The effects of low electromagnetic field and lead acetate combination on some hemato-biochemical and immunotoxicological parameters in mice

Farelerdeki bazı hemato-biyokimyasal ve immunotoksikolojik parametrelerde düşük elektromanyetik alan ile kurşun asetat kombinasyonunun etkileri

Mohamed Abd El-azim Hashem[1], Nabela Imam El-sharkawy[2]

[1]*Department of Clinical Pathology, Faculty of Vet. Medicine, Zagazig University, El-Zeraa Street, Sharkia Province, Egypt*

[2]*Department of Forensic Medicine and Toxicology, Faculty of Vet. Medicine, Zagazig University, El-Zeraa Street, Sharkia Province, Egypt*

Abstract

Objective: 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.

Material and Methods: A total of 90 female mice were equally divided into six groups. (Gp. 1) kept as control, (Gp. 2) exposed to EMF of 2 millitesla (mT) intensity and 50 Hz frequency (4h/day) for 30 days, (Gps. 3 and 4) were administered lead acetate orally at doses 1 and 5 mg/kg BW, respectively for 30 days. The last 2 groups (5, 6) were exposed to EMF-lead combination for the same period.

Results: EMF exposure induced a significant increase in RBCs ($p<0.001$), WBCs ($p<0.01$) and platelets ($p<0.001$) counts, compared to control. However, anemia and leukopenia were recorded with oral administration of Pb acetate. The phagocytosis % and phagocytic index were significantly ($p<0.05$) increased in mice exposed to EMF for 30 days, but decreased ($p<0.01$) in the animals given the highest dose of lead. Comparing to unexposed mice, significant variation in biochemical parameters (glucose, enzymes, and protein profiles) were noticed. Combined lead and EMF treatments had antagonizing effect on some previous parameters, whereas mice given the highest dose of lead with EMF aggravated hemato-biochemical and pathological findings.

Conclusion: We concluded that combined exposure to ELF-EMF and Pb acetate produced significant changes in the hemato-biochemical and immune parameters which were both real and inconsistent. *(Turk J Hematol 2009; 26: 181-9)*

Key words: Low electromagnetic field, lead acetate, hemato-biochemical parameters, phagocytic index

Received: July 19, 2009 *Accepted: September 1, 2009*

Özet

Amaç: Mevcut çalışma farelerdeki bazı hemato-biyokimyasal, immun ve patolojik değişkenler üzerinde aşırı düşük frekansta elektromanyetik alanların (ELF-EMF) ve kurşun asetatın potansiyel etkilerini araştırmak amacıyla gerçekleştirilmiştir.

Yöntem ve Gereçler: Toplam 90 dişi fare eşit sayıda altı gruba ayrıldı. 1. Grup kontrol olarak tutuldu, 2. Grup 30 gün boyunca 2 militesla (mT) yoğunlukta ve 50 Hz frekansta (4 saat/gün) EMF'ye maruz bırakıldı, 3. ve 4. Gruplara 30 gün boyunca belirtilen sırayla oral olarak 1 ve 5 mg/kg BW dozlarda kurşun asetat verildi. Son 2 grup (5, 6) aynı süre boyunca EMF-kurşun kombinasyonuna maruz bırakıldı.

Bulgular: EMF maruz kalım kontrol ile kıyaslandığında eritrosit'lerde ($p<0.001$), lökosit'lerde ($p<0.01$) ve trombositlerde ($p<0.001$) sayımlarında önemli bir düşüş görüldü. Ancak Pb asetatın oral tatbiki ile anemi ve lökopeni kaydedildi. Fagositoz

Address for Correspondence: Assoc.Prof. Mohamed Abd El-azim Hashem, 44511 Zagazig, Egypt
Phone: 0020552273680 E-mail: mhashem_vet@yahoo.com

yüzdesi ve fagositik indeksi 30 gün boyunca EMF'ye maruz bırakılan farelerde önemli ölçüde ($p<0.05$) yükseldi fakat yüksek dozlarda kurşun verilenlerde düştü ($p<0.01$). Maruz bırakılmayan farelerle kıyaslandığında biyokimyasal parametrelerde (glukoz, enzimler ve protein profilleri) önemli değişiklik fark edildi. Kombine kurşun ve EMF tedavileri daha önceki bazı parametreler üzerinde antagonize edici etkiye sahipti, oysa EMF ile birlikte en yüksek dozda kurşun verilen fareler hemato-biyokimyasal ve patolojik bulguları şiddetlendirdi.

