International Journal of

Zoological Research

ISSN 1811-9778

Academic Journals Inc.

www.academicjournals.com

International Journal of Zoological Research 7 (6): 393-400, 2011
ISSN 1811-9778 / DOI: 10.3923/ijzr.2011.393.400

Chronic Exposure to Extremely Low Frequency Electromagnetic Field Induces Mild Renal Damages in Rats

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ABSTRACT

The kidney is a major potential route for the absorption of hazardous materials encountered in the environment. The aim of this study was to evaluate the influence of ELFEMF on function and structure of kidney in rats. Experimental adult male Wistar rats were exposed to a 50 Hz ELFEMF, 1 mT (emitted from solenoid) for 24 h daily during 135 days. The sham rats were subjected to sham exposure and the control rats were kept in animal room. In final, blood samples collected for the determination of the creatinine, urea nitrogen, albumin, globulin and total protein concentration in the plasma. The kidney was examined using light microscopy. Results showed that in EMF exposed group plasma concentration of creatinine and urea nitrogen was increased ($p<0.001$) which was accompanied by marked vascular congestion in the renal cortex and reduction in red blood cell count in glomerular capillaries but there were no changes in total protein, albumin and globulin levels. In conclusion, long term exposure to ELFEMF impacts renal function by influencing the perfusion of kidney.

Key words: Long term exposure, electromagnetic field, kidney, creatinine, urea nitrogen, vascular congestion

INTRODUCTION

Biological effects of exposure to Extremely Low Frequency Electromagnetic Fields (ELFEMFs) have been reported by several authors (Lacy-Hulbert *et al.*, 1998; Lazetic *et al.*, 1997; Blank and Goodman, 2002; Mostafa *et al.*, 2006; Al-Akhras *et al.*, 2006; Roushangar and Rad, 2007; Khaki *et al.*, 2008; Kilicalp *et al.*, 2009; Zamanian *et al.*, 2010; Zare *et al.*, 2007; Gholampour *et al.*, 2011). According to the report of Ongel *et al.* (2009), electromagnetic sources can be classified into natural electromagnetic sources (sun, some distant stars, atmospheric discharges like thunder, or human body) and unnatural or human made sources (cables that carry electrical currents, Television (TV) and computers, electrical home gadgets, radio and TV base stations, cell phones, mobile phone base stations and phone equipment). Electromagnetic field reduces the speed of destroying free radical compounds thus allowing them to affect longer periods of time (Ongel *et al.*, 2009). Factors, such as frequency and amplitude of EMF and the exposure time are crucial for the determination of the possible negative effects of EMF, including a low protein level in serum, disturbances in protein synthesis at chromosal levels and damage of kidney and liver tissues (Oktem *et al.*, 2005). The kidney is a major potential route for the absorption of hazardous materials encountered in the environment (Irmak *et al.*, 2002). Disturbance in kidney functions is reported to enhance filteration of proteins leading to reduction in plasma protein levels (Eraslan *et al.*, 2004).

The status of intrarenal circulation determines in part renal excretion, affects body fluid homeostasis and has a role in long term control of arterial blood pressure. The vascular resistance in the renal cortex and medulla is determined by interaction of a vast array of vasoactive hormones and paracrine factors. Renal medulla constitutes a relatively small fraction of the whole kidney mass and receives less than 10% of the blood delivered to the kidney via the renal artery (Sadowski and Badzynska, 2006).

Since there are no reports in the published works concerning the effect of chronic exposure to ELFEMF on function and structure of kidney, the current study was undertaken to further evaluate the influence of long-term exposure to ELFEMF on functional-morphological features of kidney in rat.

MATERIALS AND METHODS

This study was conducted in Shira University laboratory in autumn, 2009. EMF exposure unit (at a frequency of 50 Hz and 1 mT) was an open wooden box (100×100×35 cm). The distribution of EMF flux density was measured using a gauss meter.

The research material consisted of 45 male rats of the Wistar strain (234.4±12.6 g). During the experiment, rats were kept in either magnetic field chamber or a similar chamber without a magnetic field, or in ordinary cages in the same animal room under controlled temperature of 21-22°C for 135 days. The lighting was turned off or on under a 12 h cycle. The rats were fed with standard granulated feed and had free access to water. The animals were randomly divided into three equal groups of 15 rats each; the groups had no significant differences in body weight. The local ethics committee approved the study.

At the end of experiment animals were weighed, anaesthetized with ethyl ether and blood (average 5 mL) was obtained from the right ventricle of heart and then decanted and centrifuged at 10000 rpm. The plasma was used to determine creatinine, urea nitrogen, total protein, albumin and globulin concentrations. After opening the abdominal cavity, left kidney was taken which after appropriate fixation and preparation was used for structural examination in light microscope. Plasma concentrations of creatinine, urea nitrogen, total protein, albumin and globulin were measured using the automatic analyzer (Technicon, RA-1000, America) and related kits.

