



## Effect of Noise Stress Exposurse on Heart Histopathology of White Rat (*Rattus Norvegicus*)

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### Abstract

Noise is one of stressors that can occur over a long period and adversely affect health if it exceeds the ability to adapt. The heart plays a significant role and is the main organ in distributing oxygen, substances, minerals, and other organic substances in the blood. The samples used in this study amounted to 24 white male rats divided into four groups. Group K1 is a control rat that does not give treatment, Group P1 is given noise treatment for 6 hours of exposure, Group P2 with 9 hours of exposure, and Group P3 with a dose of 12 hours of exposure. Heart samples were collected and histology preparations were obtain by Hematoxylin-Eosin (HE) staining, and microscopic observe were made with 10 $\mu$ m and 40 $\mu$ m magnification. The results of the study were then adjusted to degree of damage and analyzed using the Kruskal-Wallis test and the Mann-Whitney test to showing changes in the form of necrosis and inflammation due to noise stress with different levels of damage and obtained a value below the critical value ( $p < 0.05$ ). The degree of damage that occurs is in line with the length of noise exposure given. Severe damage occurred in Group 3 with noise exposer of 12 hours/day for 29 days.

**Keywords:** *Noise, Stress, Heart, Histopathology, White Rat.*

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### Introduction

In modern urban society, noise is considered harmful to the environment. Most of the non-auditory effects of noise are due to the systemic impacts of high-intensity noise, which can damage nerves, blood vessels, the immune system, and the heart (Xue *et al.*, 2014). Noise is a huge problem for residents of large cities. Therefore, noise is a pollutant that often causes public outrage and is generally a by-product of the use of these technologies (Redza, 2010). An increase in sound with irregular and complex waves is called noise (Carwadi and Juwita, 2019).

Rats have a distinct sound spectrum with maximum sensitivity at intensities inaudible to humans. Rodents hear an amplitude inaudible to humans (above 20 kHz), perceiving

sounds up to 80 kHz (Zymantiene *et al.*, 2017). Noise exposure in excess of 90 decibels (dB) can be a stressor by contributing to the genesis and manifestation of several multifactorial diseases, chronic disorders, and permanent behavioral changes (Helal *et al.*, 2011).

Noise causes the release of various stress hormones that affect the pattern of cardiovascular disease risk factors. Noise stress can cause hypertension and congestive heart failure (Abu-Amara *et al.*, 2013). The cardiovascular system is assumed complex due to the relationship between noise and heart disease. Some experts have investigated the acute effects of short-term loud noise on blood pressure and cardiovascular parameters (Rahma, 2011). Stress can trigger the cardiovascular system by releasing catecholamines that increase heart rate and induce vasoconstriction (Widyasari *et al.*, 2021).

Based on the explanation above, the author then raised the title "Effect of Exposure to Noise Stress on Histopathology of the Heart of White Rats (*Rattus norvegicus*)" to examine changes to the heart organ exposed to noise based on its microscopic appearance. This study will focus on histopathological changes caused such as cell necrosis and inflammation of heart.

## Materials and Methods

This type of research is a laboratory experimental conducted to see the effect of noise stress exposure on the histopathology of white rat heart (*Rattus norvegicus*). In addition, this study is included in descriptive analysis, it describes the changes that occur in the histopathology of the heart organs of the rats studied. This study was conducted from December 2022–January 2023 in the Surgical Room and Histopathology Laboratory of Hasanuddin University Veterinary Teaching Hospital. This study used 24 male white rats with age surrounded between 12-14 weeks with weight (200–250) gram. The animals were subdivided into 4 groups (six rats/group), first group as K1 is control group rat that does not give treatment 0 hours/day, while the second group as P1 treated with 6 hours/day, third group as P2 with 9 hours/day, and the last group as P3 with 12 hours/day of noise exposure for 29 days.

