

THE INFLUENCE OF THE INFRASOUND ON THE IMMUNOLOGICAL PROPERTIES OF RATS BLOOD

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Abstract. Recent interest in adverse effects of infrasound on organisms arises from health concerns. Infrasonic sound or infrasound is the sound produced below audible frequency range. Generation of infrasound can be found everywhere on the planet. During the 1970's infrasound was a fairly popular topic within the scientific community. In spite of the loss of interest over the years, recently it has been gaining importance. In order to study the effect of infrasound on immunological properties of rats we measured the changes in the general and differential counts of white blood cells. For this purpose 20 albino rats, each of average weight 200–250 g, were exposed to infrasonic wave in an infrasonic radiator with an exit of sound fluctuations from 13 to 30 Hz with intensity 10.9 to 14 dB. The animals were exposed to both infrasound directly for 10 minutes and indirectly irradiated through water exposed to infrasound for 10 minutes. The samples of blood collected before irradiation from rats as control and after exposure for 3, 6, 12, 30, 60 days. The obtained results show that the general leukocytic count significantly increases after direct and indirect exposure to infrasonic wave in all intervals groups; this is accompanied by significant increase in immature precursors. It can be concluded from the obtained results that infrasound from 13 to 30 Hz with intensity range 10.9 to 14 dB can induce immunological changes. These changes are represented in the disturbance of the production of leukocytic cells especially appearance of immature cells. This can be due to the effect of the intense infrasound on the resonant frequencies of body organs.

Key words: Infrasound, leukocytes, immunity.

INTRODUCTION

Infrasound (0.0001–20 Hz) is acoustic energy with physical characters of strong penetration and less attenuation in long distance propagating. With the rapid development of modern industry and transport, more and more infrasound sources have been determined, which contribute to the dramatic increase of noise pollution

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in our environment [22]. Environmental infrasound is the result of transportation vehicles, industrial processes, and the operation of machinery. Car noise has been measured at 92, 117 dB Sound Pressure Level (SPL) at frequencies less than 1 Hz up to 28 Hz [2, 17]. There are also natural occurrences of infrasound resulting from earthquakes, waves in water, waterfalls, air turbulence, and thunder. Activities pursued by human beings will also produce infrasonic energy. Walking, jogging, and swimming all produce infrasound that has been measured at levels up to 140 dB [19]. Wars have brought new technology to the battle field making military scientific research on new types of weapons and ways to avoid attacks from enemies more attractive. Doctors and psychologists have been interested in trying to understand the effects of low frequencies on the natural resonances of the organs of the human body [3].

Experimental studies have been 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 [10, 13]. Mice exposed to 8 Hz at 120 dB showed erythrocyte-filled acini and thickening of the inter-alveolar septa of the lungs. Most studies reported some effects attributed to infrasound exposure. Mice exposed to 8 Hz at 120 dB showed erythrocyte-filled acini and thickening of the inter-alveolar septa of the lungs. Exposures of 8 and 16 Hz at 140 dB ruptured blood vessel walls and destroyed acini [16]. Guinea pig short-term studies reported ear damage. Exposure to 4 Hz at 110 dB for 40 days increased alkaline phosphatase concentrations in vessels of tympanic membranes [1]. Exposures to 8 or 16 Hz at 90 to 120 dB for up to 25 days induced morphological changes in receptor cells and hair cells of the inner ear. These changes and changes in the endoplasmic reticulum and mitochondria recovered after exposure ended [12].

Rabbits exposed to 10 Hz at 100 to 110 dB for 24 days showed disturbances of enzyme levels of the mitochondria and reduced contractile function of the myocardium [14]. Rats exposed to 10 to 15 Hz at 135 to 145 dB for 45 days showed arterial constriction, nuclear deformation, and mitochondrial damage [8]. Rats exposed to 8 Hz at 120 dB for up to 45 days showed myocardial cell pathology, microcirculation disturbances, ischemia, and mitochondrial destruction in capillaries [5, 13].

