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Treatment of 40 kHz Continuous Ultrasound towards Blood Cells of Mice (Mus musculus L)

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Abstract

The application of ultrasound in mice has increased the activity of mice and showed anxious behavior that affects the blood cells of mice. This study aims to observe the effect of 40 kHz ultrasound exposure on the content of leukocytes and erythrocytes in blood cells of mice. For this purpose, five ultrasound treatments with sound pressure levels were 70, 75, 80, 85, and 90 dB respectively and one control in six sample groups, each consisting of five mice with the same weight and age. Sound treatment is carried out with a 40 kHz continuous ultrasound exposure of the same duration for two hours. Taking blood samples of mice is carried out as soon as the sample is given a sound treatment. Based on the results of blood tests of mice it was found that the average increase in the number of leukocyte mice varied between 40-1,070 cells/mm^3 for variations in sound pressure levels from 70 to 90 dB with an increase in the highest average number of leukocyte (1,070 cells/mm^3) at the sound pressure level 75 dB. With the same level of sound pressure level, the decrease in the average number of erythrocytes varied between 920,000-190,000 cells/mm^3 with a decrease in the highest average number of erythrocytes (920,000 cells/mm^3) at an 80 dB sound pressure level. The optimum level of sound pressure that can affect the average number of leukocytes and erythrocytes in blood cells of mice are still unknown.

Contents

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1. Introduction

Ultrasound is a high-frequency sound wave (20 kHz and above) above the normal human hearing frequency threshold. Like sound waves, ultrasound can only propagate if there is an intermediate medium (solid, liquid, and gas). This is because sound waves are mechanical waves that propagate longitudinally. In its propagation, sound energy is transmitted through medium particles which oscillate in the direction of wave propagation without the transfer of the medium particles. In addition to sound frequencies, important physical quantities associated with the sound is the sound intensity. Sound intensity is usually expressed in the sound pressure level in dB (deciBell) (Harris, 1995; Parker, 1988, Walker *et al.,* 2014).

Ultrasound application of animal behavior has been widely studied. The effect that can be caused is the occurrence of anxiety in the animal. Bird studies have been investigated by Ogochukwu *et al.,* (2012), where birds become uneasy and rush away after being given ultrasound treatment. Ultrasound application in a group of rats has also been studied by Simeon *et al.,* (2013) and as a result, the mice showed a shocked response and tried to escape. Previously, Dahlan *et al.,* (2007) had examined the effect of audiosonic and ultrasonic sound waves on mouse activity. They report that the application of sound in mice has resulted in an increase in the activity of mice with varying sound pressure levels and sound frequencies. This is understandable because sound waves propagate mechanical energy so that interaction between waves and living cells will occur. When the energy distributed by the wave across the elasticity limit of the cell can result in cell wall rupture.

Like mammals in general, the ear of the mouse also consists of three main parts, namely the outer ear, the middle ear, and the inner ear. The three parts of the ear work as a whole so that the hearing function can work well, especially in responding to the sound that passes through it. The hearing range is strongly influenced by the movement of the earlobe, mammals whose earlobe can move tend to have a wider range of hearing than those that cannot move. This is because the movement of the earlobe can direct the ear to the sound source. Especially for a family of mice, the frequency of hearing may exceed the capabilities of human hearing, the Norway rat even at 42 kHz (Heffner and Heffner, 2007).

One physical quantity that affects the hearing process and can affect the hearing function is the level of sound pressure. In mice, the mechanical effect that can be generated by the level of sound pressure is the acceleration of particles in the body of the mouse as a result of the energy carried by sound, in this case including ultrasound. In fact, ultrasound treatment with a low sound pressure level can affect the activity of connective tissue cells (Herle *et al.,* 2001).

Increased activity of mice associated with the treatment of ultrasound exposure is important for further research. The study emphasized the content of leukocytes and erythrocytes in the blood of mice after being given 40 kHz ultrasound treatment with the same duration of exposure but with variations in sound pressure levels. Observation of these two blood contents was carried out with the consideration that leukocytes play a role in cellular defense and humoral organisms against foreign substances while erythrocytes play a role in oxygen exposure. The selection of mice as the object of research because these animals are relatively sensitive to ultrasound and in terms of taking blood samples is relatively easier to do.

