



# THE EFFECT OF *CURCUMINOID* TO NOISE EXPOSURE VIEWED FROM THE EXPRESSION OF HEAT SHOCK PROTEIN-70 (HSP-70) IN COCHLEAR FIBROBLASTS OF *RATTUS NORVEGICUS*

TENGKU SITI HAJAR HARYUNA<sup>1\*</sup>, RAMSI LUTAN<sup>1</sup>, NYILO PURNAMI<sup>2</sup>, SUPRAPTO MA'AT<sup>3</sup> AND WIBI RIAWAN<sup>4</sup>

<sup>1</sup>Department of Otorhinolaryngology-Head and Neck Surgery, Faculty of Medicine, Universitas Sumatera Utara, Medan 20155, Indonesia

<sup>2</sup>Department of Otorhinolaryngology-Head and Neck Surgery, Faculty of Medicine, Universitas Airlangga, Surabaya 60131, Indonesia

<sup>3</sup>Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya 60131, Indonesia

<sup>4</sup>Department of Biochemistry, Faculty of Medicine, Universitas Brawijaya, Malang 65145, Indonesia

## ABSTRACT

Numerous epidemiological and experimental studies have been carried out so far, from which empirical evidences were obtained and various theories were suggested to elucidate a series of Noise-Induced Hearing Loss (NIHL) process at the molecular level. One of them is the increased expression of HSP-70 due to noise exposure. This study was conducted to demonstrate *curcuminoid* as the safe and effective phytopharmacy in order to prevent the damage of supporting tissues within the cochlear lateral wall which may lead to NIHL viewed from the expression of HSP-70. The samples were 40 *Wistar* strain white rats (*Rattus norvegicus*) divided into 8 groups: The control group/K1, Group 2/K2 noise (+) for 2 weeks, Group 3a/K3a noise (+) 50 mg/day *curcuminoid* (+) for 2 weeks, Group 3b/K3b noise (+) 100 mg/day *curcuminoid* (+) for 2 weeks, Group 4a/K4a noise (+) 50 mg/day *curcuminoid* (+) for 2 weeks, untreated for the next 2 weeks, Group 4b/K4b noise (+) 100 mg/day *curcuminoid* (+) for 2 weeks, untreated for the next 2 weeks, Group 5a/K5a 50 mg/day *curcuminoid* (+) for 2 weeks, noise (+) and 50 mg/day *curcuminoid* (+) for the next 2 weeks and Group 5b/K5b 100 mg/day *curcuminoid* (+) for 2 weeks, noise (+) and 100 mg/day *curcuminoid* (+) for the next 2 weeks. This study used *curcuminoid* derived from *Curcuma longa* L. (Turmeric) with *curcuminoid* content levels  $[28.1 \pm 1.0]\%$  w/w compared to Standard. All samples were examined for the expressions of HSP-70 in cochlear fibroblasts by Immunohistochemistry method. The data were processed with Analysis of Variance (ANOVA) using the Statistical Analysis System (SAS). The results obtained showed significant differences for the expressions of HSP-70 ( $p < 0.05$ ) in all treatment groups, except in Group 3a and 3b. *Curcuminoid* proved to be potentially effective in the prevention and treatment for the damage of supporting tissues within the cochlear lateral wall regarding the decreased expression of HSP-70.

**Keywords:** Curcuminoid, HSP-70, NIHL, Fibroblast, Cochlea

## 1. INTRODUCTION

In 2001, WHO indicated that there were 250 million people worldwide who suffered from moderate or severe hearing impairment, which increased to more than 275 million people in 2004, 80% of them were found in developing countries. These numbers

continue to rise progressively since the first study carried out by WHO in 1986 (World Health Organization, 2006). According to WHO's report (2004), it was estimated that almost 14% of the total labor forces in industrialized countries were