Leukocytes are the mobile of the body's protective system; they are formed partially in the bone marrow (the granulocytes and monocytes, and a few lymphocytes) and partially in the lymph tissue (lymphocytes and plasma cells), but after formation they are transported in the blood to the different parts of the body where they are to be used. The real value of the white blood cells is that most of them are specifically transported to areas of serious inflammation, thereby providing a rapid and potent defense against any infectious agent that might be

present [6]. The granulocytes and monocytes have a special capability to seek out and destroy any foreign invader. The adult human being has approximately 7000 white blood cells per cubic millimeter of blood. The normal percentages of the different types of white blood cells are approximately the following: polymorphonuclear neutrophils 62.0%, polymorphonuclear eosinophils 2.3%, polymorphonuclear basophils 0.4%, monocytes 5.3% and lymphocytes 30.0% [7].

Although there have been some studies on infrasonic toxicology, relative little is known about the adverse effects of infrasound on immunity, which prompted us to conduct the study, which aims to describe the direct and indirect influence of infrasonic waves on the immunological response.

MATERIALS AND METHODS

INFRASOUND EXPOSURE

In this work 20 albino rats, each of average weight 200–250 g, 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. Rats were exposed to infrasound in an infrasonic radiator EFC-1 with an exit of sound fluctuations from 13 to 30 Hz with intensity 10.9 to 14 dB [9]. The animals were divided into 2 groups the first group exposed to infrasound directly for 10 minutes, the second group irradiated through water exposed to infrasound directly for 10 minutes. The samples of blood collected before irradiation from rats as control and after irradiation through 3, 6, 12, 30 and 60 days, general count of white blood cells and total differential count were studied, results treated with SPSS program.

TOTAL LEUKOCYTIC COUNT

The improved Neubauer chamber was used for counting the white cells after diluting with Turk's solution (2 ml Glacial acetic acid, 1ml Gentian violet 1% solution and 100 ml distilled water). Leukocytes were counted in four corner squares of the hemocytometer. The obtained value was multiplied by the factor 50 to get the count/ μL [20].

TOTAL DIFFERENTIAL COUNT

Blood films for total differential count are made by placing a drop of blood on one end of a slide, and using a spreader slide to disperse the blood over the

slide's length. The aim is to get a region where the cells are spaced far enough apart to be counted and differentiated. The slide is left to air dry, after which the blood is fixed to the slide by immersing it briefly in methanol. The fixative is essential for good staining and presentation of cellular detail. After fixation, the slide is stained by Romanowsky stain to distinguish the cells from each other [18].

The shift index of blood leukocytes can be calculated by the following equation:

$$x = \frac{\text{myelocytes} + \text{metamyelocytes} + \text{band neutrophils}}{\text{segments neutrophils}}$$

RESULTS AND DISCUSSION

With the development of modern industry and transportation, infrasound plays a more and more important role in noise pollution [21]. Although natural sources such as thunder, earthquake, etc., always generate lower frequency (<2 Hz) and interval infrasound, relatively higher frequency (1–20 Hz) infrasound may be produced by some artificial environment and has great effects on the exposed workers. In the present study we focused on the effect of infrasound on the immunological response as well as the possible explanations.

Table 1

General count of WBC (C/ μ L) after exposure to infrasound and exposed water

Group	Group A		Group B	
	Mean \pm SE	LSD	Mean \pm SE	LSD
Control	14802.5 \pm 302.45	–	15552.5 \pm 467.49	–
3 days	21625 \pm 1624.99	0.007**	20812.5 \pm 1062.5	0.038*
6 days	41937.5 \pm 1562.5	0.000***	24075 \pm 1649.99	0.005**
12 days	37350 \pm 1150	0.000***	20162.5 \pm 1162.5	0.059*
30 days	37700 \pm 1300	0.000***	16650 \pm 0	0.599
60 days	38300 \pm 699.9	0.000***	41512.5 \pm 2512.5	0.000***
ANOVA				
<i>F</i> -ratio	83.39		46.265	
<i>P</i> -value	0.000***		0.000***	
Where * at $P \leq 0.05$ (Sig.); ** at $P \leq 0.01$ (Highly sig.); *** at $P \leq 0.001$ (Very highly sig.)				

