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Osmotic resistance of blood erythrocytes at rats unover *in vitro* infrasonic waves action

Effect of infrasonic waves from 13 to 30 Hz with intensity range 10.9 to 14 dB on the permeability of erythrocytes' membrane exposed to infrasonic waves with different times nas gfuetea and introduced to rats for 24 hours. The results showed that significantly high increase in the hemolysis rate, dispersion of the hemolysis process and the maximum rate of hemolysis (dH/dC) max. It can be concluded that infrasonic waves induced the increase of the permeability of erythrocyte' membranes.

Keywords: infrasonic waves, permeability of erythrocyte' membranes, hemolysis rate

Introduction

Experimental studies reported that humans or various species of animals such as rats and mice exposed to infrasound at 90 dB and higher for short or long terms up to several months exhibited significant toxicological effects [1-3]. Altman in his studies explores the relationship of vibration of the human body with infrasound, by the simple principle of resonance. Also the difference in dimension of the large waves compared to human bodies makes him explore the idea of pressure.

At low frequencies where the body dimensions are smaller than the wavelength (2m for frequencies below 170 (Hz), the same momentary pressure applies everywhere, and the tissue behaves as a viscoelastic fluid with much lower compressibility than air" [4].

Negative impacts of infrasound are perceived as subjective feelings of tiredness, sleepiness and lowering of psychomotor activity, disturbed body equilibrium and physiological functions, These subjective feelings are corroborated by changes in the central nervous system, characteristic of the reduced waking state, particularly dangerous for machine operators, Empirical investigations reveal the occurrence of the driving response effect, where by the fundamental frequency of the exciting signal or its harmonic becomes the dominating frequency of bioelectric activity of the brain [5].

The impact of infrasound on the individual and other environmental organisms has been studied insufficiently, but in most cases it is negative [6].

In this study we have investigated the effect of infrasound on the erythrocyte's membrane by method of exposure of water to different times to infrasound.

Materials and methods

In this work 56 albino rats, each of average weight 200-250 g. Rats were kept under standard conditions along the experimental period, 12/12 h light-dark regimen. Food and water were supplied daily ad libitum. All animals were housed according to the ethic rules in compliance with institutional guidelines. Water is exposed to infrasound in an infrasonic radiator EFC-1 with an exit of sound fluctuations from 13 to 30 Hz with intensity 10.9 to14 dB for different times of exposure and then introduced to rats for drinking [7].The animals were treated with drinking water exposed to infrasound for 3, 10, 30, 60, 180, 600, 1800, 3600 seconds for 24 hours. The samples of blood collected from animals before exposure which treated with clean water as control and after drinking water which treated with infrasound for different times for 24 hours after exposure for studying the permeability of erythrocytes membrane permeability and electrical conductivity of the blood.

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Infrasound facility

Installation is intended for non-operating medical treatments such diseases, as uterus, cyst ovary, fibrous adenoma mammary glands, stones of kidneys and bilious bubble, inflammatory diseases of internal bodies.

Installation is calculated on operation and physiotherapeutic, urological and other branches of clinics and hospitals at temperature of air from 18° C to 30° C Relative humidity of 80 % at temperature 25°C and atmospheric pressure (101 ±4) k pa (760±30) mm Hg.

Weight of installation in the complete set no more than 44 kg. Range of radiated frequencies – from 13 to 30 Hz.

Relative error of established frequency No more ± 2 %. Level of sound pressure generated by installation at frequency from 13 to 30 Hz is from10, 9 to 14, 0 dB.

Permeability of the red blood cells membrane

This was measured by the degree of hemolysis in a mixture of isotonic solutions of urea and NaCl after an incubation of 3 min, the samples were centrifuged at 2500 r.p.m for 10 min, 4°C the optical density was measured by means of (UV- Vis- Spectrophotometer 303-PD) at 540 nm. The level of hemolysis was presented as the percentage of hemolysis caused by a 0.1% solution of Na2Coз [8].

The hemolysis curve can be evaluated by the average hemolysis (H50-the NaCl Concentration producing 50% hemolysis). The differentiation of the hemolysis curve will represent a Gaussian curve (the rate of hemolysis dH/dC versus NaCl concentration as shown in Figure 2). The parameters area, width, height and position of the peak can obtain from Gaussian curve.

The area under curve represents the rate of hemolysis of red blood cells. The width at half maximum reflects the dispersion of hemolysis process (low dispersion than normal indicates sudden rupture of the RBCs, while higher values of dispersion reflects the abnormal increase in the membrane elasticity).