**Animal and Study Design** (The method used in sampling is simple random sampling. Samples are pick by taking rats that are healthy and relatively the same body weight. The repeatability is quantified to obtain valid data according to Federer's formula (1977):

$$(n - 1)(t - 1) \geq 1$$

Notes:

n = number of samples

t = number of groups/treatments

This study has 4 treatments consisting of 1 control group and 3 treatment groups. Therefore, the t-value used is 4. When entered in the formula above, the number of samples per treatment can determined, which are:

$$(n - 1)(t - 1) \geq 15$$

$$(n - 1)(4 - 1) \geq 15$$

$$3(n - 1) \geq 15$$

$$3n - 3 \geq 15$$

$$3n \geq 18$$

$$n \geq 6$$

**Experimental Protocol** The noise given as part of the treatment in rats (*Rattus norvegicus*) is a noise that comes from the sound of an oscilloscope soundcard application that is

rendered a speaker with a noise exposure level of 93-113 dB. According to Antunes *et al.*, (2013) the use of noise exposure levels  $\geq 90$  dB has a significant effect on enlargement of heart mitochondria and a decrease in connexin 43, which indicates damage to mitochondria and fibrosis in the heart. The type of noise used is a fire alarm sound effect using the VLC media player application. The noise used with the variation of exposure time interval for the 4 treatments is 0 hours, 6 hours, 9 hours, and 12 hours exposure/day for 29 days.

**Histological Analysis** Preparation of histology begins with the necropsy process carried out by euthanizing rats by taking blood directly from the heart. An incision is made on the medial abdomen to take the heart organ. The heart organ put into 10% formalin in the sample cup. The process of making histology preparations follows the standard operational procedure of the Pathology Laboratory of the Veterinary Teaching Hospital, Hasanuddin University, in which the heart organ is cut and arranged into a blue cassette. Then, the dehydration process done using graded alcohol (70%, 80%, 90%, 95%) with a time of 1 day each. Then, samples placed in 100% alcohol 1 for 1 hour, then 100% alcohol 2 for 1 hour; the tissues were then subjected to a clearing process using xylol 1 for 30 minutes, then xylol 2 for 30 minutes. After that, the tissue is embedded in liquid paraffin for infiltration and put into an incubator at about 56°C for 2 hours. Then it through the embedding process (planting the organ in paraffin). The incision is done using a microtome with a thickness of 4µm and then put on a glass object into an incubator. After that all process above, staining process using *Hematoxylin-Eosin* (HE).

**Statistical Analysis** Based on histopathology observation, data were analyzed statistically and nonparametrically using *Kruskal-Wallis* test. If there is a significant difference ( $p < 0.05$ ), it will be continued with *Mann-Whitney* test to identify differences in the effect of noise stress exposure on cardiac histopathology of white rats (*Rattus norvegicus*).

## Results and Discussion

Heart histopathology findings of male white rats (*Rattus norvegicus*) were obtained from observation of histological preparations using eight views for each treatment group. Observations can be magnification of 10µm and 40µm by examining changes in the presence of necrosis and inflammation.

**Table 1.** Heart histopathology observation results

| Groups                       | Scores |   |   |   |   |   | Average Scores |
|------------------------------|--------|---|---|---|---|---|----------------|
| Negative control (K1)        | 0      | 0 | 0 | 0 | 0 | 0 | 0              |
| 6 hours noise exposure (P1)  | 1      | 2 | 2 | 1 | 1 | 1 | 1,3            |
| 9 hours noise exposure (P2)  | 2      | 2 | 2 | 3 | 3 | 2 | 2,3            |
| 12 hours noise exposure (P3) | 3      | 3 | 3 | 3 | 2 | 3 | 2,8            |

Description:

Score 0 : No change

Score 1 : There is a change of about <25% in 8 visual fields

Score 2 : There is a change of about 26-50% in 8 field of view

Score 3 : There are changes around >51% in 8 field of view

The results of the degree of histopathological damage were then processed using the Statistical Program for Social Science (SPSS). Non-parametric statistical tests are statistical tests that do not require assumptions about the circulation of population data. *Kruskal-Wallis* test is one of the non-parametric statistical tests that can be use to test whether there is a significant difference between group of independent and dependent variable. This test

is needed to compare more than 2 population groups with data to see significant differences between groups (Jamco and Abdul, 2022). The results of the *Kruskal–Wallis* non-parametric statistical test showed a significant change between the negative control group (K1) and each treatment group (P1, P2, and P3) on necrosis and inflammation that caused in the heart organ.

The *Kruskal–Wallis* test results showed a chi-square of 19,855 with a significant value of 0.000. From this value, it shows that the value is below the critical value ( $p < 0.05$ ), so it can be construed that there is a real significant difference between the negative control group and other treatment groups that are given noise stress exposure treatment with different time intensities, so the test can be continued using the *Mann–Whitney* test to determine significant differences in the effect between noise stress exposure given to all treatments.

The results of data analysis using the *Mann–Whitney* test on the category of necrosis and inflammation between control groups with each treatment showed a significant difference in value ( $p < 0.05$ ), in accordance with what is present in Table 2. In this test, there was a significant difference in the value of the white rat heart organ in the comparison of group K1 with P1, P2, and P3 as the comparison of group P1 with P2 and P3, which showed a value below the critical value ( $p < 0.05$ ). Based on the results of the data analysis, it is possible to see that the comparison between K1 and P3 has a high significant value of 0.001, that it can be stated that there is a change or influence due to the provision of noise stress with different time vulnerabilities. While the comparison between groups P2 and P3 shows a value above  $p < 0.05$  that there is no significant difference. This can be interpreted that the heart muscle undergoes changes due to the provision of noise stress with an intensity of 93-113 dB.

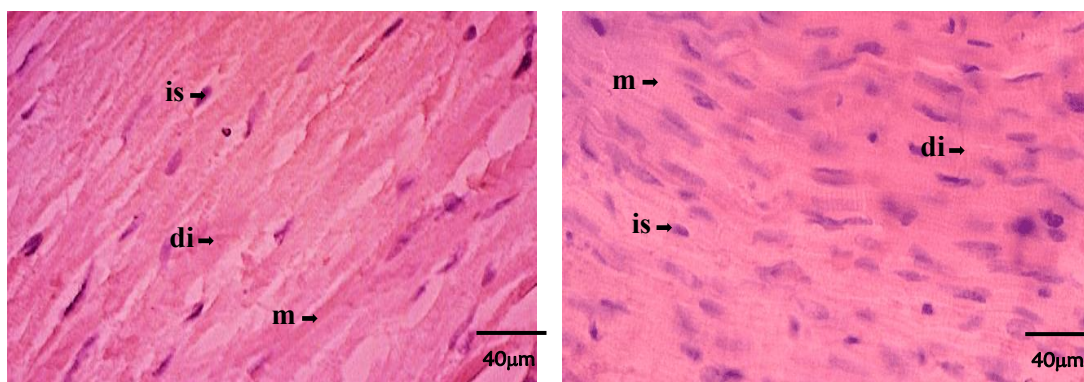
**Table 1.** *Mann–Whitney* test results

| Groups                |                  | Asymp. Sig. |
|-----------------------|------------------|-------------|
| Negative control (K1) | Treatment 1 (P1) | 0.002       |
|                       | Treatment 2 (P2) | 0.002       |
|                       | Treatment 3 (P3) | 0.001       |
| Treatment 1 (P1)      | Treatment 2 (P2) | 0.014       |
|                       | Treatment 3 (P3) | 0.004       |
| Treatment 2 (P2)      | Treatment 3 (P3) | 0.093       |