Sonuç: ELF-EMF ve Pb asetatın birlikte maruziyeti hemato-biyokimyasal ve immun parametrelerde gerçek ama tutarsız ağır değişiklikler üretti. *(Turk J Hematol 2009; 26: 181-9)*

Anahtar kelimeler: Düşük frekans elektromanyetik alan, kurşun asetat, hemato-biyokimyasal parametre, fagositik indeks

Geliş tarihi: 19 Temmuz 2009 Kabul tarihi: 1 Eylül 2009

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 at 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] and long-term significant occupational exposure to ELF-EMF 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]. Previous data showed that EMFs are of minor importance in controlling heart rate, blood pressure, and biochemical parameters [6]. Exposure of mice to static magnetic field [SMF) increased the blood urea nitrogen, glucose and creatinine concentrations [7].

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 lead are the hematopoietic, nervous, and renal systems; there are also reports in support of its impairment effects on the hepatic, reproductive and immune systems [8, 9]. A significant decreased RBCs counts, hemoglobin levels and hematocrit values were reported in male and female mice given dietary lead [10]. Phagocytic cells, such as macrophages, may be used as a biomarker of immunotoxicity in wildlife studies [11]. There are only a few publications on medical examinations of workers exposed simultaneously to electromagnetic fields and chemicals [12].

Considering the lack of consensus on the biologic effects of static magnetic fields especially in combination with pollutants, this work 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

A total of 90 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. After 3 days of habituation to the laboratory environment, the animals were housed in metallic cages (50 cm length, 25 cm width and 30 cm height), 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. They were removed daily from their cages for cleaning the cages and renewing their food and water supply.

Exposure system

Electromagnetic field generator 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 0C). 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.

Treatments

The 90 mice were divided randomly into six groups including fifteen animals each: control group (Gp. 1) which received only water as a vehicle; EMF-exposed group (Gp. 2) which was exposed to EMF of 2 mT intensity and 50 Hz frequency, 4h/day, for 30 days; Pb acetate-treated groups

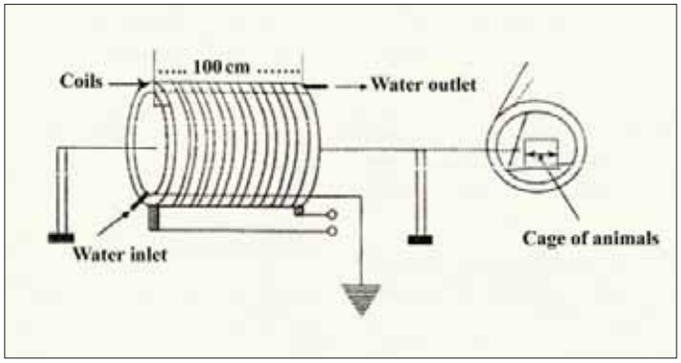

Figure 1. Magnetic field exposure facility

(Gps. 3 and 4), administered Pb acetate orally at doses of 1 and 5 mg/kg BW, respectively for 30 days [13]; EMF + Pb acetate groups (Gps. 5 and 6) administered Pb acetate and exposed to EMF as Pb acetate and EMF groups. The animal experiments have been approved by the Committee of Animal Experimental Ethics of the Faculty of Veterinary Medicine, 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 RBCs, hematocrit, hemoglobin, WBCs, neutrophils, eosinophils, lymphocytes, monocytes and platelets analysis, according to [14]. 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 kit 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 [15] for the evaluation of the cellular immunity. Materials used for assessment of phagocytic activity were heparinized tubes for blood collection, Hank's solution, phosphate buffer saline, Leishman's stain [16], 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.) according to [17].

Statistical analysis

The data were expressed as means ±standard errors (SE). Differences between group means were estimated using a one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test was done for multiple comparisons using the SPSS 12.0 for Windows. Results were considered statistically significant at $p<0.05$.

Results

Blood hematology

As shown in Table 1, EMF (2 mT intensity and 50 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 Pb acetate at doses of 1 and 5 mg/kg BW alone (Gps. 3 and 4) or in combination with EMF (Gps. 5 and 6) 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 leukocyte counts did not significantly differ in (Gps. 3, 5 and 6) in comparison with the values of control mice, but significantly ($p<0.01$) decreased in groups 4. However, eosinophil count showed insignificant change in all groups. EMF exposure significantly ($p<0.001$) increased the platelets count, but Pb administered mice (Gp. 4) decreased it ($p<0.05$). It showed insignificant change in mice of gps. 3, 5 and 6.