exposed to noise exceeding 90 dB at their working places. The consultation meeting of WHO-SEARO (South East Asia Regional Office) Intercountry Meeting (2002) stated that noise has been listed as one of the main problems inducing hearing impairment. Occupational Noise-Induced Hearing Loss (ONIHL) has occupied the highest proportion compared to other noise-induced impairments (Bashiruddin and Soetirto, 2007). Numerous epidemiological and experimental studies have been carried out so far, from which empirical evidences were obtained and various theories were suggested to elucidate a series of Noise-Induced Hearing Loss (NIHL) process at the molecular level. As a matter of fact, until now, there is still no drug available that can be used on the basis of molecular biology for the preventive and protective measures against the effects of noise exposure resulting in hearing impairment yet (Cappaert et al., 2000; Altschuler et al., 2002; Le Prell et al., 2003; Henderson et al., 2006; Kujawa and Liberman 2006). A recent study found the role of supporting tissues within the cochlear lateral wall which may lead to NIHL despite there is no damage of sensory cells, outer hair cells and stereocilia (Purnami, 2009). Noise exposure inflicts cellular stress that furthermore triggers *chaperone* activity and activates inflammatory signaling pathways subsequently. (Wang et al., 2002; Hirose and Liberman, 2003; Purnami, 2009). Extracellular matrix that serves to maintain the elasticity of the basal membrane located in the organ of Corti, has an important role in the process of sound transduction. Excessive noise exposure affects the cochlear fibroblasts, leading to an increase in the permeability of cell membrane and an increase in intracellular  $Ca^{2+}$  (Purnami, 2009). The exaggerated increase of  $Ca^{2+}$  in cell triggers cell death and activates intracellular signaling pathways. Activation of HSF-1 gene promotes an increase in HSP-70 (a family of molecular *chaperones*) synthesis, which at certain level acts as the cell protector in order to maintain homeostasis against stress response (Le Prell et al., 2003; Purnami, 2009). HSP-70 may serve as an agonist ligand for TLR-2 and TLR-4 in the transmission of intracellular signaling pathways by the activation of a transmembrane receptor and then transmits the subsequent signal excitation to the underneath effector molecules. Extracellular HSP-70 (eHSP-70) activates NF $\kappa$ B in the cell surface and induces NF $\kappa$ B activation (30 minutes) and regulates the expression of proinflammatory cytokines in the

human monocytes (2 hours after exposure). eHSP-70 molecule acts as a mediator in the production of proinflammatory cytokines (Asea, 2005; Calderwood et al., 2007; Purnami, 2009). Turmeric (*Curcuma domestica* Val.) also known as *Curcuma longa* L. is one of the spice plants originated from Asia, particularly Southeast Asia. In Asia, turmeric has been used as a medicine since 2000 BC. The use of turmeric in the world of medicine is rapidly increasing after the discovery of phenolic compounds commonly called *curcuminoid*. Turmeric contains three active substances of *curcuminoid*, namely *curcumin*, *bisdemethoxycurcumin* and *demethoxycurcumin*. They share similar scientific classification and are reported to possess anti-inflammatory and therapeutic effects. Protection mechanism against inflammation and oxidative damage serves *curcumin* as a natural agent against tissue damage. *Curcumin* can down-regulate the expression of cytokines and chemokines, also play a role in the suppression of NF $\kappa$ B activation (Thaloor et al., 1999; Surh et al., 2001; Wright, 2002; Hong et al., 2004; Wyke et al., 2004; Johnson and Mattia, 2006). This study used *curcuminoid* derived from *Curcuma longa* L. since it is expected to be able to treat and even prevent the damage of cochlear fibroblasts as stressed cells due to noise exposure. This study was conducted to demonstrate that *curcuminoid* is able to decrease the expression of HSP-70; a dose of *curcuminoid* 100 mg per day was more preferable than a dose of *curcuminoid* 50 mg per day in the noise-exposed cochlear fibroblast cells at frequency range of 1-10 kHz 100 dB SPL for 2 hours.