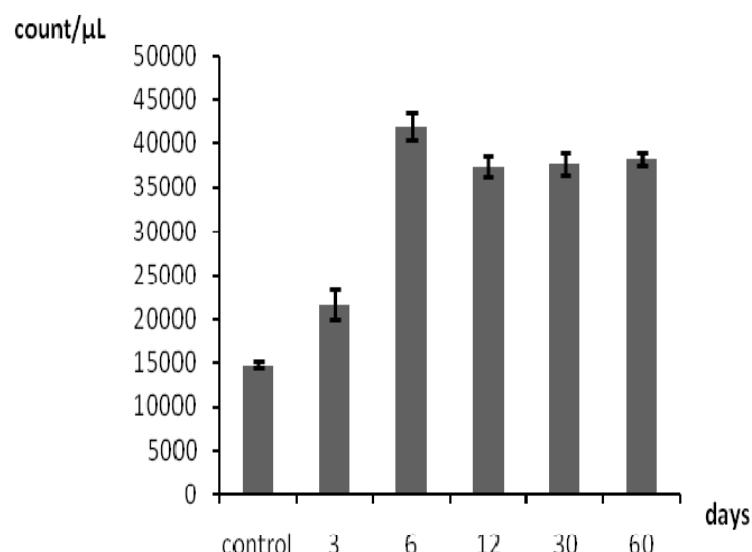
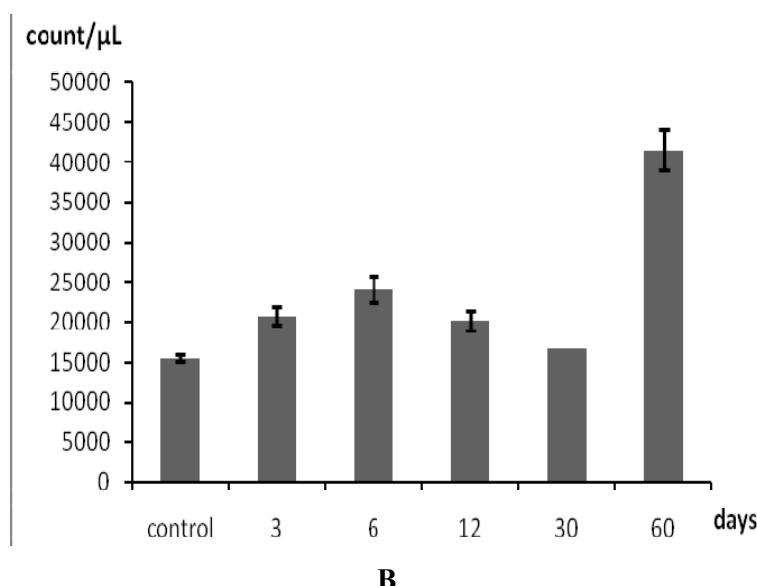
**A****B**

Fig. 1. General count of white blood cells for control and after 3, 6, 12, 30, 60 days after exposure for 10 minutes. A. General count after exposure to 10 min infrasound; B. General count after drinking water exposed to 10 min infrasound.