Blood chemistry

As illustrated in Table 2, EMF and lead acetate-treated mice (Gps. 2 and 4) showed significant ($p<0.01$) higher levels of serum enzymes (ALT, AST), creatinine and urea than that of control. In contrast, significant ($p<0.01$) hyperglycemia (Gp. 2) and hypoglycemia (Gp. 4) were recorded, when compared with control mice. Insignificant change in these parameters was observed in (Gp. 3). Compared with the control mice, exposure to EMF- Pb acetate combinations (Gps. 5 and 6) resulted in the same findings for ALT, AST, creatinine and urea, while serum glucose showed insignificant change.

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. The serum total proteins, albumin, and globulin fractions were significantly ($p<0.01$) decreased in mice given the highest dose of Pb acetate (5 mg/kg BW), but they showed no statistical change with the lowest one (1 mg/kg BW). Combined EMF- Pb acetate treatments produced insignificant change.

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

Table 1. Hematological parameters (Mean values ± SE) in different mice groups

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

Note: Values are expressed as means ±SE; n = 15 for each treatment group. ^{ABCDE} Within rows, means with different superscript letters differ significantly ($P < 0.05$)

Table 2. Serum biochemical changes (Mean values ± SE) in different mice groups

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

Note: Values are expressed as means±SE; n = 15 for each treatment group. ^{ABCDE} Within rows, means with different superscript letters differ significantly ($P < 0.05$)

Table 3. Proteinogram (Mean values ± SE) in different mice groups

Groups / Parameters	Gp. 1	Gp. 2	Gp. 3	Gp. 4	Gp. 5	Gp. 6
Total protein (g/dl)	6.10 ^{B}	6.90 ^{A}	6.32 ^{B}	4.12 ^{C}	5.99 ^{B}	5.90 ^{B}
	±1.42	±1.50	±1.22	±1.03	±1.05	±1.12
Albumin	3.10 ^{A}	3.04 ^{A}	3.00 ^{A}	1.95 ^{B}	2.60 ^{A}	2.60 ^{A}
(g/dl)	± 0.22	± 0.19	± 0.14	± 0.06	± 0.12	± 0.10
α1-Globulins (g/dl)	0.42 ^{A}	0.44 ^{A}	0.45 ^{A}	0.35 ^{B}	0.45 ^{A}	0.44 ^{A}
	± 0.19	± 0.12	± 0.11	± 0.19	± 0.15	± 0.07
α2-Globulins (g/dl)	0.61 ^{A}	0.62 ^{A}	0.61 ^{A}	0.34 ^{C}	0.60 ^{A}	0.55 ^{AB}
	±0.11	±0.13	±0.05	±0.11	±0.13	±0.09
β-Globulins (g/dl)	1.14 ^{A}	1.09 ^{A}	1.12 ^{A}	0.85 ^{B}	0.99 ^{AB}	1.06 ^{A}
	±0.50	±0.71	±0.80	±0.02	±0.06	±0.25
γ-Globulins (g/dl)	1.19 ^{B}	1.85 ^{A}	1.21 ^{B}	0.64 ^{C}	1.23 ^{B}	1.11 ^{B}
	±0.50	±0.65	±0.23	±0.45	±0.43	±0.36
Total globulins (g/dl)	3.00 ^{B}	3.80 ^{A}	3.40 ^{B}	2.47 ^{C}	3.29 ^{B}	3.30 ^{B}
	±1.00	±1.10	±1.03	±0.50	±1.14	±1.16

Note: Values are expressed as means±SE; n = 15 for each treatment group. ^{ABC} Within rows, means with different superscript letters differ significantly ($P < 0.05$)

