# BIOLOGICAL EFFECTS OF WATER TREATED USING STATIC ELECTRIC FIELD AS REVEALED BY RAT BLOOD TESTS

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Abstract. Static electric field (SEF) is permanently present in the environment and is associated with the presence of electrical charges. The aim of this work is to study the effect of tap water treated by static electric field on the liver and kidney function and on the complete blood count of albino rats. The examined rats were divided into two groups, with 8 animals in each group, along 6 weeks. Group-1, the control group, consisted of animals that consumed untreated tap water. Group-2 was composed of animals that consumed treated tap water that was exposed to a static electric field of 160 kV/m for 24 hours. The results indicated that the mean level of aspartate transaminase (AST) and alanine transaminase (ALT) activities and total bilirubin concentration were slightly increased in group-2 which amounted to 13.73%, 1.86%, and 4.16% respectively compared to group-1. On the other hand, there was a slight decrease in Hemoglobin concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), as well as erythrocyte counts (RBC) and platelet (PLT) count in blood samples of group-2. Our results revealed a very significant change in liver enzymes ALT, AST (p < 0.0001 and 0.0029, respectively) and no significant change in the total bilirubin. Also, no significant differences were noted in kidney function test urea and creatinine (p > 0.05).

Key words: Electrostatic field, liver and kidney function, complete blood picture, tap water.

## INTRODUCTION

The biological effects of static electric fields (SEF) on laboratory animals have been observed under artificial conditions and at exceptionally strong fields 40–100 kV/m [1]. Several studies on the biological effects of external SEF were performed at different levels and in different directions. The importance is due to the fact that external SEF is one of several environmental factors under the constant influence of which all biological objects exist and develop. SEF of natural

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origin is generated by the charge redistribution between the upper layers of the atmosphere and the Earth's surface. The intensity of natural SEF near the Earth's surface in fair weather is about 150 V/m [2].

Nowadays, the background level of the external SEF is increased due to the widespread use of electric appliances, high-voltage DC transmission lines and production of synthetic fabrics by the textile industry. In certain cases, the intensity of the external SEF of technological origin can reach up to 500 kV/m [3]. Furthermore, an external SEF of exactly 200 kV/m alters the balance in pro-/antioxidant processes. Whereas, the examination of the oxidative processes in plasma and *RBC* (hemolysate and membranes) shows that the biological effects depend on exposure time and the acute action of SEF characterized by activation of the pro-oxidant processes [4, 5].

The water hydrogen bond structure improved with increasing strength of the electric field that has been deduced from the radial distribution functions [6].

As shown by previous work, external SEF of 200 kV/m strength leads to alterations in the pro-/antioxidant system of blood of rats. Acute action of SEF characterized by the change of *RBC* content coincided with the activation of the pro-oxidant processes in blood plasma and *RBC*. After long-term SEF exposure, the situation changed: the *RBC* content increased and the antioxidant processes prevailed [6].

In a study performed on mice, full-body exposure to SEF did not cause a significant influence on learning ability, but caused memory impairment of receptors. This impairment was dose-dependent and not causally linked to the glutamate and gamma-aminobutyric acid levels in the hippocampus [7].

The impact of SEF on human bone marrow-derived mesenchymal stem cells (hBM-MSCs) was studied for exposure times of up to 15 hours [8]. In this study, the rate of cell migration towards the cathode was controllable depending on the SEF intensities, providing a new opportunity for the utilization of SEFs in directed scaffold colonization *in vivo* post implantation or *in vitro* for tissue engineering applications [8]. The increase in superoxide dismutase (SOD) and thiobarbituric acid reactive substances (TBARS) in the plasma, liver, lung, and kidney tissues were found to depend significantly on the type of electric field and the exposure period [9].

Theoretical and experimental results regarding cell membrane permeabilization by externally applied electric fields show that the extent of permeabilization is not only a function of electric field intensity and cell size, but also of cell shape. The theoretical results were confirmed by experimental data on ovary cells, which are elongated [10].