2. Materials and Methods

The research procedures that have been carried out include three main activities, namely the assembly of ultrasound generators, ultrasound treatment of mice samples, and blood sampling to determine the number of leukocytes and erythrocytes in the blood cell of mice. The ultrasound treatment process was carried out in two stages, namely the first stage of inserting the mouse sample into the treatment box of 20x20x40 cm2 plywood material, the second stage, except control (without ultrasound treatment), all samples were given a 40 kHz continuous ultrasound treatment with sound pressure level 70, 75, 80, 85, and 90 dB each two hours continuously without stopping. A total of 25 mice were divided into 5 sample groups in one treatment box of the same number of mice per box. Each box consists of five healthy mice of the same age (2 months). After treatment, blood samples of mice were immediately taken as much as 0.5 mL for the calculation of the number of leukocytes and erythrocytes. The same blood sample was taken for control.

3. Results and Discussions

The ultrasound generator consists of a series of oscillators, amplifiers, and transducers which are diagrammatically described in Figure 2.1. The oscillator functions as an electric pulse generator whose frequency can be regulated. The oscillator output must be amplified by both current and voltage using an amplifier before finally being fed to a 40 kHz ultrasound transducer to convert electrical signals into ultrasound waves. Oscillator and amplifier circuits are usually known as signal generators.

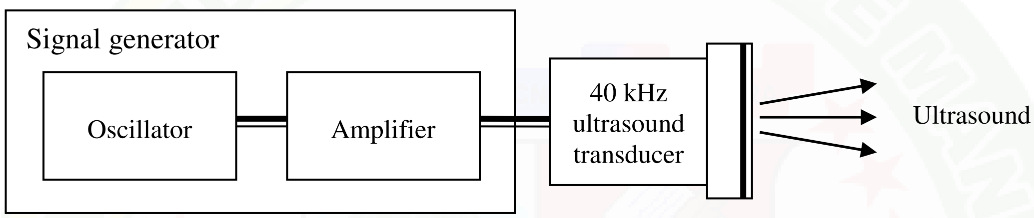

Figure 1. Block diagram of the ultrasound generator

Ultrasound transducers can be used as transmitters or receivers (Parker, 1988). This device works based on piezoelectric effects (Ackerman *et al.,* 1979). In this research, the transducer functions as a transmitter to convert electrical signals into mechanical signals (ultrasound). The 40 kHz ultrasound transducers are usually made from piezoelectric materials that work optimally in these frequencies.

The process of carrying out 40 kHz ultrasound treatment in the sample group and blood sampling of mice is shown in Figure 2. All experiments were carried out in a laboratory atmosphere with almost the same conditions, namely in the 25^{o} C and 60% humidity.

(a)

(b)

Figure 2. Research implementation (a) 40 kHz ultrasound treatment process in the sample group and (b) mice blood sampling

Graphically, the effect of ultrasound pressure levels on mouse leukocyte concentrations is shown in Figure 3.

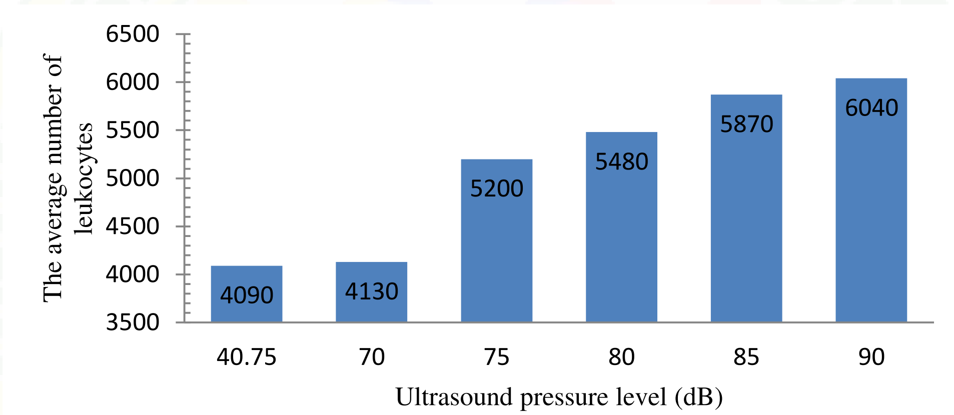

Figure 3. Effect of ultrasound pressure levels on mouse leukocyte concentration (cell/mm^3)

The higher the level of ultrasound pressure given, the average number of leukocytes in the blood of mice also increased. Before the ultrasound treatment, the average of the sound pressure level was recorded at 40.75 dB (quiet state/speech whispered). This condition is used as a control (without ultrasound treatment). For a pressure level of 70 dB, the average number of leukocytes increased by 40 cells/mm^3 compared to controls. For an increase in the pressure level from 70 dB to 75 dB, the average number of leukocytes increased by 1070 cells/mm^3. When the pressure level is increased again from 75 dB to 80 dB, the average number of leukocytes also increases by 280 cells/mm^3. Likewise, for pressure levels from 80 dB to 85 dB and from 85 dB to 90 dB, the average number of leukocytes increased by 390 cells/mm3 and 170 cells/mm^3, respectively.