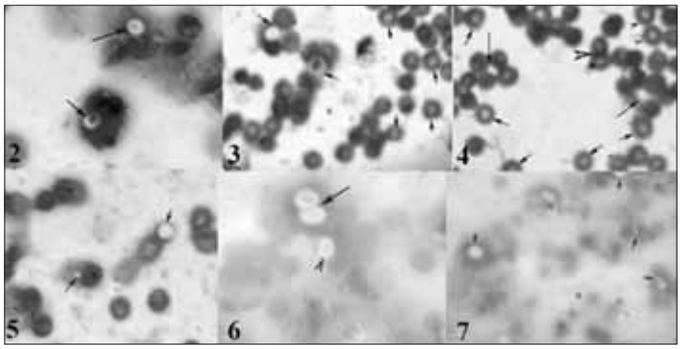

Figure 2. Phagocytosis of C. albicans by neutrophils of control mice (gp.1). One cell of C. albicans was engulfed by neutrophils (arrow), Leishman's stain, X100.
Figures 3-4. Phagocytosis of C. albicans by neutrophils of EMF exposed mice (gp.2). One and two C. albicans engulfed by neutrophils were represented by arrow (Fig. 2) and arrow-head (Fig. 3) respectively, Leishman's stain, X100.
Figures 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.
Figures 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.
Figures 7. Phagocytosis of C. albicans by neutrophils of mice administered lead acetate (gp.5) 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.

administration of Pb acetate exerted a significant ($p<0.01$) decrease (Gp. 4), in comparison with (Gp. 1). These tests were insignificantly changed in mice of groups 3 and 6, but increased significantly ($p<0.05$) in mice of (Gp. 5).

Pathological findings

The liver, kidney and spleen sections from a control animal showing normal parenchyma (Figs. 8, 9 and 10) respectively. The liver from exposed mice to EMF (Gp. 2) showed focal centrolobular necrosis of the hepatic cells surrounded by severe hydropic degeneration involving the majority of hepatic parenchyma (Fig. 11). The kidneys of EMF exposed mice showed congestion of renal blood vessels, contracted glomerular tufts of some glomeruli and focal leukocyte aggregation (Fig. 12). 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). Mice given 1mg/kg BW of Pb acetate (Gp. 3) showed mild lesions in comparison with Pb acetate group given the high dose. The liver sections from mice given 5mg/kg BW of Pb acetate (Gp. 4) showed congestion of blood vessels, portal leukocyte infiltration and hydropic degeneration (Figs. 14), 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. Kidneys revealed focal replacement of renal parenchyma by lymphocytes (Fig. 15). Spleen showed the same lesion of (Gp. 2). In addition, few hemosiderin granules were scattered in the white and red pulps. The spleen showed severe lymphoid depletion, rudimentary white pulps, and hemosiderosis in red and white pulps, beside thickened splenic trabeculae (Fig. 16). Mice exposed to a combination of lead and EMF (Gps. 5 and 6)

showed more severe lesions than previous groups. Liver (Gp. 6) showed portal and interstitial lymphocyte aggregations, hyperplastic Kuffer cells, hydropic degeneration, hyperchromatic nuclei and disorganized hepatic cords, (Fig. 17). Periglomerular lymphocytic infiltrations with individual coagulative necrosis were noticed in the renal tubules (Fig. 18).

Discussion

For several decades, researchers have been concerning about the bioeffects of low-frequency, low-intensity EMFs, which are comparable to both residential and occupational exposure levels in many work fields [18]. The choice of 2 mT intensity/50 Hz magnetic fields which they are below ICNIRP standard [19] and since they are to a certain degree realistic in terms of human and animal exposure.

Our data demonstrated that 30 days (4h/day) of MF exposure was associated with an increase in the count of RBCs, platelets, Hb content, Ht values and leukocyte count with neutrophilia, lymphocytosis and monocytosis, compared to control. Previous study reported that the increase in Hb and RBCs may be explained by the hypoxia-like status induced by static MF resulting probably from the oxygen-binding impairment of Hb [6]. By contrast, hematocrit remained unchanged [20]. The differences of hematocrit values may be related to duration (number of h/day) of EMF- exposure. The same results were observed in rats after exposure to SMF of 128 mT for 1h/day during 30 consecutive days [21]. Contrarily, mice exposed to magnetic wave field of 60 Hz with a 0.11-mT intensity showed leukopenia and hemoglobin decrease [22]. They added that the bioeffects described could be correlated with spleen hyperfunction, which could have been produced by chronic exposure to EMF. Besides, rats exposed to low EMF reveal a decrease in platelets count [23]. Similarly, no EMF effects on the hematologic variables of rats exposed to 20 kHz MF at 6.25 microT, 8h/day, for 12 or 18 months [24].This discrepancy may be explained by the intensity and the duration of the exposure, as well as, type of experimental animal.