Description: Mann-Whitney test is meaningful if Asymp. Sig.  $p < 0.05$

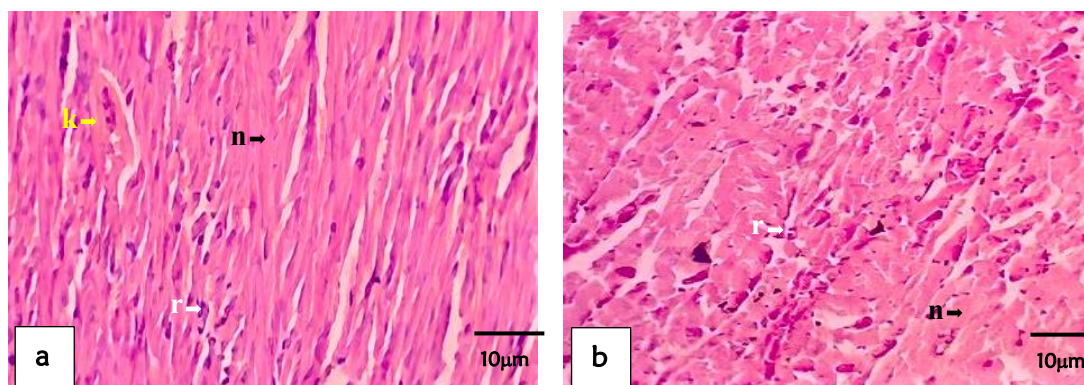
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Histopathological observations of the heart of male white rats (*Rattus norvegicus*) were made under a microscope using 10 $\mu$ m and 40 $\mu$ m magnification through an optical lens.



**Fig. 1** In picture a and b histopathology of the heart of the negative control group (K1) rats (HE, 40 $\mu$ m). (di) Dictus intercalaris, (is) cell nucleus, and (m) musculus.

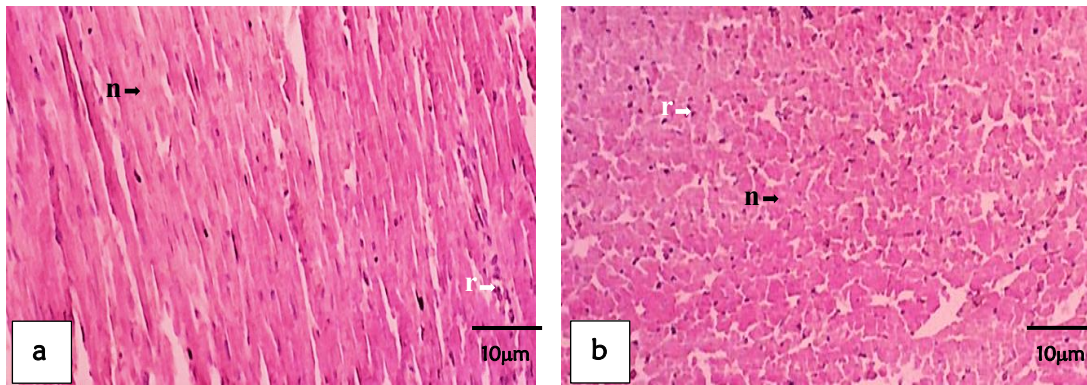
Microscopic observations (Fig 1) in the negative control group (K1) showed no morphological changes in the heart muscle. The shape of the heart muscle is not damage due to no noise exposure given. This is indicate by the intact morphology of the heart muscle, and the cell nucleus that varies in color and size. Heart muscle cells appear striated with basophilic cell nuclei in the center, acidophilic cytoplasm, and existence of a link between heart cells, namely Discus intercalaris. As described by El-Desouki *et al.*, (2012) that heart muscle fibers are connect by Discus intercalaris, there is an acidophilic cytoplasm with an oval nucleus located in the middle. The heart muscle fibers are separate by a layer connective tissue layer with apparent myocardial blood capillaries. According to Okasha and Ayah (2015), the histological structure of normal heart muscle cells with the presence of cardiac myocytes with acidophilic sarcoplasm and cell nucleus boundaries in the middle. The degree of damage to heart histopathology in the negative control group (K1) is 0 due to no visible find.