The Gaussian peak represents the maximum rate of hemolysis (dH/dC) max reached by the sample. The position on the x-axis is equivalent to the average hemolysis (H50) [9].

Determination of the electric conductivity of blood

The electrical properties of RBCs can be investigated by measuring the electrical properties of blood suspension, which has the benefit of measuring viable cells close to its physiological state, and to avoid any induced changes in the sample during preparation or rouleaux formation during settling in the measuring tubes.

The blood samples were diluted in isotonic buffered saline (pH 7.4 and conductivity 0.627 S/m), and the hematocrit was adjusted at 3%. The samples were incubated in water bath at 37°C during measurement. The electrical conductivity was determined by means of 32000 conductivity instrument ISTC Property K1117-15 [10].

Statistical analysis

All data were expressed as mean ± SE and statistical analysis was made using the Statistical Package for Social Sciences (SPSS 11.0 software and Microsoft Excel 2010). For tests, analysis of differences between groups consisted on a one-way analysis of variance (ANOVA) with repeated measures, followed by post-hoc comparisons (LSD test). Differences were considered statistically significant at $p< 0.05$ and marked as (*), highly significant at $p< 0.01$ and marked as (**), and very highly significant at $p< 0.001$ and marked as (***) [11].

Results

The results of the hemolysis curves showed that the shift of the hemolysis curves in to the left at 10, 30, 60, 180, 600, 1800, 3600 sec except in the case of 3 sec which indicate that The decrease in the average hemolysis of red blood cells as shown in table-1- and figure1A, 1B. The differential of the hemolysis curves in figure 1A, 1B will give the Gaussian curve as in figure 2A, 2B. From the results as shown in figure 2A,2B and table 1 showed high and very high significant increase in the area under the curve indicating the increase in the rate of hemolysis of red blood cells at 30, 180, 1800 sec ($p= 0.001$**, $F= 9.525$). The width at half maximum recorded high significant increase at 30, 180, 1800 sec indicating the increase in the elasticity of red blood cells membrane ($p=0.005$***, $F=6.69$). Height of the Gaussian peak very high significant increase at 3, 10, 180,1800sec indicating increase in the maximum rate of hemolysis of red blood cells (p=0.00***, F=52.89). The results also showed that the increase in the average hemolysis of red blood cells H50 (p=0.00***, F=15.1).

Table 1 – The area (A), width (W), Height (H) and H50 of the Guassian peaks for control and after different periods of exposure

	AREA	WIDTH	HEIGHT	H50
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
control	-83.00±4.88	14.36±1.25	-4.62±0.13	40.38±0.11
3.00	-87.73±3.87	12.48±0.28	-6±0.12***	41.54±0.44
10.00	-82.82±2.32	9.61±0.32	-7±0.04***	38.68±0.31*
30.00	-196.9±3.5**	34.10±0.09**	-4.61±0.09	37.29±0.60**
60.00	-58.32±2.97	9.46±0.32	-4.92±0.09	39.53±0.60
180.00	-22.9±5.1***	30.01±0.35*	-5.9±0.2***	35.66±0.42***
600.00	-124.1±63.17	23.22±11.92	-4.28±0.02	38.44±0.61*
1800.00	-206.6±3.8**	32.41±0.24**	-5.09±0.13*	36.10±0.52***
3600.00	-61.95±3.12	10.62±0.36	-4.65±0.08	39.01±0.58
Total	-124.93±15.9	19.59±2.56	-5.17±0.19	38.51±0.45
Anova				
F- ratio	9.525	6.692	52.888	15.113
P-value	0.001***	0.005***	0.000***	0.000***