Lead is one of the most common toxic metals. Pb 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 Pb acetate poisoning is in part the result of various inhibitory effects of lead on heme biosynthesis [25]. Besides, excessive lead exposure inhibits heme synthesis, leading to anemia and erythrocytes degeneration or destruction [26]. Lead may inhibit 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 [27]. Our findings were in coincidence with other reporters [9,10,28]. Dose dependent changes in total and differential WBCs, as well as platelets counts were detected in Pb acetate alone or with EMF-treatment groups. Leucopenia, neutropenia, lymphopenia, monocytopenia and thrombocytopenia were noticed in 5 mg/kg Pb acetate treated animals. On the other hand, injection of Pb acetate for 4 days was shown to stimulate a striking leukocytosis in female mice with an increase of monocytes and neutrophils [29]. In addition, insignificant change in hematological and enzymatic parameters were reported with Pb acetate given for 30 and 60 days [30]. Leukocytosis was previously reported in mice given Pb acetate orally at doses 1 and 10 mg/kg BW for one month [13].

Present data showed that exposure to EMF increased serum glucose level and transaminases (AST and ALT) activities. The increase in the glucose level agrees well with previous findings with EMF with different strengths [31]. Moreover, exposure to EMF had increased blood glucose and decreased insulin release, leading to a diabetic-like state in pregnant rats [6]. Formerly, hyperglycemia was explained by structural and functional changes in pancreas, in response to EMF stress [20]. Serum transaminases have been widely utilized in mammalian toxicology as biomarkers of specific organ dysfunction [6]. Magnetic field induced structural changes in hepatocytes, primarily in mitochondria [32]. In addition, a significant increase in ALT activity indicates citotoxic effect of non-ionizing radiation on hepatocytes inducing apoptosis and necrosis [33]. These results were in accordance with previous findings [7,21]. EMF- exposure increased the serum creatinine and urea concentrations. These results may be due to the renal dysfunction associated with congestion of renal blood vessels, contracted glomeral tufts of some glomeruli and focal leukocyte aggregation by pathologic examination. Similarly, mice exposed to MFs (5 T) for 48 h increased significantly blood urea nitrogen, whereas creatinine levels remained unchanged [34]. Contrarily, subchronic exposure of rats to MF (128mT, 1h/day for 30 days) had no effect on serum creatinine and urea levels [21]. This discrepancy could be related to the difference of the intensity of the SMF and the exposure time (h/day) and duration.

Lead acetate administration (5, 10 mg lead acetate/kg, daily for 30 days) resulted in a dose- dependent increase in serum ALT, AST, creatinine, and urea; 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 [35]. Increases in serum enzyme activities are attributed to their release from the cells and this may be related to the tissue injury induced by lead. Moreover, an elevation of transaminases at 2-week intervals in male albino rats receiving Pb (500 mg/kg diet 3 times per week) for a period of 6 weeks was encountered [36]. Similar results were observed in albino rats orally administered 200 or 400 ppm Pb acetate [8], 1000 or 2000 ppm Pb acetate for 60 days [28] or in rabbits orally given 80 or 160 ppm Pb for 15 days [9]. The observed elevation in creatinine concentrations indicates impairment in kidney function [37]. Enhanced protein catabolism together with accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpret the elevated levels of urea [28]. The latter authors added that the elevated serum urea levels may be due to the previously reported destruction of RBCs. Similarly, the increased concentrations of urea and creatinine in serum indicated a nephropathy possibly induced by lead [9,38]. Therefore, creatinine and urea could be considered as suitable prognostic indicators of renal dysfunction in case of lead exposure [39].

Table 4. Percentage and index of phagocytosis (Mean values ± SE) in different mice groups

Groups / Parameters	Gp. 1	Gp. 2	Gp. 3	Gp. 4	Gp. 5	Gp. 6
Phagocytic %	43.10 ^{C}	56.90 ^{A}	43.31 ^{C}	36.20 ^{D}	50.20 ^{B}	43.90 ^{C}
	±5.30	±4.11	±5.60	±3.82	±6.12	±4.72
Phagocytic index	0.71 ^{B}	0.95 ^{A}	0.70 ^{B}	0.65 ^{C}	0.85 ^{A}	0.75 ^{B}
	± 0.22	± 0.19	± 0.14	± 0.17	± 0.12	± 0.13

Note: Values are expressed as means±SE; n = 15 for each treatment group. ^{ABCDE} Within rows, means with different superscript letters differ significantly ($P < 0.05$)