A high-voltage electric field (333 kV/m) has been shown to raise the water activity in bread dough, so ensuring a more efficient hydration of the gluten [11]. Rather unexpectedly, such electric fields (~1 MV/m) apparently increase water's surface tension by about 2% [12]. Electric fields were harmless up to transmission voltages of 400 kV [13].

# MATERIALS AND METHODS

#### STATIC ELECTRIC FIELD EXPERIMENTAL SETUP

The schematic diagram of SEF setup is represented in Figure 1 as a source of high voltage. The parallel-plate capacitor is used to produce a homogeneous electrical field. The distance between the plates can be adjusted up to a maximum of 7 cm, measured with an accuracy of 0.1 cm.

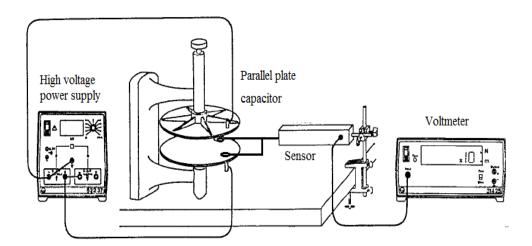


Fig. 1. The external electrostatic field setup.

In this study, the distance between the plates was fixed to 6 cm and the value of the applied voltage was fixed to 10 kV, which resulted in an electric field strength of approximately E = 160 kV/m. The water container filled with tap water of pH 7 was placed between the capacitor plates of homogeneous electric field E. As a pre-study, we measured the conductivity and pH of treated water for different

durations. A resistor of 100 M $\Omega$  electrical resistance was used to kill the current and to obtain a static electric field.

# CONDUCTIVITY AND pH MEASUREMENTS

Figure 2 shows the instruments that were used to measure the conductivity and the pH of control fresh tap water and of the fresh water treated by SEF.

Figure 2 (A, B and C) shows the pH sensor, electric conductivity sensor and Xplorer GLX (PS-2102 and PS-2116A, PS-2002, Pasco, USA). The pH Sensor was found to be well-suited for continuous recording as well as for discrete measurements.

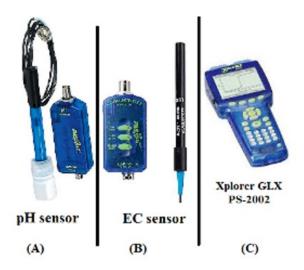


Fig. 2. PASCO PASSPORT-compatible interface (Xplorer GLX).(A) to measure pH-values. (B) to measure electrolytic conductivity. (C) the Xplorer GLX data logger [18].

## BLOOD SAMPLING AND PROCESSING

All animal experiments were performed under the provision of local Ethical Authority of Zagazig University. Adult male albino rats, weighing 90–120 g, were obtained from the Experimental Animal Care Center and were housed in metabolic cages under controlled environmental conditions (25 °C and a 12 h light/dark cycle) one week before starting the experiment as acclimatization period. The animals were fed a standard diet provided *ad libitum*.

After an acclimatization period of one week with standard basal diet, a total of 16 rats were divided into two groups with 8 animals in each group.

Group-1 (control): Animals were provided with tap water for 6 weeks. Group-2: Animals were provided with treated water (electrostatic fields, E = 160 kV/m) for 6 weeks.

## COLLECTION AND SAMPLING OF BLOOD

At the end of the experimental period, the animals were fasted for 12 hours and weighed. Animals were killed by cervical decapitation and blood (serum and plasma) was collected according to the procedure described in ref. [15].

#### BIOCHEMICAL ANALYSIS

The liver enzymes alanine transaminase (ALT) and aspartate transaminase (AST) were measured using a colorimetric assay kit [16]. Also, bilirubin level was measured using the method presented in ref. [17]. Kidney function tests urea and creatinine were performed via the methods described in references [18, 19]. Automated complete blood count (*CBC*) was also determined.

