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Correlation of Noise Intensity to *Heat Shock Response* with *Hsp 70, p53, Cytochrome C, Caspase 3* expressions and ultrastructure region of *Rattus norvegicus*'s cochlea.

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Abstract: Introduction: Noise is one of the stress factors that mostly found in our environment. The regular and continuous noise exposure will cause permanent damage to cochlea as hearing organ.
Method: Hsp 70, p53, Cytochrome C and Caspase 3 expression are the specific protein markers due to the continuous environment noise exposure. Trearment consist of 5 groups are; P0 = control group/noise <25 dB; P1 = noise by 30-50 dB; P2 = noise by 60-70 dB; P3 = noisy at 80-90 dB and P4 = noisy at 100-110 dB. Each groups has been done for 8 days and 8 hours/day. Invivo experimental method with white rat *Rattus norvegicus* for the purpose to measure the stress protein marker in cochlea tissue with Hsp 70, p53, Cytochrome C and Caspase 3 to evaluate the apoptosis of the involved cochlea. Cochlea structure with Scanning Electron Microscope (SEM) has been done after the noise various intensity for 8 hours per day as long as 8 days and was terminated.
Results: This study shown that Hsp 70 expression tend to increased due to P0-P4, but other markers tight increased such as expression of p53, Cytochrome C and Caspase 3 with the score 4-6 (moderate intensity). All apoptosis biomarker groups have shown significant difference compare to experiment group ($p<0.01$). The results of SEM on the organ of corti at the group P1 to P4 showed the gradual destruction (P4>50%).
Keywords: Noise, Hsp 70, p53, Cytochrome C, Caspase 3, Organ of Corti.

Introduction

Noises in our daily lives often interfere with hearing, Noise Induced Hearing Loss (NIHL) often found in industrial workers. Hearing loss is usually bilateral but unilateral is not uncommon. Average noise exposure allowed per day should not exceed 85 dB or 40 hours a week. The nature of hearing loss that occurs is the cochlear type of sensorineural deafness. According to the Occupational Safety & Health Administration [1] the safe limit of exposure to noise depends on the length of its exposure, the frequency and intensity of the noise and sensitivity of individuals and several other factors. Noise is one of the causes of stress are most commonly found in municipal and industrial environments.

Hsp 70 as an active protein that plays a role in the noisy is closely related to the formation of an antibody against Hsp 70 expression induced by autoantibodies Hsp 70 which is significantly caused NIHL pathogenesis. Damage to the outer hair cells that is triggered by the continuous expression of Hsp 70 would be cell death through multiple apoptotic pathways[2].

Cell damage internally in the mitochondria may be caused by the cycle of ROS, which causes the release of Apaf-1 so that Hsp 70 penetrate the mitochondrial membrane causing the release of cytochrome C and caspase-3. Cell death is preceded by translocation of cytochrome C that activates caspase 3 and eventually causes cell damage and apoptosis in cochlear tissue. Continuous noise exposure will cause expression of caspase-8 and caspase-9, and triggers caspase-3 as an executor of the Program Cell Death (PCD) apoptosis.

Material and Methods

This research was purely experimental in vivo study with the design following the research design of Completely Randomized Design (CRD). The design of this study is an experimental study in the laboratory that is designed to follow the completely randomized design (CRD). This study consists of five (5) treatment groups, and 25 rats of *Rattus norvegicus* as replicates (150-200g; 8-11 weeks), namely: Group I (P0) = consists of 5 adult male rats that did not receive treatment of noise <25 dB (control group). Group II (P1) = consists of 5 adult male rats were given noise by 30-50 dB for 8 hours each day. Group III (P2) = consists of 5 adult male rats were given noise by 60-70 dB for 8 hours each day. Group IV (P3) = consists of 5 adult male rats were given noisy at 80-90 dB for 8 hours each day. Group V (P4) = consists of 5 adult male rats were given noisy at 100-110 dB for 8 hours each day.