**Fig. 2** In picture a and b histopathological of the heart of rats in the 6-hour treatment group (P1) (HE, 10 $\mu$ m). n (necrosis) and r (inflammation).

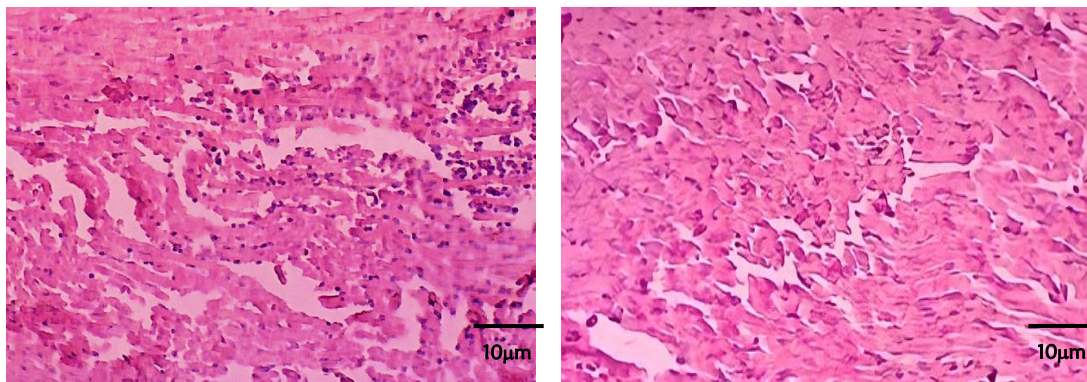
In group P1 rats (Figure 2) given exposure to noise stress for 6 hours/day for 29 days, it can be observe that the tissue shows necrosis and inflammation. Inflammation is the body's response to foreign agents entering the body. Inflammatory cells are form due to the body's response to trauma to the tissue. Inflammation plays a role in destroying, diluting, or blocking harmful agents, as well as stimulating the formation of a series of processes that attempt to restore and transform damaged tissues (Herdiani and Endah, 2018). The degree of damage to heart histopathology in this group is score 1 with changes that occur <25%.





**Fig. 3** In picture a and b histopathological of the heart of rats in the 9-hour treatment group (P2) (HE, 10µm). n (necrosis) and r (inflammation).

Histopathological analysis of the heart give exposure to noise stress for 9 hours/day (Figure 3) of 29 days, experience necrosis and inflammation in several cell nuclei. Necrosis that can be found are pyknosis, karyorrhexis, and karyolysis. Necrosis that experiences shrinkage of the nucleus and an increase in basophils and compacted DNA so that the mass becomes dark and then shrinks (pyknosis), cell nuclei that experience fragmentation or rupture of cell nuclei and chromatin damage (karyorrhexis), and cell nuclei that disappear due to DNA digestion by DNase activity (karyolysis) (Elmore *et al.*, 2016). The degree of damage to heart histopathology in this group is with a score of 2 with damage changes that occur around 50%.



**Fig. 4** In picture a and b histopathological of the heart of rats in the 9-hour treatment group (P3) (HE, 10µm). n (necrosis) and r (inflammation).

In Figure 4, the P3 group was given noise stress exposure for 12 hours/day within 29 days. This treatment group shows that the damage mark by the number of cell nuclei that experience changes in necrosis damage and severe inflammation. Necrosis is the death of cells or tissues due to an irreversible degeneration process. Macroscopically, cells and tissues that experience necrosis are mark by whiteness, softened tissue, and there is demarcation (barrier) with healthy tissue (Nugraha *et al.*, 2021). Necrosis can arise by several things, one of them including trauma (Muhsi *et al.*, 2020). The degree of damage to heart histopathology in this group with damage changes that occur more than 50% with a score of 3.