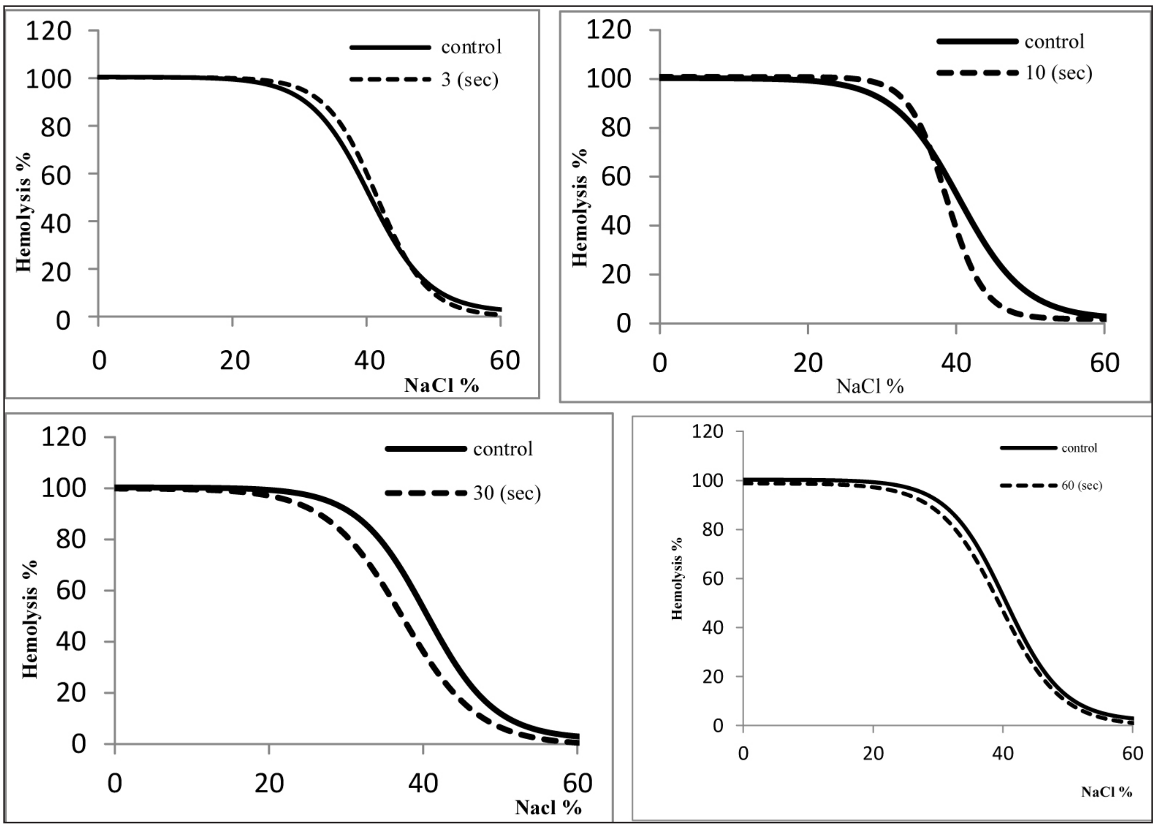

Figure-1 A-Hemolysis curves at different concentrations percent of NaCl (0.9%) for control and after 3,10,30,60 seconds of exposure

