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Effect of 50-Hz-Powerline-Exposed Water on Hematological Parameters in Rats

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ABSTRACT

Double distilled water samples were exposed for 24 hr and 48 hr to 50 Hz-powerline electromagnetic field (EMF) strength of 51.2 μT (36.2 RMS). This EMF exposed water was made available to experimental adult Charles-Foster male rats for drinking ad libitum for 90 days. Blood samples were collected on day 0 (base line), 30, 60, and 90. Hematological parameters (e.g., total leukocyte counts [TLC], differential leukocyte counts [DLC], and platelet counts) were analyzed statistically. There was significant increase in TLC platelet counts on day 30, whereas significant reduction was observed on day 60. On day 90, significant increase in TLC and significant decrease in platelet counts was observed. For DLC, neutrophils exhibited nonsignificant alteration on day 90, whereas, although lymphocytes and monocytes showed significant increase throughout the experiment though on day 90, there was a slight tendency to decline in monocytes. The present study clearly demonstrates a strong influence of EMF exposed water on day 30 (leukocytosis and thrombocytosis) and day 60 (leukopenia and thrombopenia) with differential results on day 90. This study provides further evidence for EMF bioeffects connected to EMF-exposed water.

Key Words: Magnetic fields; Powerline; Thrombocytopenia; Leucocytosis.

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INTRODUCTION

Although a number of experimental and epidemiological studies (Auvinen et al., 2000; Electromagnetic fields (300 Hz to 300 GHz) and Environmental Health Criteria 137, 1993; Interim guidelines on limits of exposure to 50/60 Hz electric and magnetic fields, 1990; Tenforde, 1996) have been conducted to evaluate the potential biological influence of electromagnetic field (EMF) exposure, and many known physical mechanisms for direct EMF, exposure have been proposed (ion cyclotron resonance, parametric resonance, radical pair mechanisms), still none of the proposed mechanisms is widely accepted (Nair and Morgan, 1990; Rai; Ryan et al., 1996; Smith and Best, 1989). Lacking an essentially complete definition of the EMF exposure/environment for both control and experimental groups, no single unambiguous bioeffect mechanism for EMF exposure has been put forward so far.

The bioeffect of direct exposure to powerline fields (i.e., the effect resulting from exposure of a biosystem itself) has been studied and a few attempts have been made to study indirect influences on biosystems through EMF-exposed water. Water, being a dielectric, was not thought to sustain EMF influences for long periods. Recently, it has been speculated that EMF may induce changes in the structure of water, which appears to produce significant biological effects (Fesenko and Glustein, 1995; Rai et al., 1994a,b; Singh et al., 1994). The structure of water—ice polymers (Kamb, 1968; Rai et al., 1995) and water clusters (Del Guidice et al., 1988; Fesenko and Glustein, 1995; Smith, 1994; Watterson, 1988; Wiggins, 1990)—under various EMF exposures as detected by different instrumental detection procedures (e.g., x-ray diffraction (Rai et al., 1995) and electrophotography (Singh et al., 1995)), has already been reported.

Bioeffects of indirect EMF exposure through EMF-treated water on fungi, bacteria, and plant systems are well-documented (Cerbonell et al., 2000; Dilova et al., 1987; Jerman et al., 1996; Rai et al., 1994a,b; Singh et al., 1995) but these effects have not been studied thoroughly in animal systems except for a few reports (Berden et al., 1997; Garg et al., 1995; Pandey et al., 1996). So, a need exists to establish the nonthermal bioeffect mechanism(s) of powerline exposure in biological systems. Therefore, the present study has been planned to study the mechanism of indirect exposure of 50 Hz powerline EMF using EMF-treated water on hematological parameters in adult rats.

MATERIALS AND METHODS

Animals

In the present study, laboratory inbred male rats (approximately 10 weeks old) of the Charles-Foster strain were used. All experimental rats weighing 180 ± 10 gm were housed under standard laboratory conditions (25 ± 2°C and 60% relative humidity) in a natural dark cycle. Two animals were housed in each polyvinyl animal cage measuring 30 × 23 × 23 cm wherein rice husk was used as the bedding material. The animals were fed pelleted animal food and water (control and treated) ad libitum. The experimental rats (number = 30) were randomly selected and divided into three

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groups; Group A (Control, $n = 10$), Group B (24-hr exposed, $n = 10$), and Group C (48-hr exposed, $n = 10$).