## RESULTS

Figure 3 shows the values of the water conductivities and pH values during exposure time from 20 min to 220 min. The measured conductivities, expressed in micro Siemens/cm ( $\mu$ S/cm), were comparable to 298  $\mu$ S/cm, the conductivity of normal tap water (control). Moreover, the mean and standard deviation (SD) of the treated water's pH was  $8.4 \pm 0.082$ , whereas the pH of control is 7.2.

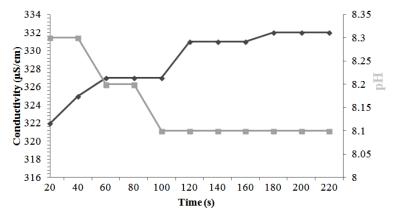


Fig. 3. The pH and EC versus SEF exposure time. Black diamonds refers to EC, whereas light gray squares represent pH values according to the scale displayed on the right side.

## LIVER FUNCTION TESTS

This study also evaluated the effect of treated tap water on the rats. Table 1 indicates the systemic elimination or clearance of treated water from the rats. The mean level of ALT, AST activities, and total bilirubin concentration shown to be slightly increased in group-2 compared to the control group-1. ALT concentration differed between the two groups with very high statistical significance (p < 0.0001, change = 13.73%). AST also displayed a highly significant difference in means (p < 0.0029, change = 1.86%) compared to control group-1.

In contrast, the mean total bilirubin of the treated group-2 was found to be  $0.50 \pm 0.02$  mg/dL, not significantly different from the mean total bilirubin of the control group-1.

Table 1
The results of liver function tests

Groups	ALT (U/L)	AST (U/L)	T. bilirubin (mg/dL)
Group-1 (control) Mean ± SD	$68.05 \pm 16.9$	$160.\ 5 \pm 24.37$	$0.48 \pm 0.02$
Group-2 Mean ± SD	$77.40 \pm 14.04$	$163.45 \pm 11.27$	$0.50 \pm 0.02$
% change	13.73	1.86	4.16
<i>p</i> -value	0.0001	0.0029	0.405

p < 0.05 refers to significance; p < 0.005 indicates high significance; p < 0.0005 indicates very high significance with respect to the control group-1.

## KIDNEY FUNCTION TESTS

Data in Table 2 indicate that the mean levels of urea and creatinine concentration were just slightly increased for group-2 compared to the control group 1; no significant differences in kindey function were found between the two groups (p > 0.05).

Table 2
Urea and creatinine concentrations

Groups	Urea (mg/dL)	Creatinine (mg/dL)	
Group-1 (Mean ± SD)	$18.50 \pm 0.5$	$0.54 \pm .048$	
Group-2 (Mean $\pm$ SD)	$19.25 \pm 1.03$	$0.55 \pm 0.02$	
% change	4.05	1.8	
<i>p</i> -value	0.189	0.452	

p < 0.05 refers to significance with respect to the control group 1.

## COMPLETE BLOOD COUNT (CBC)

Table 3 shows that there was a slight but not significant decrease in hemoglobin concentration (Hb), RBC, and MCH count in blood samples using water treated group-2 in comparison to the control group-1 (p > 0.05). There was a slight decrease and slight significance in HCT, MCV and MCHC count in blood samples using water treated group-2 (p < 0.011 and p < 0.02, respectively).

White blood cell count showed a highly significant increase in treated water group-2 which amounted to 26.5 % in comparison to the control group-1 (p < 0.0004). Also platelet counts suffered a highly significant increase (p < 0.00006).