In percentage terms, the increase in the average number of leukocytes for the treatment given compared to the control is given in Figure 4. The percentage is calculated by dividing the difference between leukocytes at each level of sound pressure and control by the mean number of leukocytes in the control. Based on the graph, it can be seen that the percentage of increasing average number of leucocytes increases with increasing levels of ultrasound sound pressure.

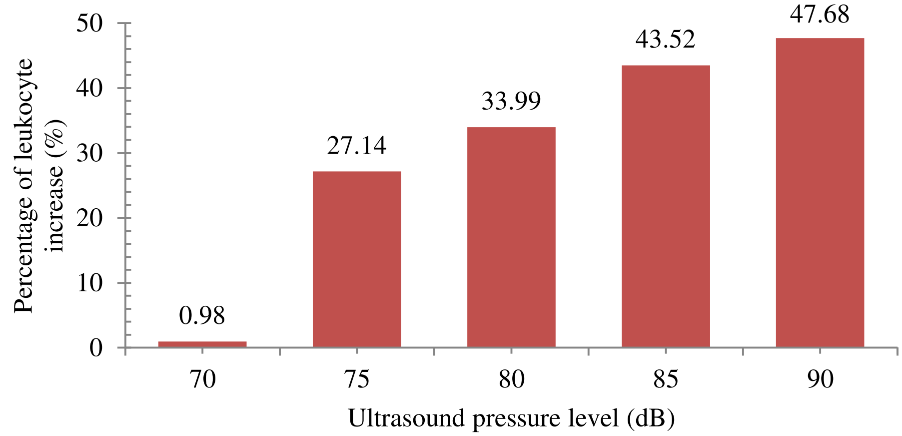

Figure 4. Graph increase in the average number of leukocytes to controls in percent

The effect of ultrasound sound pressure level on the number of erythrocytes of mice in the form of graphs is shown in Figure 5. Based on the graph it can be seen that the increase in ultrasound pressure levels resulted in a decrease in the average erythrocytes number of the mouse. When compared with the control, at a pressure level of 70 dB, the average number of erythrocytes decreased by 440,000 cells/mm^3. For an increase in the pressure level from 70 dB to 75 dB, the magnitude of the reduction in the average number of erythrocytes is 190,000 cells/mm^3. When the pressure level is increased again from 75 dB to 80 dB, the reduction in the average number of leukocytes is 920,000 cells/mm^3. Likewise, for pressure levels from 80 dB to 85 dB and from 85 dB to 90 dB, the average number of erythrocytes is 220,000 cells/mm^3 and 210,000 cells/mm^3, respectively.

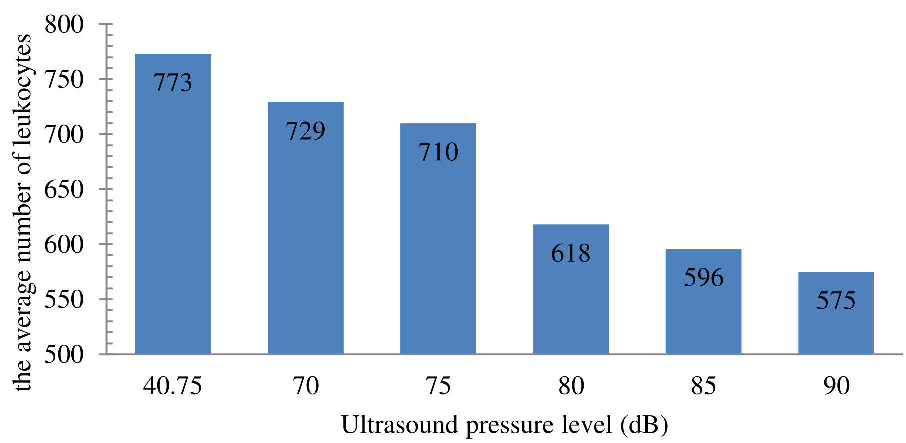

Figure 5. Effect of ultrasound pressure levels on mouse erythrocyte concentration (cell/mm^3)

In percentage terms, the decrease in the average number of erythrocytes for the treatment given compared to control is given in Figure 6. The percentage is calculated by dividing the difference between erythrocytes at each level of sound pressure and control by the mean number of erythrocytes in the control. Based on this graph, it can be seen that the percentage of decreasing the average number of erythrocytes increases with increasing levels of ultrasound sound pressure.