Histopathological examinations: One half of each kidney was fixed in the buffered 10% formaldehyde (Merck, USA), embedded in paraffin and 5 μm sections were obtained by microtome (Erma, Japan). Sections were subjected to routine staining with hematoxylin and eosin (H and E).

In a blinded fashion, each section was examined in at least 10 randomly selected non-overlapping fields under light microscope. The renal histopathology were quantified for the degree of reduced number of RBCs in glomerular capillaries and vascular congestion. The level of each pathological manifestation was graded according to the changes involving: none with 0, less than 20% with 1, 20-40% with 2, 40-60% with 3, 60-80% with 4, greater than 80% with 5. The sum of all numerical scores in each group was taken as the total histopathological score.

Statistical analysis: The results of plasma creatinine, urea nitrogen, total protein, albumin and globulin concentrations, as well histopathological scores in different groups, are presented as mean Values±SEM and were statistically analyzed with the ANOVA test, followed by the post hoc Donkan test. All data analyses were performed using SPSS ver. 17 software and significance was taken at $p<0.05$.

RESULTS AND DISCUSSION

This study investigated the impact of exposure to ELF electromagnetic fields over 135 days on functional-morphological features of kidney in rat. All measurements were carried out in rats exposed for 135 days to EMF that is about one-fifth of their life span. The field strength level used for this study (50 Hz and 1 mT) cover the range of values detected close to power lines and in some occupational settings (Mader *et al.*, 1990; Kavet *et al.*, 1992; Sobel *et al.*, 1995; Zecca *et al.*, 1998). Blood urea nitrogen and creatinine play important role in the determination of renal functions (Buemi *et al.*, 2001). The problem encountered in elimination of these compounds, produced endogenously within the body, results in their increased levels in the blood (Eraslan *et al.*, 2004).

We found increase in plasma creatinine and BUN level (Table 1) as well vascular congestion in cortex (Fig. 1, Table 2) and reduction of Red Blood Cell (RBC) count in glomerular capillaries

Table 1: Mean plasma concentration of creatinine, urea nitrogen, total protein, albumin and globulin of EMF exposed male rats

Groups	Creatinine (mg mL^{-1})	Urea nitrogen (mg dL^{-1})	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})
Control	0.69±0.01^{a}	21.47±0.43^{a}	6.26±0.26^{a}	3.91±0.19^{a}	2.51±0.16^{a}
Sham operated	0.68±0.0^{a}	22.00±0.48^{a}	5.54±0.25^{a}	3.76±0.16^{a}	2.64±0.12^{a}
EMF exposed	0.78±0.03^{b}	25.33±0.83^{b}	5.71±0.29^{a}	3.96±0.18^{a}	2.74±0.23^{a}

Values are as Mean±SEM. Means in a column with no common superscript differ ($p<0.05$)

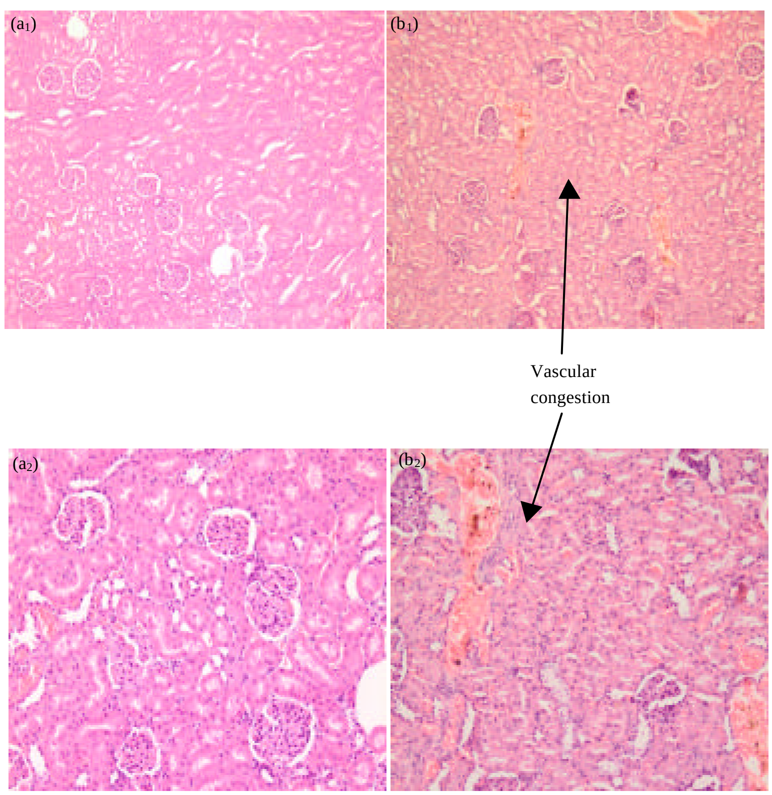

Fig. 1(a-b): Representative light microphotographs of the renal cortex obtained from the control group (a_1, a_2 and a_3), EMF exposed group (b_1, b_2 and b_3); (H and E staining; a_1 and b_1, x100; a_2, b_2, a_3 and b_3, x200)