Powerline Exposure of Water

A powerline step down transformer of 2500 KVA was used as the source for exposure of water. The total height of the transformer was 3.65 m. Double distilled water ($0.32 \times 10^{-4}\ \Omega^{-1}\ \text{cm}^{-1}$) samples in cotton-plugged 250 mL conical flasks were placed undisturbed on a 12.5 cm projected portion of the body called ''Rib,'' surrounded with three layers of fins, at a height of 1.4 m for 24 and 48 hr when the treatment was terminated.

The sine wave and magnetic flux strength at the site of exposure was uniformly 36.2 (RMS) and 51.2 μT. It was measured by coil and a voltmeter and calibrated in a solenoid measuring 10 cm in diameter $\times$ 15 cm in length supplied with a 50 Hz AC signal. The time of starting the exposure to water was fixed around 9 A.M. everyday. All metallic contacts to the exposed water were avoided. Unexposed double-distilled water from the same source was used as control (geomagnetic field = 30 μT) under the same laboratory conditions.

Animal Treatment

The powerline-exposed water samples were transferred to feeding bottles fitted with glass tubes for free access to experimental rats. Water was replaced every day with fresh EMF-exposed water samples at approximately 9:30–10 A.M. The control rats were treated similarly with double-distilled water. The experiment continued for 90 days. During the treatment, the leftover water in each group was observed every day to determine the amount of water used to rule out any dehydration of animals from reduction in consumption of water.

Hematological Procedures

Blood (1 mL) was obtained by retro-orbital puncture by a glass capillary under light ether anesthesia and collected in 2% ethylene diamine tetrachloracetic acid (EDTA) via (5 mL) on days 0 (baseline), 30, 60, and 90. For preparing EDTA vials, 2% solution of EDTA was prepared in distilled water, 0.5 mL of the solution was poured in every vial, and then it was air dried. The blood was collected on each experimental day at the same time (i.e., around 10 A.M.). These blood samples were analyzed using a cell counter (Thoma Chamber) for counting the total leucocytes as a routine procedure in the laboratory. DLC were carried out after May-Grunwald-Giemsa staining of the slides. Platelet counts were done in a Neubauer Chamber.

Statistical Analysis

All data represent mean ± standard deviation SD value for each group. For the total leukocyte counts (TLC), DLC, and platelet counts data were analyzed by one-way analysis of variance (ANOVA) for different exposure days and analysis of variance for

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Table 1. Total leukocyte counts in control and EMF-treated water groups.

		White blood cells (cells/mm^3)			
Groups	*N*	Day 0	Day 30	Day 60	Day 90
A (control)	10	6905.000	6924.500	6937.000	6903.000
		117.154	115.638	107.708	101.000
B (24 hr)	10	6960.000	10330.000	7080.000	8030.000
		672.607	1247.437	742.698	830.723
C (48 hr)	10	6880.000	9590.000	5586.000	6950.000
		437.721	1732.311	633.849	768.440
Total	30	6915.000	8948.167	6534.333	7294.333
		469.423	1913.723	880.188	837.399

Values are mean and standard deviation. A = control, B = 24 hr exposed water group, C = 48 hr exposed water group.

two factors (exposure time and days) with repeated measurements in control and EMF-treated water groups.

RESULTS

Using analysis of variance (one-way ANOVA), no significant alterations were found among exposure days 0, 30, 60, and 90 control groups nor between baseline data (day 0) of EMF exposure time (groups A, B, and C) in TLC, DLC, (neutrophils, lymphocytes, and monocytes), and platelet counts. The F value (range 0.20–2.73) for all these analyses remained below the level of significance [F (2, 27) = 2.96 to $P < .05$)].

Table 1 shows significant ($P < .01$) increase in TLC on days 30 and significant ($P < .01$) decrease on day 60. However, on day 90 it again increased significantly

Table 2. Differential leucocyte counts in control and EMF-treated water groups.