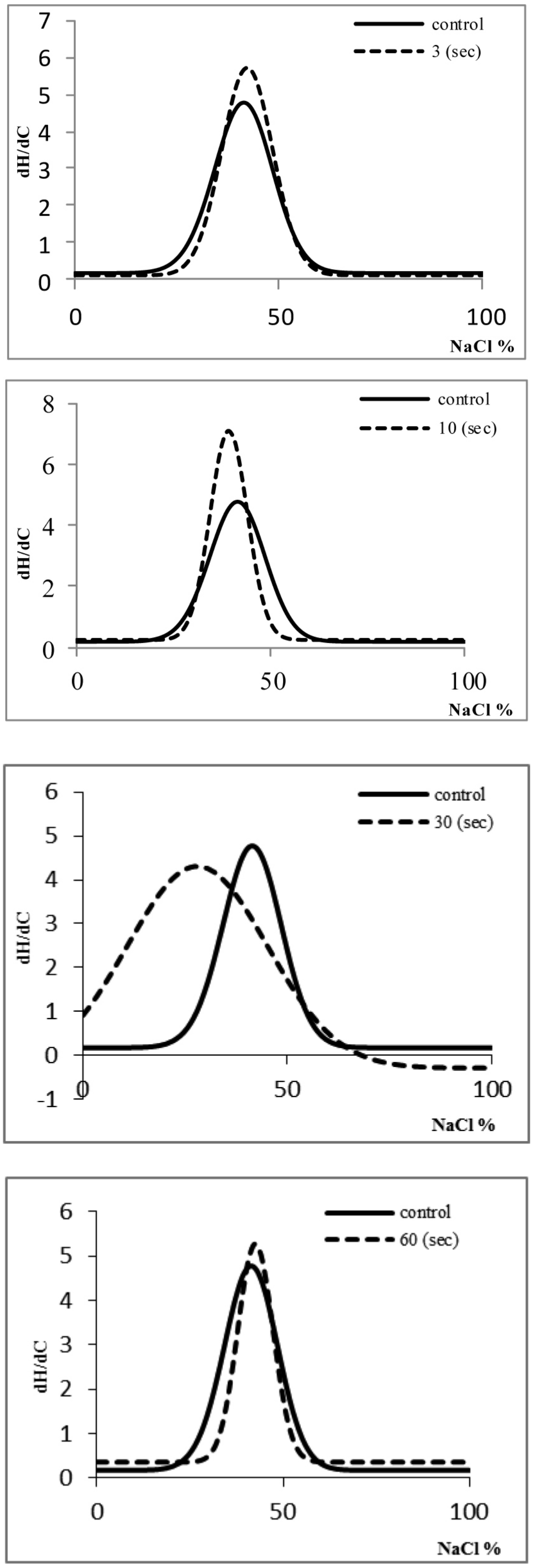

Figure 2 A – The rate of hemolysis versus NaC l% for control and after 3,10,30,60 seconds of exposure

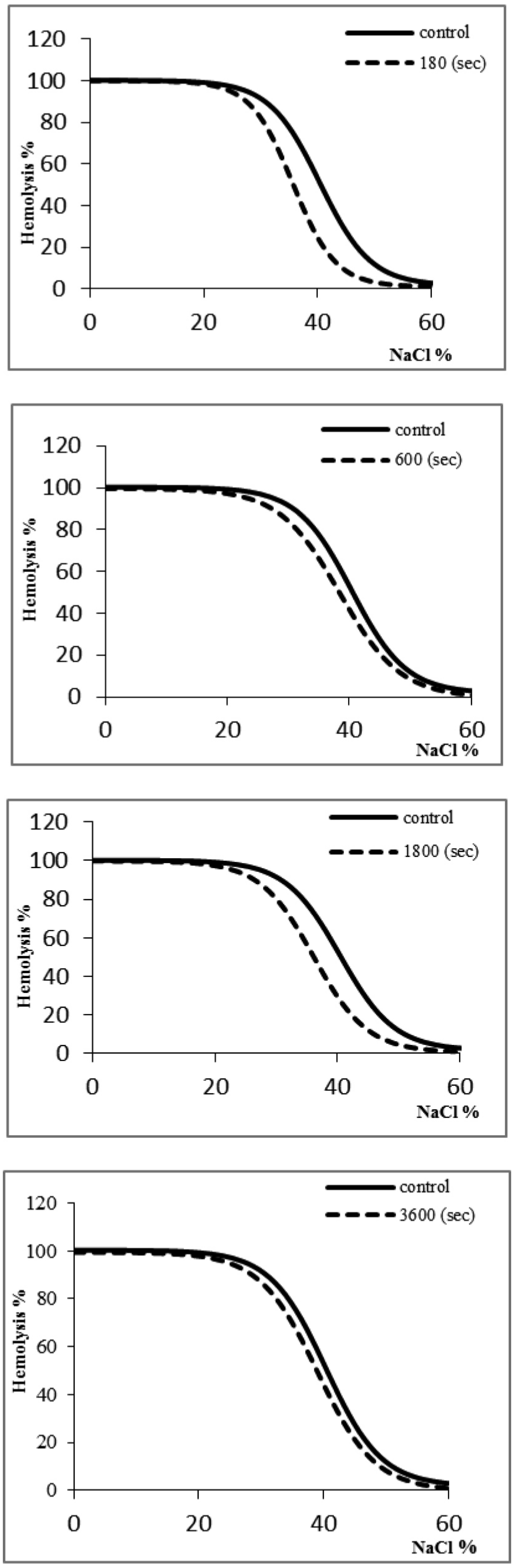

Figure-1 B-Hemolysis curves at different concentrations percent of NaCl (0.9%) for control and after 180,600,1800,3600Seconds of exposure