Table 2: Histopathological score of EMF exposed male rats

Groups	Histopathological score
Control	0^a
Sham operated	0^a
EMF exposed	5^b

Values are Mean±SEM. Means in a column with no common superscript differ ($p<0.05$)

(Table 2) after exposure to ELFEMF. These results indicated that exposure to ELFEMF caused renal damage. The severe reductions of RBC in glomerular capillaries of EMF exposed group can be taken as an indication of sustained renal hypoperfusion during the exposure time. The renal hypoperfusion probably resulted from an imbalance between the production of vasodilators and vasoconstrictors. It is well established that free radicals function in the induction of the cellular responses upon EMF exposure (Lupke *et al*., 2004; Zmyslony *et al*., 2004; Bediz *et al*., 2006; Simko *et al*., 2006). Free radicals target structural molecules such as lipids, proteins and nucleic acids (Stadtman and Levine, 2003; Cumaoglu *et al*., 2007).

It is reported that, electromagnetic field reduces the speed of destroying free radical compounds thus allowing them to affect longer periods of time (Ongel *et al*., 2009). Reactive Oxygen Species (ROS) have been implicated in tissue injury. One of the main ROS that have to be considered is superoxide anion (O_2^-) which is predominantly generated by the mitochondria. Combination of superoxide anion (O_2^-) and NO generates peroxynitrite ($ONOO^-$) which is a cytotoxic species implicated in both the origin and the progression of protein oxidation (Stadtman and Levine, 2003). Nitric Oxide (NO) is a small lipophilic signalling molecule, produced by Nitric Oxide Synthase (NOS) which has been revealed to play important roles in renal function both under normal and pathological conditions. There are three isoforms of NOS, including neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS) which all of them have been identified in the kidney. The nNOS and eNOS act as constitutive Ca^{2+}-dependent NOS. But, iNOS is characterized by Ca^{2+}-independency and high basal activity (Gabbai, 2001) and is mostly expressed in the kidney after induction by appropriate stimuli, such as ELFEMFs (Patruno *et al*., 2010; Hao *et al*., 2010; Yoshikawa *et al*., 2000; Yang *et al*., 2010). It has been suggested that high NO levels secondary to increased iNOS activity may inhibit eNOS and through this mechanism lead to more renal vasoconstriction and reductions in GFR (Gabbai, 2001). Thus, decreased renal blood flow was likely to be responsible for the reduced GFR following the exposure to ELFEMF in EMF exposed group.

We also, observed vascular congestion in the cortex of the kidney. Congestion of the renal microcirculation contributes to deficits in renal perfusion (Sutton *et al*., 2002). Endothelial-leukocyte interactions mediated through complementary adhesion molecules on endothelial cells and leukocytes play a key role in the local accumulation of leukocytes (Molitoris and Sutton, 2004). Studying of Jonai *et al*. (1996) on immune response to 50 Hz ELFEMF exposure in vitro by assaying cytokines produced by human Peripheral Blood Mononuclear Cells (PBMCs) showed that some cytokine levels changed in response to exposure to EMF. Specifically, a significant decrease in tumor necrosis factor α (TNF-α) and a significant increase in interleukin 1β (IL-1β) levels were observed. It is well established that changes in cytokine production induce specific cellular and molecular responses in target cells. In the case of leukocytes, some cytokines (IL-1β, TNF-α) can stimulate the up-regulation of E-selectin and L-selectin ligands which mediate subsequent leukocyte rolling (Spertini *et al*., 1991). In addition, TNF-α, IL-1β and interferon γ (IFN-γ) enhance the expression of ICAM-1 and VCAM-1 in endothelial cells which mediate leukocyte adhesion to the endothelium (Haraldsen *et al*., 1996; Waldman and Knight, 1996; Silverman *et al*., 2001). Thus, exposure to ELFEMF leads to vascular congestion by changing some cytokine levels and therefore, to reduction in renal perfusion.

It is reported that, disturbance in kidney function enhances filteration of proteins leading to reduction in plasma protein levels (Levin, 2000) but in our study EMF exposed rats did not show significant changes in total protein, albumin and globulin levels.

All the changes observed in the present study were occurred in the renal cortex. On the other hand, the studies involving the effects of cell phones on kidneys show that cortical renal tubular epithelium is affected more than the medullary tubules (Kang *et al*., 1997; Pyrpasopoulou *et al*., 2004; Oktem *et al*., 2005; Ozguner *et al*., 2005). Thus, it is concluded that exposure to ELFEMF, probably affects renal cortex more than renal medulla.

In conclusion, ELFEMF of 50 Hz frequency and 1 mT applied on adult rats, for 24 h daily during 135 days induced changes in the blood level of creatinine and nitrogen urea as well vascular congestion in renal cortex, without changing the blood levels of total protein, albumin and globulin.

ACKNOWLEDGMENTS

We wish to acknowledge the Research Council of Shiraz university, Shiraz, Iran, for the financial support of this study (grant No. 88-GR-SCST-117). We also thank Dr. Aminollah Bahaoddini, assistant professor of Shiraz University.

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