Results

Research before being given exposure to continuous noise from 25 dB to 110 dB is measured by Oto Acoustic Emission (OAE) pre- and post-treatment of noise for 8 hours a day. Then the results of the OAE assessed according to the value of Signal to Noise Ratio (SNR). Thus the value of SNR ≥ 6 is Pass, and SNR <-6 is Refer, and shown below:

Table 1. Distribution of SNR at frequency 2 KHz, 3 KHz, 4 KHz Noisy P4 treatment intensity (100 dB s / d 110 dB)

Days	Right	Left	Right	Left	Right	Left
1	6	6	6	6	6	6
2	-5	4,8	-6	6,3	0	2,6
3	-6	2,7	-6	3,5	-7	0,2
4	-5	1,7	-8	-0,1	-6	-5.3
5	-9	-7,3	-6	0,8	-3	-6
6	-3	-6,3	-9	-5,1	-8	-1,4
7	-5	-1,9	-3	-0,3	-4	-2,7
8	-7	-16,4	-7	-8,1	-5	-6.3

From the graph we can see the frequency at 2KHz on P4 decreased below SNR <6 at day 2 until day 8. It decreased from SNR <6 until SNR -7 on the right ear meanwhile the decreased on the left ear happened from SNR <6 until -1,64. At the 3 KHz frequency on P4 decreased happened from SNR <6 until SNR -7,1 on the right ear, when on the left ear SNR decreased from <6 until 0,3. At the 4 KHz frequency on P4 SNR decreased <6 on day 2 until day 8. The decreased started from SNR <6 until SNR -5 on the right ear meanwhile on the left ear SNR decreased f

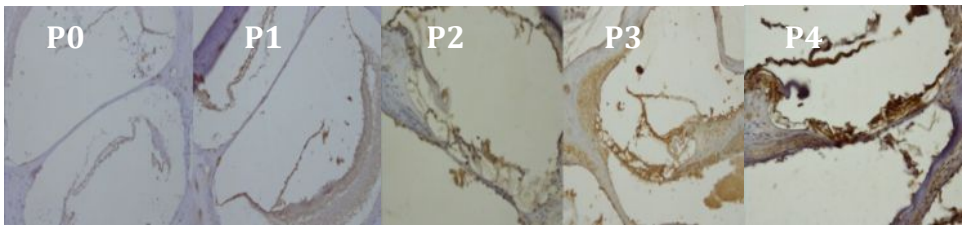

Figure 1. Expression of Hsp 70 antibodies with treatment intensity noisy 25 dB to 110 dB (400x).

In the control group (P0) there is not appear brown color it means there is no expression of Hsp 70 and damage in the outer hair cell and cell organ of corti. In the group (P1) and group (P2) there is appear brown color with a score 2-4, it means there has been expression of Hsp 70 in the outer hair cells and the cell organ of corti. In

the group (P3) there is appear more brown color with a score 4-7 (moderate intensity), it means there is increase of the expression of Hsp 70, and there is moderate damage in the organ of corti and the cell organ of corti. From the group (P4) there is appear increase of the expression of Hsp 70 with a score 8-9 (severe intensity). P4 group occurred Hsp 70 expression and there is appear brown color is more pronounced in all cells organ of corti. It means the total damage has occurred entirely on the hair cells and[2] cell organ of corti[3,4].

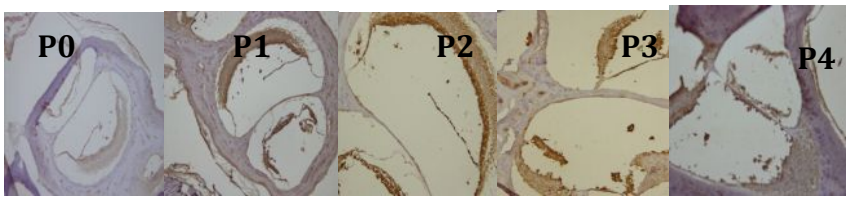

Figure 2. Expression of p53 antibodies with treatment intensity noisy 25 dB to 110 dB (400x).

In the control group (P0) there is not appear brown color it means there is no expression of P53. In the group (P1) and group (P2) there is appear brown color, it means there has been expression of P53 in the outer hair cells and the cell organ of corti. In the group (P3) there is appear more brown color with a score 7-8 (severe intensity), it means there is increase of the expression of P53, and there is severe damage in the organ of corti and the cell organ of corti. From the group (P4) there is appear increase of the expression of Hsp 70 with a score 8-9 (severe intensity). P4 group occurred P53 expression with a score 9 (severe intensity) and there is appear brown color is more pronounced in all cells organ of corti. It means the total damage has occurred entirely on the hair cells and cell organ of corti. The role of p53 is a regulator of the cycle of apoptosis, in which phosphorylation of p53 would occur[5]. This phosphorylation will activate and stabilize p53, which then converts the proapoptotic groups Bcl 2, Bax and the transcriptional level, and activating cytochrome C and caspase 3 which leads to cell death[6].