		Neutrophils			
Groups	*N*	Day 0	Day 30	Day 60	Day 90
A (control)	10	20.80	21.30	21.10	20.90
		0.87	1.10	1.30	0.94
B (24 hr)	10	20.90	35.30	26.00	25.30
		2.74	3.87	8.41	3.41
C (48 hr)	10	22.20	21.00	26.30	21.50
		4.17	7.43	4.67	7.62
Total	30	21.300	25.867	24.467	22.567
		2.991	8.265	6.092	5.226

Values are mean and standard deviation. A = control, B = 24 hr exposed water group, C = 48 hr exposed water group.

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Table 3. Differential leucocyte counts in control and EMF-treated water groups.

		Lymphocytes			
Groups	*N*	Day 0	Day 30	Day 60	Day 90
A (control)	10	61.10	61.90	61.90	61.20
		1.30	1.81	1.37	1.47
B (24 hr)	10	61.70	62.10	70.70	73.40
		6.94	8.51	6.28	9.10
C (48 hr)	10	62.30	75.10	70.20	76.10
		5.80	7.61	4.87	7.60
Total	30	61.700	66.367	67.600	70.233
		5.299	9.094	6.162	9.465

Values are mean and standard deviation. A = control, B = 24 hr exposed water group, C = 48 hr exposed water group.

($P < .01$). Analysis of variance for two-factor experiment with repeated measurements indicated significant effect of both in EMF exposure time [F (2, 27) = 7.54, $P < .01$] and days [F (3, 81) = 127.19, $P < .01$]. The interaction between exposure time and days was also found to be significant [F (6, 81) = 35.13, $P < .01$]. In DLC, one way ANOVA for neutrophils showed significant ($P < .01$) increase in EMF-treated water groups on day 30, whereas on day 60 and 90 the increase was nonsignificant (Table 2). However, analysis of variance for the two-factor experiment with repeated measurements showed significant effects of exposure time [F (2, 27) = 4.86, $P < .01$] and days [F (3, 81) = 16.21, $P < .01$]. There was also significant [F (6, 81) = 20.07, $P < .01$] interaction between exposure time and day.

In EMF-treated water groups, there was substantial increase ($P < .01$) in lymphocytes on day 30, 60, and 90 (Table 3). Two-way analysis of variance exhibited significant effects of both EMF exposure time [F (2, 27) = 6.29, $P < .01$] and days

Table 4. Differential leukocyte counts in control and EMF-treated water groups.

		Monocytes			
Groups	*N*	Day 0	Day 30	Day 60	Day 90
A (control)	10	0.40	0.80	0.60	0.50
		0.49	0.75	0.49	0.67
B (24 hr)	10	0.60	0.70	1.60	1.30
		0.66	0.78	1.02	1.19
C (48 hr)	10	0.70	2.70	1.80	1.40
		0.46	1.10	1.17	0.80
Total	30	0.567	1.400	1.333	1.067
		0.559	1.281	1.075	0.998

Values are mean and standard deviation. A = control, B = 24 hr exposed water group, C = 48 hr exposed water group.

Table 5. Platelet counts in control and EMF-treated water groups.

		Platelets (10^{-5})			
Groups	*N*	Day 0	Day 30	Day 60	Day 90
A (control)	10	4.92	4.92	4.95	4.90
		0.53	0.57	0.54	0.52
B (24 hr)	10	5.69	12.51	4.71	2.95
		1.89	1.18	1.10	0.69
C (48 hr)	10	5.21	10.49	2.47	3.60
		0.96	1.41	0.63	1.20
Total	30	5.273	9.308	4.043	3.817
		1.300	3.397	1.372	1.177

Values are mean and standard deviation. A = control, B = 24 hr exposed water group, C = 48 hr exposed water group.

[F (3, 81) = 72.93, $P < .01$]. The interaction between exposure time and days was also significant [F (6, 81) = 35.58, $P < .01$].

One-way ANOVA for different days displayed significant increase of monocytes on day 30 ($P < .01$) and 60 ($P < .05$), whereas nonsignificant alteration was found on day 90 as it was increased in comparison to control, but showed a tendency to decrease when compared with day 30 and day 60 groups (Table 4). Two-way ANOVA revealed significant effects of both EMF exposure time [F (2, 27) = 4.61, $P < .01$] and days [F (3, 81) = 21.65, $P < .01$]. The F value was also significant [F (6, 81) = 13.26, $P < .01$] for interaction between exposure time and days. The platelet counts on day 30 were found to be significantly ($P < .01$) increased, whereas a significant ($P < .01$) decrease was observed on days 60 and 90 (Table 5). Two-factor ANOVA demonstrated significant alterations by EMF exposure time [F (2, 27) = 6.28, $P < .01$] and days [F (3, 81) = 805.13, $P < .01$]. The interaction between exposure time and days was also observed significant [F (6, 81)–220.77, $P < .01$].

