of peripheral blood neutrophil leucocytes in lead-exposed workers. Int Arch Occup Environ Health 2006; 79: 491-498.
43. Kim YO, Pyo MY, Kim JH. Influence of melatonin on immunotoxicity of lead. Int J Immunopharmacol. 2000; 22: 821-832.
44. Rosenberg CE, Fink NE, Arrieta MA. Salibian A. Effect of lead acetate on the in vitro engulfment and killing capability of toad (Bufo arenarum) neutrophils. Comp Biochem Physiol C Toxicol Pharmacol. 2003; 136: 225-233.
45. Rader JL, Celesk EM, Peeler JR, Mahaffey KR. Retention of lead acetate in wealing and adult rats. Toxicol Appl Pharmacol. 1983; 67: 100-109.
46. Victery W, Miller CR, Zhu SY, Goyer RA. Effect of different levels and periods of lead exposure on tissue levels and excretion of lead, zinc and calcium in the rat. Fundam Appl Toxicol. 1987; 8(4): 506-516.
47. Vyskocil A, Semecky V, Fiala Z Cizkova M, Viau C. Renal alterations in female rats following subchronic lead exposure. J Appl Toxicol. 1995; 15: 257–262.