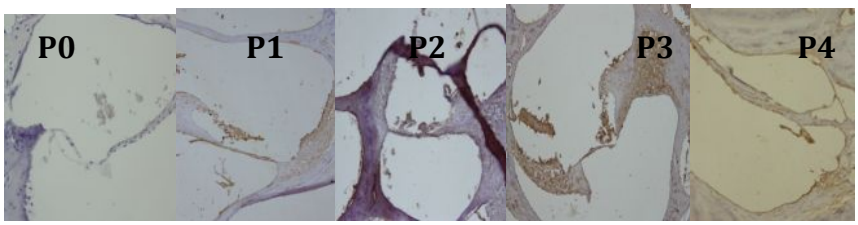

Figure 3. Expression of Cytochrome C antibodies with treatment intensity noisy 25 dB to 110 dB (400x).

In the control group (P0) there is not appear brown color it means there is no expression of cytochrome C and there is no damage in the outer hair cell and cell organ of corti. In the group (P1) and group (P2) there is appear brown color with a score 2-4, it means there has been expression of cytochrome C in the outer hair cells and the cell organ of corti. In the group (P3) there is appear more brown color with a score 5-6, it means there is increase of the expression of cytochrome C, and there is almost damage in the organ of corti and the cell organ of corti. From the group (P4) there is appear increase of the expression cytochrome C with a score 8-9. P4 group occurred cytochrome C expression and there is appear brown color is more pronounced in all cells organ of corti. It means the total damage has occurred entirely on the hair cells and cell organ of corti. Nicotera *et al.*[7], caspase activation, noise exposure caused the release of cytochrome c from mitochondria, resulting in a punctate fluorescence in the cytosol. In contrast to activation of caspases, the release of cytochrome c took place in both apoptotic and necrotic OHCs (Outer Hair Cell). Moreover, the release of cytochrome c in a subpopulation of OHCs took place early in the cell death process, prior to any outward signs of necrosis or apoptosis.

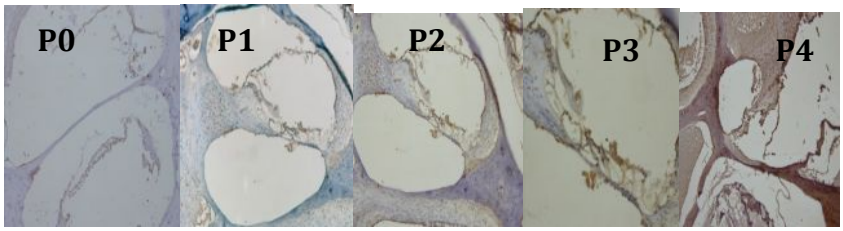

Figure 4. Expression of Caspase-3 antibodies with treatment intensity the noisy 25 dB to 110 dB (400x).

In the control group (P0) there is not appear brown color it means there is no expression of caspase 3 and there is no damage in the outer hair cell and cell organ of corti. In the group (P1) and group (P2) there is appear brown color with a score 2-4, it means there has been expression of caspase 3 in the outer hair cells and the cell organ of corti. In the group (P3) there is appear more brown color with a score 6-9 (moderate until severe intensity), it means there is increase of the expression of caspase 3, and there is moderate until severe damage in the organ of corti and the cell organ of corti. From the group (P4) there is appear increase of the expression of caspase 3 with a score 9 (severe intensity). It means the total damage has occurred entirely on the hair cells and cell organ of corti.

In the graph above it appears that in P0 group (controls) were found no cell damaged to organ of corti in all biomarker, in P1 group were found cell damaged organ of corti with intensity 3-4 (weak intensity), in P2 group found cell damage to organ of corti with 4-5 intensity (weak intensity), in the treatment group P3 were found cell damaged to organ of corti with the intensity of 7-8 (moderate intensity), in the treatment group P4 were found cell damaged to organ of corti reaching intensity 9 (strong intensity) at all biomarkers. Generally the graph above shows existence of cell damaged organ of Corti characterized by increased expression at all biomarker (Hsp 70, p53, Cytochrome C, Caspase 3) from the treatment group P0 to P4. The lowest level of damage in the organ of corti was found in the treatment group P0, and a high level of damage is found in the treatment group P4 of the noisy intensity was 100 to 110 dB.