Table 2
The shift index and differential count of blood leukocytes for groups A

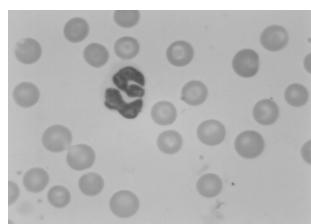
	Shift index	Myelocytes	Neutrophils		Monocytes	Basocytes	Lymphocytes
			Band	Segments			
Control	0.036±0.001	0 0	296.05±6.04 2±0	8141.37±314.39 55±1	222.03±78.54 1.5±0.5	1258.21±99.72 8.5±0.5	148.02±3.024 1±0
3 days	0.493±0.053	2162.5±162.499 10±0	324.4±132.5 1.5±0.5	5081.87±57.49** 23.5±1.5	216.3±16.24 1±0	2919.37±105* 13.5±1.5	4108.75±24.99** 19±1
6 days	0.504±0.104***	5661.56±1259.37** 13.5±2.499	419.375±15.62 1±0	1216.1±33.74*** 29±1	0±0 0±0	3145.31±746.24* 7.5±1.5	4822.81±449.37** 11.5±1.5
12 days	3.131±0.669	12512.25±548.49*** 33.5±2.49	560.3±169.4 1.5±0.5	4295.25±692.5*** 11.5±1.5	0±0 0±0	4855.5±896.49*** 13±1.99	2614.5±292.99** 7±1
30 days	0.216±0.067	5089.5±1495** 13.5±3.99	0±0 0±0	23939.5±493.99*** 63.5±3.49	1131±337.99** 3±1	0±0 0±0	565.5±207.99 1.5±0.5
60 days	0.234±0.008	2681±48.99 7±0	0±0 0±0	11490±173*** 30±1	383±6.99 1±0	574.5±202 1.5±0.5	6974.5±428.99* 18.5±0.5
F-ratio	17.65	27.28547	6.534718	358.3114	8.603541	14.27595	43.86038
P-value	0.002**	0.000468***	0.020365*	0.000***	0.01041944**	0.00279674**	0.000120251***
							0.000***

Where * at $P \leq 0.05$ (Sig.); ** at $P \leq 0.01$ (Highly sig.); *** at $P \leq 0.001$ (Very highly sig.).

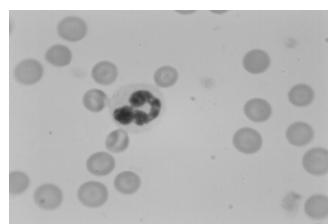
Table 3
The shift index and differential count of blood leukocytes for group B

	Shift index	Myelocytes	Neutrophils		Eosinophils	Monocytes	Basocytes	Lymphocytes
			Band	Segments				
Control	0.045±0.010	0±0 0±0	<u>388.81±66.07</u> <u>2.5±0.5</u>	<u>8787.2±497.4</u> <u>56.5±2.5</u>	<u>233.28±84.77</u> <u>1.5±0.5</u>	<u>1244.2±118.12</u> <u>8±1</u>	<u>233.3±70.74</u> <u>1.5±0.5</u>	<u>4665.8±140.3</u> <u>30±0</u>
3 days	1.004±0.049*	<u>3954.4±624.**</u> <u>19±1</u>	<u>312.18±120</u> <u>1.5±0.5</u>	<u>4266.56±94.37.**</u> <u>20.5±1.5</u>	<u>624.37±176.24</u> <u>3±1</u>	<u>3850.31±508.74.**</u> <u>18.5±1.5</u>	<u>1665.12±293.12.**</u> <u>8±1</u>	<u>6139.68±417.5</u> <u>29.5±0.5</u>
6 days	0.707±0.007	<u>3490.87±359.6.**</u> <u>14.5±0.5</u>	<u>0±0.**</u> <u>0±0</u>	<u>4935.37±458.62.**</u> <u>20.5±0.5</u>	<u>0±0</u> <u>0±0</u>	<u>3611.25±247.49.**</u> <u>1.5±0</u>	<u>3250.12±102.37.**</u> <u>13.5±0.5</u>	<u>8787.37±481.87</u> <u>36.5±0.5</u>
12 days	1.321±0.179**	<u>5242.25±907.12.**</u> <u>26±2.99</u>	<u>201.62±11.62</u> <u>1±0</u>	<u>4133.31±137.5.**</u> <u>20.5±0.5</u>	<u>1008.12±143.5.**</u> <u>5±1</u>	<u>3528.43±300.62.**</u> <u>17±2.5</u>	<u>604.9±34.9</u> <u>3±0</u>	<u>5443.9±515.5</u> <u>27±1</u>
30 days	0.448±0.073	<u>1831.5±332.99.**</u> <u>11±1.99</u>	<u>0±0.**</u> <u>0±0</u>	<u>4079.3±83.24.**</u> <u>24.5±0.5</u>	<u>832.5±166.5.**</u> <u>5±1</u>	<u>416.3±83.24.</u> <u>2.5±0.5</u>	<u>166.5±0</u> <u>1±0</u>	<u>9324±499.49</u> <u>56±3</u>
60 days	1.329±0.529**	<u>5189.06±521.62.**</u> <u>12.5±0.5</u>	<u>0±0.**</u> <u>0±0</u>	<u>4566.375±1384.1.**</u> <u>11±4</u>	<u>0±0</u> <u>0±0</u>	<u>0±0*</u> <u>0±0</u>	<u>415.12±25.12</u> <u>1±0</u>	<u>31341.94±3349.874.**</u> <u>75.5±3.45</u>
F-ratio	4.873496	18.89602	9.785908	8.362033	12.79026	42.69561	84.23495	51.25834
P-value	0.039824*	0.001305***	0.007525**	0.011186**	0.003749**	0.00013***	0.0000***	0.000***