Based on the results of heart histopathology observations above, could be seen that each treatment group experienced changes in damage with different levels. Group K1 was not give noise stress treatment 0 hour/day had a normal appearance in which no damage are

detect. Meanwhile, in the P1 treatment group with 6 hours/day of noise stress, heart organ damage is found in the presence of changes in cell nuclei that experienced pyknotic nuclei in the mild category. The P2 group with 9 hours/day of noise stress showed changes in damage in the moderate type of necrosis and inflammation with the start of the number of cell nuclei experiencing cell nucleus fragmentation and loss of cell nuclei in the heart organ. The P3 group experienced the most severe changes in heart organ damage compared to the other treatment groups with 12 hours/day of noise stress, showing damage in the form of pyknotic nuclei, karyorrhexis, and karyolysis and inflammation. Pyknotic nuclei are the initial stage of necrosis, so changes in the nuclei of heart organ cells are characteristic of necrosis. The varying time of exposure to noise stress provides the body's reaction to causing trauma in increased levels of cerebral oxidative stress hormone mediated by angiotensin-II which is an important biomarker of stress in noise-exposed animals. The influence of the associated changes in mitochondrial damage may lead to higher noradrenaline levels.

High-intensity noise can cause health problems. Health problems can be caused in the form of stress (Sembor *et al.*, 2013). Exposure to noise stress can increase heart rate and arterial pressure and decrease parasympathetic nervous system stimulation. Rats exposed to noise intensity ( $\geq 90$  dB(A),  $< 500$  Hz) experienced enlarged heart mitochondria, indicating mitochondrial damage, and cardiac fibrosis (Antunes *et al.*, 2013).

In addition, noise intensity (100 dB) can induce mitochondrial DNA damage, mitochondrial membrane swelling, membrane wasting and cristolysis, which can be associated with higher noradrenaline levels, MAO activity, and impaired mitophagy, all of which can contribute to the dysregulation of mitochondrial transition and calcium handling (Li *et al.*, 2019).

The histopathological changes that are severe can be found in the observation of the P3 group which was given the treatment of noise stress exposure with an intensity of 93-113 dB for 12 hours/day within 29 days. This can be seen in Table 1, while the histopathological picture of the heart organ can be seen in Figure 4, which shows that in necrosis and inflammation as many as 1 sample entered with a score of 2 and 5 samples entered with a score of 3.

In rat cardiomyocytes, exposure to loud noise (for given noise exposure 12 hours) causes a significant increase in DNA damage accompanied by mitochondrial membrane swelling, matrix dilution, and karyolysis. These changes were accompanied by increased in situ noradrenaline levels and utilization (Li *et al.*, 2019). Noradrenaline is a major substrate of MAO-A located in the outer mitochondrial membrane and ROS produced by MAO-A causes accumulation of protein 53 (p53) within the cytosol where it can inhibit parkin, an important regulator of mitophagy, resulting in mitochondrial dysfunction (Manzella *et al.*, 2018).

Cardiomyocytes isolated from rat hearts after noise exposure ( $> 100$  dB) suffered oxidative damage, including DNA damage, which explains cardiovascular dysfunction in response to noise exposure that has given. Malfunctioning or damaged mitochondria could explain the substantial impact of noise exposure on cardiovascular disease in general and ischemic heart disease in particular, as (oxidative) damage to mitochondria plays an essential role in

cardiovascular health and disease (Daiber *et al.*, 2020).

## Conclusion

Based on the result of the research that have done, it can be conclude that the provision of noise stress influences changes in damage that are different in each treatment group with different levels of damage to the heart organ. The provision of noise stress on organ damage is evidenced by HE staining which shows the presence of necrosis and inflammation. Significant changes were found in the P3 group with noise stress exposure of 12 hours/day getting the most severe level of damage compared to the control group.

## Conflict of Interest

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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