The noise exposure triggered activation of caspase-3, an important mediator of apoptosis. The noise exposure also caused the activation of caspase-8 and caspase-9, each of which is associated with a distinct signaling pathway that leads to activation of caspase-3. Caspase activation occurred only in the apoptotic OHCs and not in the necrotic OHCs. These results indicate that multiple signaling pathways leading to caspase-3 activation take place simultaneously in the apoptotic OHCs[7].

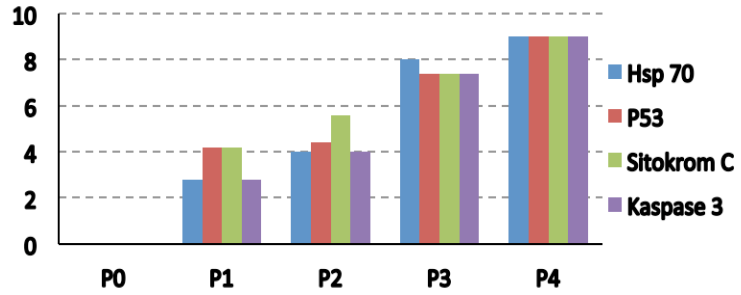

Figure 6. Histogram of the mean and SD scores of the organ of corti damage at all antibodies with various intensity noise of <25 dB to 110 dB (P0 to P4)

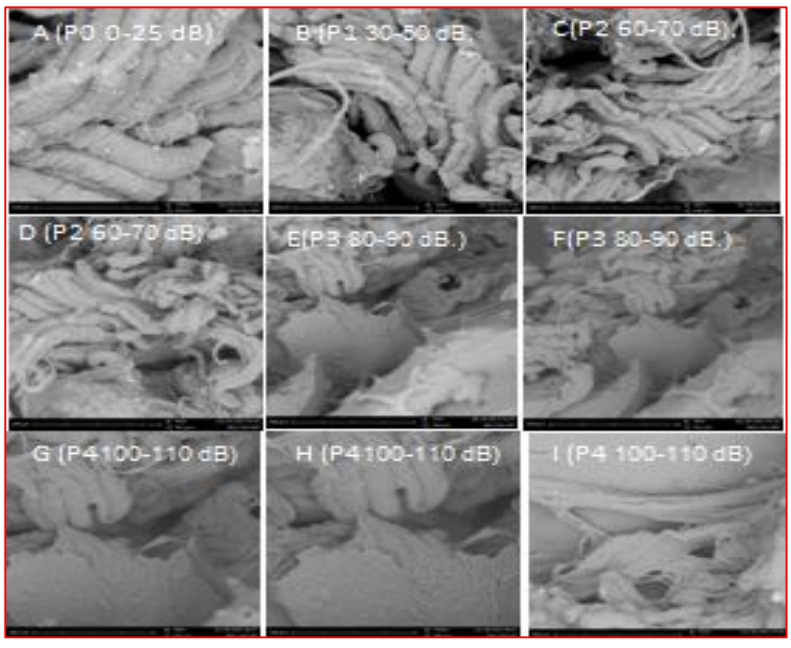

Figure 7. Ultrastructure of normal cochlea tissue to demaged region which are evaluated with Scanning Electron Microscope (SEM) (5000x).

The SEM result with 5000x magnification, ultrastructure corti organ on the cochlea such as the outer cells, inner cells, basiller membrane, Hensen cell, Deitter cell, and the supporting cell can be seen clearly. The stereocillia as part of the hair cell did not attach to the tectorial membrane in "U" shape, where as the stereocillia on the outer cell attach firmly on the upper tectorial membrane in "W" shape[8,9]. The result our study SEM to shown organ of corti at the group treatment group P0 was found lowest level <50 % of damage in the organ of corti. Group P1 to P4 showed the gradual destruction level >50%. It means to damaged ultrastructure region organ of corti to high level >50 % of damaged is found in the treatment group P4 of the noisy intensity was 100 to 110 dB. Hu *et al*.,[10] has reported that apoptosis is the major pathway by which expansion of the cochlear lesion takes place in the chinchilla after exposure to an intense noise.

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