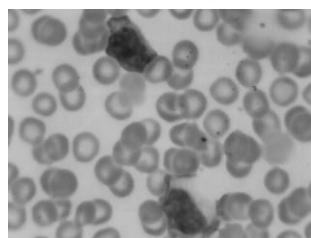
Where * at $P \leq 0.05$ (Sig); ** at $P \leq 0.01$ (Highly sig.); *** at $P \leq 0.001$ (Very highly sig.).



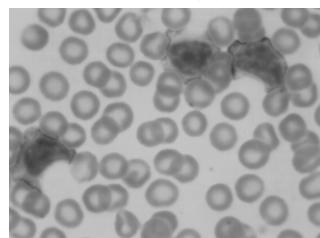
Control (A)



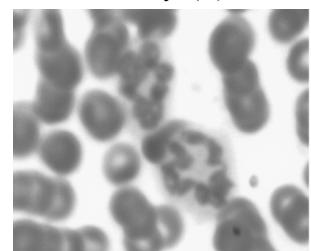
Control (B)



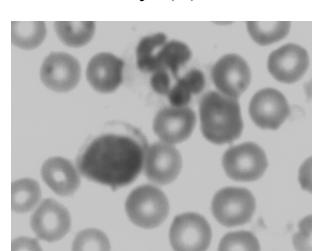
3 days (A)



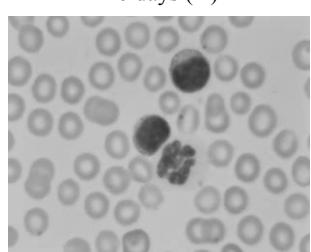
3 days (B)



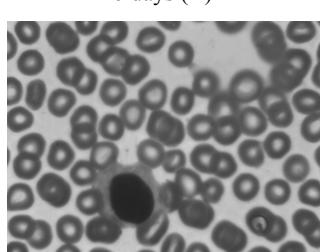
6 days (A)



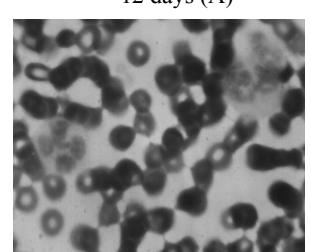
6 days (B)



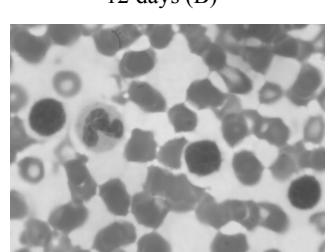
12 days (A)