Table 3

The results of complete blood counts

Parameter /Groups	Group-1 (control)	Group-2	change (%)	<i>p</i> -value
White blood cell (10 <sup>3</sup> /mm <sup>3</sup> )	$15.77 \pm 4.16$	19.95 ± 1.01	26.50	0.0004
Platelets (10 <sup>3</sup> /mm <sup>3</sup> )	$673.5 \pm 64.6$	$668 \pm 50.24$	0.82	0.00006
Red blood cells (10 <sup>6</sup> /mm <sup>3</sup> )	$7.11 \pm 0.32$	$6.73 \pm 0.57$	5.34	0.3249
Hb (g/dL) (Mean ± SD)	$13.55 \pm 1.0$	$13.45 \pm .16$	0.73	0.4521
HCT (%)	$43.95 \pm 1.97$	$42.5 \pm 0.10$	3.29	0.0549
MCV (fL)	$63.55 \pm 5.6$	$61.25 \pm 0.58$	3.62	0.0106
MCH (pg)	$21.75 \pm 4.11$	$20.35 \pm 3.26$	6.44	0.1206
MCHC (%)	$31.85 \pm 0.86$	$29.60 \pm 0.64$	7.06	0.0235

p < 0.05 refers to significance; p < 0.005 indicates high significance; p < 0.0005 indicates very high significance with respect to the control group 1.

## DISCUSSION

In a study of water exposed to high-voltage electric field (570 kV/m), the decrease of pH and the increase of the EC depended on the volume of water and the period of treatment [20].

The biological impact of external SEF is a controversial scientific topic [21]. The therapeutic effects of franklisation apparently are based on the polarization of the molecules of dielectric in biological tissues, electrification of conductive structures with the advent of micro currents, which leads to a redistribution of ions in the area of action and development of irritation effects. An additional effect is

the inhalation of negative ions, which are generated near the electrode of negative polarity [22].

Our results revealed that there was a highly significant change in liver enzymes ALT, AST and no significance occurred in the total bilirubin. Also, no significant difference was found in kidney function tests (urea and creatinine levels in blood (p > 0.05). This result is in disagreement with [23], which reported that no biological effects are possible for these fields.

A previous study [24] stated that after SEF exposure of rats their red blood cell count did not differ significantly from that of the control group. This finding is in agreement with our results on red blood cell count, although in our experiments the animals were not exposed to SEF; instead, they consumed water treated with SEF. Our study showed a significant increase in *WBC* count, which amounted to 26.5 % with very high significance.

#### **CONCLUSION**

In this work, we studied the *in vivo* effects of water treated by a static electric field of 160 kV/m in intensity, as revealed by blood tests of rats that consumed the treated water. Our experiments showed that the mean level of ALT, AST, and total bilirubin concentration increased compared to the control group (the group of animals that consumed untreated tap water). Also, there was a slight decrease in hemoglobin concentration, *HCT*, *MCV*, *MCH*, *MCHC*, *RBC* and *PLT* tests. Our experiments showed significant differences in liver function, but not in kidney function.

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#### REFERENCES

- 1. BANKS, T.A., P.S.B. LUCKMAN, J. E. FRITH, J.J. COOPER-WHITE, Effects of electric fields on human mesenchymal stem cell behavior and morphology using a novel multichannel device, *Integr. Biol.*, 2015, 7, 693–712.
- BATENI, A., S.S. SUSNAR, A. AMIRFAZLI, A.W. NEUMANN, Development of a new methodology to study drop shape and surface tension in electric fields, *Langmuir*, 2004, 20, 7589–7597.