DISCUSSION

The present study revealed that 30 days treatment of EMF-exposed water caused leukocytosis, 60 days of treatment induced leukopenia, and 90 days treatment of induced hematopoietic compensation in terms of leukocytosis. This compensation was directly related to nonsignificant increases in neutrophils and monocytes and to significant lymphocytosis. It also indicted that the treated water caused thrombocytosis on day 30 and thrombopenia on day 60 and 90.

Two-way ANOVA for times and days demonstrated significant impact of exposure time (24 hr or 48 hr) and days (30, 60, and 90) on the hematopoietic system in adult male rats indicating a potent influence of EMF-treated water on the hematopoietic system. These findings corroborate well with those workers who exposed their subjects (rodents) directly to EMF (varied field strength from 0.1 to 15 μT) at different intervals (exposure period 30, 60, or 90 days) and reported significant quantitative alterations at

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least in TLC and DLC as well as in platelet counts (possible alterations in blood coagulation system or disorders in genesis of bone marrow of exposed animals). Their field strength was lower than in our experiment but EMF exposure to subjects was direct (Bonhomme-Faivre et al., 1995; Picazo et al., 1994, 1995).

This study clearly demonstrated an indirect effect of EMF exposure on hematological parameters through a water-mediated mechanism. There are accumulating reports that EMF-exposed water also causes significant biological effects by inducing the synthesis of biomolecules affecting the spontaneously changing structural chemistry of water (Markov, 1984; Rai et al., 1994a,b; Singh et al., 1994, 1995). It has already been hypothesized that EMF radiation produces changes in structural chemistry of both ''live'' (water content of cells and tissues) and natural water (normal water) that ''remembers'' (EM-memory bits) the frequency characteristics of the field for an extended period of time (Rai et al., 1995; Singh et al., 1994). Recently, Jerman and associates also supported this hypothesis in their experiments and concluded that even the ultraweak electromagnetic emission from living beings (e.g., spruce seedlings) can change the properties of water (Cerbonell et al., 2000; Singh et al., 1995), which then showed inhibitory effects on germination and growth of seedlings. Fesenko and Glustein (1995) also suggested the bioeffects of EMF-treated water on enzyme activity, RNA, and DNA synthesis and growth.

Recently it has been suggested that a possible biomechanism of EMF-treated water (from permanent magnet, 50-Hz powerline, and microwave radiation) causes nutrient deficiency in bacteria, fungal germination, and growth changes in cytoplasmic organization, enzymatic activities, active membrane transport, and hormonal imbalances in prokaryotic and eukaryotic organisms (Dilova et al., 1987; Garg et al., 1995; Pandey et al., 1996; Rai; Rai et al., 1994a,b; Singh et al., 1994). It has been proposed that the magnetic field induced changes include membrane depolarization and increased action potential discharge, reduced uptake of Ca into cells, altered content of cyclic nucleotides, and increased volume of isolated cell bodies (Ayrapetyan et al., 1994). It is also proposed that cell hydration is a messenger through which the static magnetic field exerts its influence (Danielyan and Ayrapetyan, 1999).

The present results demonstrate that EMF-exposed water can directly or indirectly influence the inter- or intracellular ''live'' (biological) water and influence the production centres of leucocytes and bone marrow in terms of either increasing, decreasing or stabilizing the production of blood cells by affecting the cellular activities by various proposed mechanisms. Therefore, this study extends further support to the ''EM memory bits'' hyphothesis.

CONCLUSION

Exposure time and day dependent treated water effects on hematological parameters of adult rats were observed. The study revealed that water exposed to different length of time and exposure days caused significant alteration in TLC, DLC, and platelet counts. It suggests a strong influence of EMF-treated water on hematopoietic system. This study supports the hypothesis of ''biological water as the cause of EM bioeffects.'' Further investigations are required to establish the exact mechanism of such activities.

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