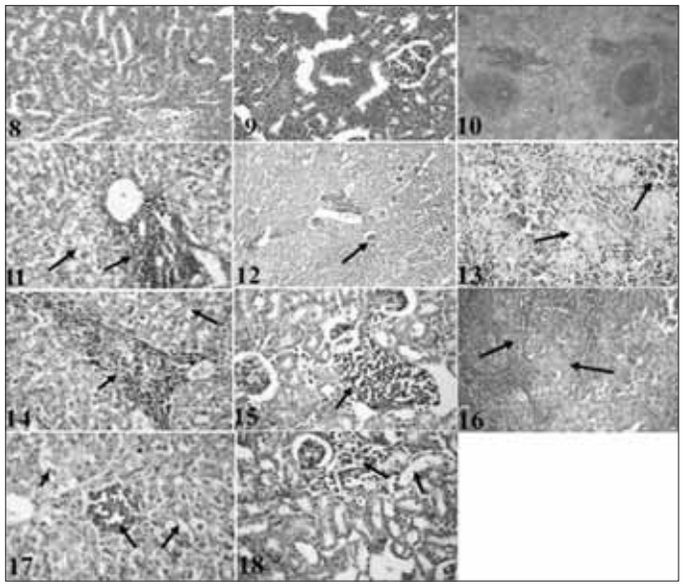

Figure 8-10. Liver, kidney and spleen sections from control mice (Gp. 1) showing normal structure respectively, H&E., x1200.
Figure 11. The liver section from mice of (Gp. 2) showed focal centrolobular necrosis of the hepatic cells surrounded by severe hydropic degeneration involving the majority of hepatic parenchyma and round cells infiltration, H&E., x1200.
Figure 12. The kidney section (Gp. 2) suffered from congestion of renal blood vessels, contracted glomerular tufts of some glomeruli and focal leukocyte aggregation, H&E., x300.
Figure 13. Spleen section (Gp. 2) showed lymphoid depletion in splenic white pulps and hemosiderosis in red and white pulps, H&E., x1200.
Figure 14. Liver section from mice given 5mg/kg BW (gp. 4) of lead acetate showed leukocyte infiltration in the portal area and hydropic degeneration, H&E., x1200.
Figure 15. Kidney section from mice given 10mg/kg BW of lead acetate (Gp. 5) revealed focal replacement of renal parenchyma by lymphocytes, H&E., x1200.
Figure 16. Spleen section from Gp. (5) showed severe lymphoid depletion, hemosiderosis in red and white pulps beside thickened splenic trabeculae, H&E., x1200.
Figure 17. Liver section of mice exposed to a combination of lead and EMF (Gp. 6) showed focal replacement of hepatic parenchyma by lymphocytes, hyperplastic Kuffer cells, hydropic degeneration, hyperchromatic nuclei and disorganized hepatic cords, H&E., x1200.
Figure 18. Kidney section from mice of Gp. (6) showed periglomerular lymphocytic infiltrations with individual coagulative necrosis, H&E., x1200.

Concerning the effect of EMF on immune parameters, present data showed that EMF exposure increased serum total protein levels, gamma, and total globulins, phagocytosis % and phagocytic index in mice. This hyperproteinemia may be attributed to the change in protein metabolism of stressed mice or the increase in the globulin component [20,40]. On the contrary, a significant decrease in the levels of total protein, β- and γ-globulins in steelworkers exposed to EMF (1.3mT intensity and 50 Hz frequency, mean 6.8h /day) for 5days was reported [7]. Significant decrease in blood levels of total protein, albumin and β-globulin were observed in rats exposed to EMF, 24 h/ day for 8 weeks [41]. This discrepancy could be attributed 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 [42]. There is no generally accepted mechanism to explain how extremely low frequency fields might initiate bioeffects, if any, on immune system [43]. In other way, some reports indicate that acute exposure to a 50 Hz magnetic field (10 microT, for 24 h) has no effect on hematological or immune parameters in healthy male volunteers [44]. The present results were supported by the pathologic lesions in the liver (Fig. 11) and kidney (Fig. 12). Similarly, experimental exposition of mice by mobile telephones showed a slightly increased number of micronuclei and discrete perivenular fatty changes in liver [45]. Highly cytoplasmic vacuolation of liver and swelling of epithelial cells of kidney tubules with subsequently cell necrosis were shown 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 [46].