12 days (B)



30 days (A)



30 days (B)

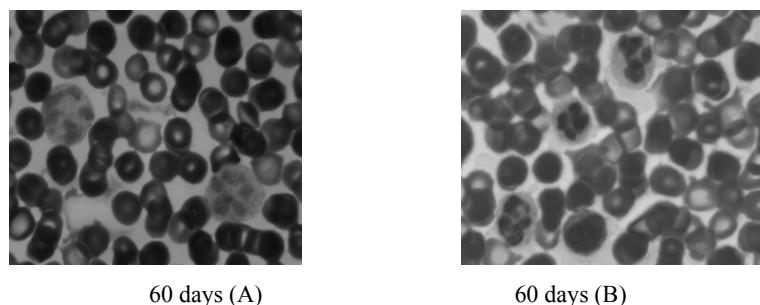


Fig. 2. Light Micrograph of white blood cells after exposure to infrasonic wave (exposure time 10 min, frequency range 13–30 Hz and intensity 10.9–14 dB) before and after exposure (3, 6, 12, 30 and 60 days). The pictures show that the nuclear deformation in neutrophil cells has occurred and appearance of immature precursors (myelocytes).

DIRECT EXPOSURE

Table 1 and Figure 1 show the average of the general counts of leukocytes after exposure to infrasonic waves (10.9 –14 dB) for intervals 3, 6, 12, 30 and 60 days. The obtained results show that the general leukocytic count significantly increased after all intervals groups; this is accompanied by a significant increase in immature precursors cells compared to the control group up to 30 days. In contrast with the previous results, there was an increase in the number of neutrophils and lymphocytes cells after two months (60 days). At the same time, it was found a highly significant increase in the number of monocytes and basophiles (Table 2).

INDIRECT EXPOSURE

Fig. 1B and Table 1 show the general count of leukocytes after indirect exposure to infrasonic wave (10 min). The recorded results show a significant increase in the average of total leukocytic count for all intervals (3, 6, 12, 30 and 60 days). This increase is accompanied by a very high significant increase in the myelocytes, monocytes, neutrophils, lymphocytes and basophiles. This increase in the number of differentiated cells indicates that there is chronic inflammation or stress due to the exposure to infrasonic wave. It is known that the infrasonic waves have effect on the decrease of specific electrical conductivity (SEC) of water, which was accompanied by the increase of pH and decrease of CO₂ solubility in water [15, 20]. Also, it is known that there is a close correlation between water dissociation and CO₂ solubility in it, and in the presence of oxygen the reactive oxygen species (ROS) generation, such as H₂O₂, takes place [4]. Based on the foregoing, it can be explained by what happened from an increase in the total and differential white blood cells after exposure to the infrasonic wave. On the other hand, the shift index of blood leucogram was calculated and it was found that after

direct and indirect exposure to infrasonic waves shift to the left side had occurred. A “left shift” refers to the presence of increased proportions of younger, less well differentiated neutrophils and neutrophil-precursor cells in the blood. This generally reflects early or premature release of myeloid cells from the bone marrow, the site where neutrophils are generated. A severe neutrophilia with left shift is referred to as a leukemoid reaction. The leucocyte alkaline phosphatase (LAP) score, which refers to the amount of alkaline phosphatase per neutrophil, will increase. In a severe infection, toxic granulation changes happen to neutrophils [11]. This increase in the immature leukocytes may be due to proliferation and release of granulocytes and monocytes precursors in the bone marrow which is stimulated by several types of inflammation.

CONCLUSION

It can be concluded that direct and indirect exposure for 10 minutes to infrasound (13 to 30 Hz with intensity 10.9 to 14 dB) can stimulate the immune system and lead to disturbance in the production of white blood cells in addition to appearance of immature products in the blood. Caution is recommended for people dealing with these low frequencies.

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