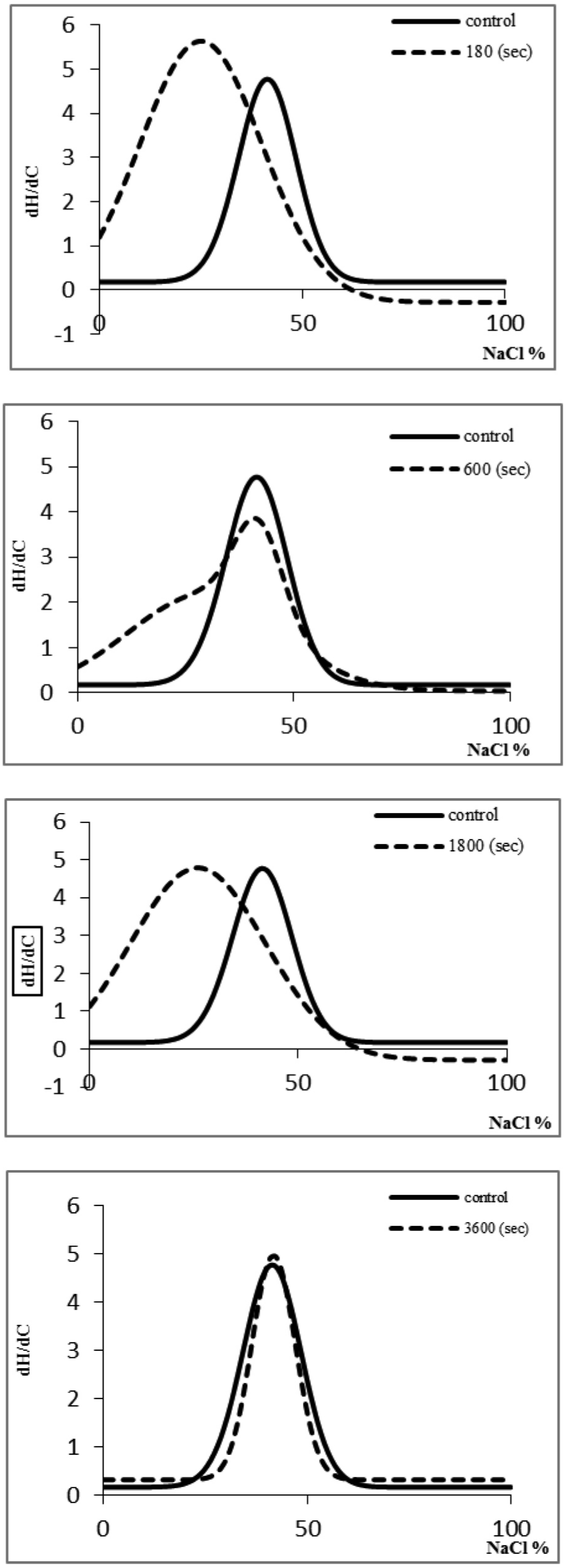

Figure 2 B- The rate of hemolysis versus NaC l % for control and after 180,600,1800,3600Seconds of exposure

Discussion

In our study we focused on the effect of infrasound biologically through the exposure of water into infrasound for different times of exposure and as shown in the results increase in the rate of hemolysis for erythrocytes membrane, increase in the width at half maximum which indicate that increase in the dispersion of hemolysis process which lead to increase in the elasticity of membrane of erythrocytes after exposure of water to infrasound and introducing this water in to rats. Also from the results showed that increase in the maximum rate of hemolysis after exposure to infrasound. Increase the permeability of erythrocytes membrane as a result of increase of penetrating urea through a cellular membrane and create inside the erythrocyte a hyperosmotic medium, which leads to swelling of the erythrocyte, infringement of the integrity of the cell membrane and leakage of hemoglobin [8]. These results are in agreement with [12, 13, 14, 15, 16 , 17] and this may be due to increase of oxidative stress produced by the noise stress [18] and which in many studies showed that several parameters of red blood cells functions and integrity are negatively affected by increased oxidative stress which is responsible for hemolysis and induces the activation of an enzyme directly involved in energy metabolism whose regulation might closely be related to the maintenance of cell integrity [19] and which may be lead to changes of erythrocyte membrane ionic permeability [20].

This is in agreement with [21] who indicated that infrasonic exposure induced organismal effect suggests that infrasound is capable of eliciting certain biological resonance responsible for a series of physical and chemical consequences. Based on the results of study and in the light of bio resonance phenomena it is believed that the mechanical energy of infrasound was absorbed and turned into thermal, biochemical and bioelectrical energy, which can directly affect the plasma membrane and mitochondrial membrane of various tissues and cells, result in a change in membrane permeability as well as affect enzyme activity. Thus, the resonant mechanical energy will finally affect the bio-oxidation process as well as the metabolism and synthesis of the energy and reduce the function of the anti-oxidation system [22].

Conclusion

Exposure to infrasound from 13 to 30 Hz with intensity 10.9 to 14 dB induced the increase in the permeability of erythrocyte' membranes.

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