Interestingly, decreased in serum proteins, albumin, and globulins may be attributed to hepatotoxicity or renal toxicity resulting from the highest dose of lead acetate. Contrarily, low dose of Pb acetate (1 mg/kg) produced insignificant change. Experiments conducted in male rats exposed to 200 ppm dietary Pb acetate for 10 weeks showed normal serum total proteins and albumin [47]. However, blood lead > or = 25 microg/dL caused a significant decrease in immunoglobulins (IgG, IgM, IgA) with dysfunctions in different organ systems of the body, such as the immune system [48].

Observations of suppression of phagocytic activity neutrophil in relationship to Pb acetate exposure were in parallel with others [49]. The former authors mentioned that occupational exposure to lead interfered with humoral and especially cell-mediated immunity even at frequently occurring (<50 microg/dl) blood lead levels and caused a primary impairment of the chemotactic and phagocytic activities of neutrophil leucocytes. Similarly, a significant decrease in phagocytic activity and the number of peripheral leukocytes in

Pb (35 mg/kg)- administered mice [50] was shown. Phagocytic and lytic functions of the polymorphonuclear cells collected from sublethal lead-injected toads and incubated with suspensions of Candida pseudotropicalis [51] were affected negatively.

The combined effects of EMF and of Pb acetate were found to potentiate the toxic effects. Similarly, the combined effects of EMF (300μT to 0.3T) and of chlorpyrifos at various concentrations (0.1,1, 50 and 100mg/kg) were found to potentiate the toxic properties of the pesticide [42].

The most common and constant findings was a portal leukocyte 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. These findings were dose-dependent. Similar histopathological lesions have been reported in experimental lead toxicity with different species [9,52-54].

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 for their assistance in the histopathology, and Dr.Magda Hanafy, Prof. of physics, Faculty of Science, Zagazig University, Egypt for supplying electromagnetic field generator.

No author of this paper has a conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included in this manuscript.

References

1. Gobba F, Bargellini A, Scaringi M, Bravo G, Borella P. Extremely low frequency-magnetic fields (ELF-EMF) occupational exposure and natural killer activity in peripheral blood lymph. Sci Total Environ 2009;407:1218-23.
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;8:127-40.
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;76:589-95.
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;16:149-56.
5. Upfal M. Liver enzymes among microelectronics equipment maintenance technicians. J Occup Med 1992; 34:384-90.
6. 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;23:182-7.
7. Boguslaw K, Andrzej S, Rozalia G, Danuta P. Effect of electromagnetic field on serum biochemical parameters in steelworkers. J Occup Health 1999;41:177-80.
8. 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;111:151-65.
9. 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;2:11-8.
10. 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;137:193-9.
11. 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;40:412-20.
12. Kamedula M, Kamedula T. Combined biological effect of electromagnetic fields and chemical substances (toxic). Med Pr 1996;47:285-92.
13. Schlick E, Friedberg KD. Bone marrow cells of mice under the influence of low lead doses. Arch. Toxicol 1982;49:227-36
14. Feldman BF, Zinkl JG, Jain NC. Schalm's Veterinary Hematology. 5th Ed., Lippincott Williams and Wilkins. A Wolters Company. Philadelphia, Baltimore, New York, London, 2000:1219-24.
15. Antley PP, Hazen KC. Role of yeast cell growth temperature on Candida albicans virulence in mice. Infect Immun 1988;56:2884-90.
16. Cruickshank R, Duguid JP, Marinion BP, Swain RH. Textbook of Medical Microbiology, 12th Ed. E.S. Living Stone Limited Edinburgh and London, 1975.
17. Bancroft GD, Stevens A. Theory and Practice of Histopathological Technique. 4th Ed., Churchill Livingstone Edinburgh, London, Melbourne and New York, 1996.
18. Arafa HMM, Abd-Allaha ARA, El-Mahdya MA, Ramadanb LA, Hamada FMA. Immunomodulatory effects of L-carnitine and q10 in mouse spleen exposed to low-frequency high-intensity magnetic field. Toxicol 2003;187:171-81.
19. ICNIRP, International Commission on Non-Ionizing Radiation Protection. Guidelines for limiting exposure to time-varying electric, magnetic and electromagnetic fields (up to 300GHz). Health physics 1998; 74:494-522.
20. Amara S, Abdelmelek H, Ben Salem M, Abidi R, Sakly M. Effects of static magnetic field exposure on hematological and biochemical parameters in rats. Braz Arch Biol Technol 2006;49: 889-95.
21. 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; 57:616-22.
22. Cabrales LB, Ciria HC, Bruzón RP, Quevedo MS, Céspedes MC, Salas MF. ELF magnetic field effects on some hematological and biochemical parameters of peripheral blood in mice. Electromagnetic Biol Med 2001;20:185-91.
23. Ciejka E, Goraca A, Michalska M, Kostka B. The effect of low magnetic field on select parameters of blood coagulation. Pol Merkur Lekarski 2005;19:148-51.
24. Kim SH, Lee HJ, Choi SY, Gimm YM, Pack JK, Choi HD, Lee YS. Toxicity bioassay in Sprague-Dawley rats exposed to 20 kHz triangular magnetic field for 90 days. Bioelectromagnetics 2006;27:105-11.
25. 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;40:23-7.
26. Patocka J, Cerný K. Inorganic lead toxicology. Acta Medica 2003;46:65-72.