- 3. BONNELL, J.A., Effects of electric fields near power-transmission plant, *J. R. Soc. Med.*, 1982, 75(12), 933–941.
- 4. CREIM, J.A., R.H. LOVELY, R.J. WEIGEL, W.C. FORSYTHE, L.E. ANDERSON, Rats avoid exposure to HV DC electric fields: a dose-response study, *Bioelectromagnetics*, 1993, **14**(4), 341–352.
- ESAKI, K., F. NINOMIYA, K. HISAKI, T. HIGASA, K. SHIBATA, K. MURATAAND, S. AIBARA, Effects of high voltage electric field treatment on the water activity of bread, *Biosci. Biotech. Biochem.*, 1996, 60, 1444–1449.
- GRANDOLFO, M., P. VECCHIA, Natural and man-made environmental exposures to static and ELF electromagnetic fields, In: *Biological effects and dosimetry of static and ELF* electromagnetic fields, Ettore Majorana International Science Series, 1985, pp. 49–70.
- GÜLER, G., N. SEYHAN, A. ARICIOĞLU, Effects of static and 50 Hz alternating electric fields on superoxide dismutase activity and TBARS levels in guinea pigs, *Gen. Physiol. Biophys.*, 2006, 25(2), 177–193.
- 8. HARUTYUNYAN, H.A., G.G. ARTSRUNI, Biological effects of the electrostatic field: Plasma/serum proteome analysis of rats, *Electromagn. Biol. Med.*, 2013, **32**(1), 79–94.
- 9. HARUTYUNYAN, H.A, L. KHACHATRYAN, A. SOGHOMONYAN, G.G. ARTSRUNI, Modification of oxidative processes in blood by the external electrostatic field, *New Arm. Med. J.*, 2013, 7, 22–32.
- HARUTYUNYAN, H.A., G.V. SAHAKYAN, Biological effects of the electrostatic field: red blood cell-related alterations of oxidative processes in blood, *Int. J. Biometeorol.*, 2016, 60(1), 99–111.
- 11. HAYK, A.H., Carbonylation of plasma proteins and blood hemolysis induced by the action of external electrostatic field *in vitro*, *Medicinal Chemistry*, 2015, **5**(5): 226–230.
- 12. KELLEY, M., The Earth's Electric Field: Sources from Sun to Mud, Elsevier, Amsterdam, 2014.
- 13. KHARELL, G., R. HASHINAGA, Changes of pH and electrical conductivity of water treated under high-voltage electric field, *Nepal J. Sci. Technol.*, 2000, **2**, 1–4.
- 14. MARTINEK, R., Improved micro-method for determination of serum bilirubin, *Clin. Chim. Acta*, 1966, **9**, 161–170.
- 15. MERCER, H., Biological effects of electric fields on agricultural animals, *Vet. Hum. Toxicol.*, 1985, **27**(5), 422–426.
- MURRAY, R., Creatinine, In: Clinical Chemistry; Theory, Analysis and Correlation, L.A. Kaplan, A.J. Pesce eds., Mosby, St. Louis, 1984, pp. 1247–1253.
- 17. OLEJNIK, V., Fundamentals of the interaction of physical fields with biological objects, *KAI*, *Kharkiv*, 2006, 17–18.
- 18. PASCO Scientific, www.pasco.com/, accessed 10.10.2017.
- 19. REITMAN, S., S. FRANKEL, *In vitro* determination of transaminases activity in serum, *Am. J. Clin. Pathol.*, 1975, **28**, 56–60.
- SUN, W., Z. CHEN, S. HUANG, Effect of an external electric field on liquid water using molecular dynamics simulation with a flexible potential, *Journal of Shanghai University*, 2006, 10, 268–273.
- 21. TABACCO, A., F. MEIATTINI, E. MODA, P. ETARLI, Simplified enzymatic/Colorimetric serum urea nitrogen determination, *Clin. Chem.*, 1979, **25**, 336–337.
- THAVASU, P., S. LONGHURST, S. JOEL, M. SLEVIN, F. BALKWILL, Measuring cytokine levels in blood. Importance of anticoagulants, processing, and storage conditions, *J. Immunol. Methods*, 1992, 153(2), 115–124.

- 23. VALIC, B., M. GOLZIO, M. PAVLIN, A. SCHATZ, C. FAURIE, B. GABRIEL, J. TEISSIE, M.P. ROLS, D. MIKLAVCIC. Effect of electric field induced transmembrane potential on spheroidal cells: theory and experiment, *Eur. Biophys. J.*, 2003, **32**(6), 519–528.
- 24. XU, Y., S. WU, G. DI, P. LING, J. JIANG, H. BAO, Influence of electrostatic field on cognition in mice, *Bioengineered*, 2016, 7(4), 241–245.