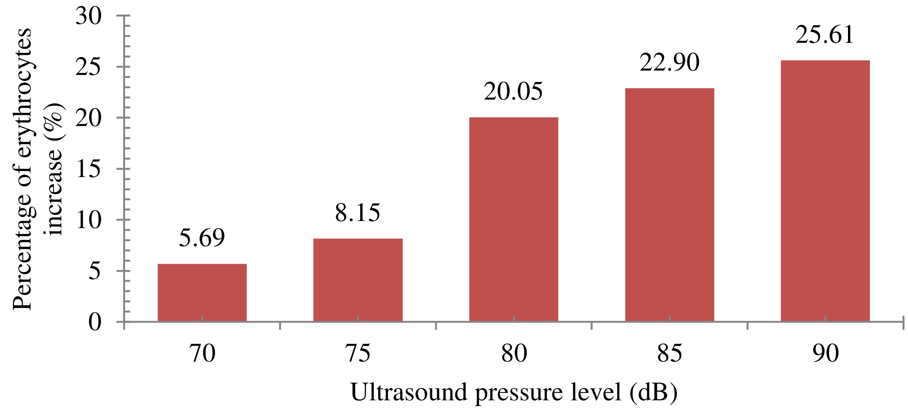

Figure 6. Graph increase in the average number of erythrocytes to controls in percent

Statistically, the data is processed using Statistical product and service solutions (SPSS) program for Windows version 14. For the number of leukocytes and erythrocytes, the calculation results are obtained, namely F_{count} of 11.740 and 26.963 for leukocytes and erythrocytes respectively, while the F_{table} value is 2.62. For both leukocytes and erythrocytes, it was found that $F_{count} \geq F_{table}$ so Ho was rejected and Ha was accepted, or the effect of ultrasound pressure level on the on the average number of both leukocytes and erythrocytes was said to be significant.

Ultrasound exposure of 40 kHz on mice with varying levels of sound pressure levels has affected the concentration of leukocytes and erythrocytes in the blood of mice. An increase in the level of ultrasound pressure can result in an increase in the average number of leukocytes and vice versa can lead to a decrease in the average number of erythrocyte mice compared to controls. This situation may be caused by a disturbance in the body's metabolism due to the stress experienced by mice as a result of the ultrasound noise it receives (Jalali *et al.,* 2012). This is understandable because the higher the level of ultrasound pressure received by the mouse, the higher the sound energy it receives. This sound energy can cause damage to cell membranes if it exceeds the ability of the cell itself. The blood cells can be damaged due to cell tearing by cavitation effects. One of the causes of the cavitation effect is the presence of ultrasound that has a high level of sound pressure. This pressure effect can cause cavitation bubbles and if they occur continuously for a long enough duration (in this case, 2 hours), ultrasound energy will cause the bubble to burst.

The effect of the optimal ultrasound sound pressure level for this study is unknown. Based on the graph in Figure 3, the average increase in the blood leukocyte number of mice is in line with the increase in the level of ultrasound sound pressure given. In contrast, according to the graph in Figure 5, an increase in the ultrasound sound pressure level results in a decrease in the average number of erythrocytes. Based on the graph of the percentage increase in the average number of leukocytes to the control (Figure 4), the level of ultrasound sound pressure level of 75 dB has an impact on the increase in the average number of leukocytes of more than 27% and tends to be linear up to 47% at 90 dB. The average percentage reduction in the number of erythrocytes to the control (Figure 6) increased by 20% for the 80 dB ultrasound sound pressure level and tended to be linear to close to 26% at 90 dB. When compared with the level of sound pressure in the human ear, the sound pressure level of 80 dB (loud voice/radio noise) is enough to make an acoustic noise effect that can interfere with the body's metabolism of mice.

4. Conclusion

Based on the results of research on the effect of 40 kHz ultrasound sound pressure on leukocytes and erythrocytes of mice (Mus musculus L) that have been done, it can be concluded that the increase in the average number of mouse leukocytes varies between 40 cells / mm3-1070 cells / mm3 for variations in pressure levels sound from 70 to 90 dB with the highest increase in the number of leukocytes (1070 cells /

mm3) at a sound pressure level of 75 dB. With the same sound pressure level variation, the average reduction in the number of erythrocytes varied between 920,000 cells / mm3 - 190,000 cells / mm3 with the highest reduction in the average number of erythrocytes (920,000 cells / mm3) at an 80 dB sound pressure level. In this study, the level of optimal ultrasound sound pressure that can affect the average number of leukocytes and erythrocytes of mice has not been known.

Acknowledgments

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