27. Gurer-Orhan H, Sabir HU, Ozgüneş H. Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. Toxicol 2004;195:147-54.
28. 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.
29. Hogan GR, Adams DP. Lead-induced leukocytosis in female mice. Arch Toxicol 2004;41:295-300.
30. Helena C, Alejandro S, Renata C, Lilian N, Cinthia M, Sibelle C, Paulo H, Aleksander R, Emillien P, Ciro A. Subchronic toxic effects of tributyltin (TBT) and inorganic lead (PbII) in rats. Environ Toxicol Pharmacol 2005;19:113-20.
31. Armario A, Marti J, Gil M. The serum glucose response to acute stress is sensitive to the intensity of the stressor and to habituation. Psychoneuroendocrinol 1990;15:341-7.
32. 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;30:119-23.
33. Djindjić B, Sokolović D, Radić S, Najman S, Krstić D, Marković cV. 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., 2003:1063-6.
34. Tsuji Y, Nakagawa M, Suzuki Y. Five-tesla static magnetic fields suppress food and water consumption and weight gain in mice. Ind health 1996;34:347-57.
35. Herman DS, Geraldine M, Venkatesh T. Influence of minerals on lead-induced alterations in liver function in rats exposed to long-term lead exposure. J Hazard Mater 2009;166:1410-4.
36. 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. Toxicol 2005;206:1-15.
37. 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;109:457-63.
38. Cledes J, Allain P. Chronic lead nephropathy. Epidemiology and diagnosis. Presse Medi 1992;21:759-62.
39. Weaver VM, Jaar BG, Schwartz BS, Todd AC, Ahn KD, Lee SS, Wen J, Parsons PJ, Lee BK. Associations among lead dose biomarkers, uric acid, and renal function in Korean lead workers. Environ Health Perspect 2005;113:36-42.
40. Kumosani TA, Qari MH. The effect of magnetic field on the biochemical parameters of mice blood. Pak J Med Sci 2003;19:36-40.
41. 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.
42. Chernykh AM. Hygienic evaluation of the combined effect of electromagnetic fields and pesticides in experimental conditions. Gig Sanit 2003;2:56-8.
43. 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;222:374-8.
44. 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;8:506-16.
45. 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;11:34-7.
46. Zare S, Alivandi S, Ebadi AG. Histological studies of the low frequency electromagnetic fields effect on liver, testes and kidney in guinea pigs. World Appl Sci J 2007;2:509-11.
47. 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;98:463-81.
48. Ayatollahi M. Study of the impact of blood lead level on humoral immunity in humans. Toxicol Ind Health 2002;18:39-44.
49. 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;79:491-8.
50. Kim YO, Pyo MY, Kim JH. Influence of melatonin on immunotoxicity of lead. Int J Immunopharmacol 2000;22:821-32.
51. 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;136:225-33.
52. Rader JL, Celesk EM, Peeler JR, Mahaffey KR. Retention of lead acetate in wealing and adult rats. Toxicol Appl Pharmacol 1983; 67:100-9.
53. Selmaoui B, Bogdan A, Auzeby A, Lambrozo J, Touitou Y. Acute exposure to 50 Hz magnetic field does not affect hematologic or immunologic functions in healthy young men: a circadian study. Bioelectromagnetics 1996;17:364-72
54. Vyskocil A, Semecky V, Fiala Z, Cizkova M, Viau C. Renal alterations in female rats following subchronic lead exposure. J Appl Toxicol 1995;15:257-62.