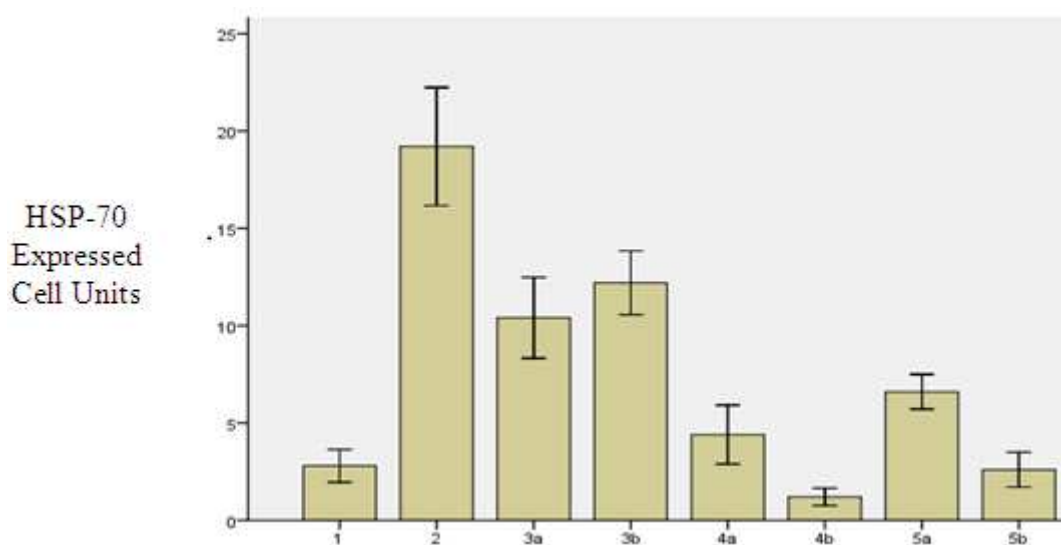
## 2. MATERIALS AND METHODS

This study was an experimental study with randomized post test only control group design using *Wistar* strain white rats (*Rattus norvegicus*). A noise frequency within the range of 1 kHz to 10 kHz was used based on the consideration of the noise characteristics influenced by the sensitivity range of interception capability of sense of hearing in humans and rats (Heffner and Heffner, 2007). The noise exposure dose given was 100 dB SPL for 2 hours since significant differences were found in protein expressions for HSP-70, NF $\kappa$ B, TLR-2, TLR-4, MMP-9 and Type IV Collagen at that noise exposure dose (Purnami, 2009). The samples were 40 rats divided into 8 groups: The control group/K1,

Group 2/K2 noise (+) for 2 weeks, Group 3a/K3a noise (+) 50 mg/day *curcuminoid* (+) for 2 weeks, Group 3b/K3b noise (+) 100 mg/day *curcuminoid* (+) for 2 weeks, Group 4a/K4a noise (+) 50 mg/day *curcuminoid* (+) for 2 weeks, untreated for the next 2 weeks, Group 4b/K4b noise (+) 100 mg/day *curcuminoid* (+) for 2 weeks, untreated for the next 2 weeks, Group 5a/K5a 50 mg/day *curcuminoid* (+) for 2 weeks, noise (+) and 50 mg/day *curcuminoid* (+) for the next 2 weeks and Group 5b/K5b 100

mg/day *curcuminoid* (+) for 2 weeks, noise (+) and 100 mg/day *curcuminoid* (+) for the next 2 weeks. This study used *curcuminoid* derived from *Curcuma longa* L. (Turmeric) with *curcuminoid* content levels  $[28.1 \pm 1.0]\%$  w/w compared to Standard. All samples were examined for the expressions of HSP-70 in cochlear fibroblasts by Immunohistochemistry method. The data were processed with Analysis of Variance (ANOVA) using the Statistical Analysis System (SAS).

## 1. RESULTS



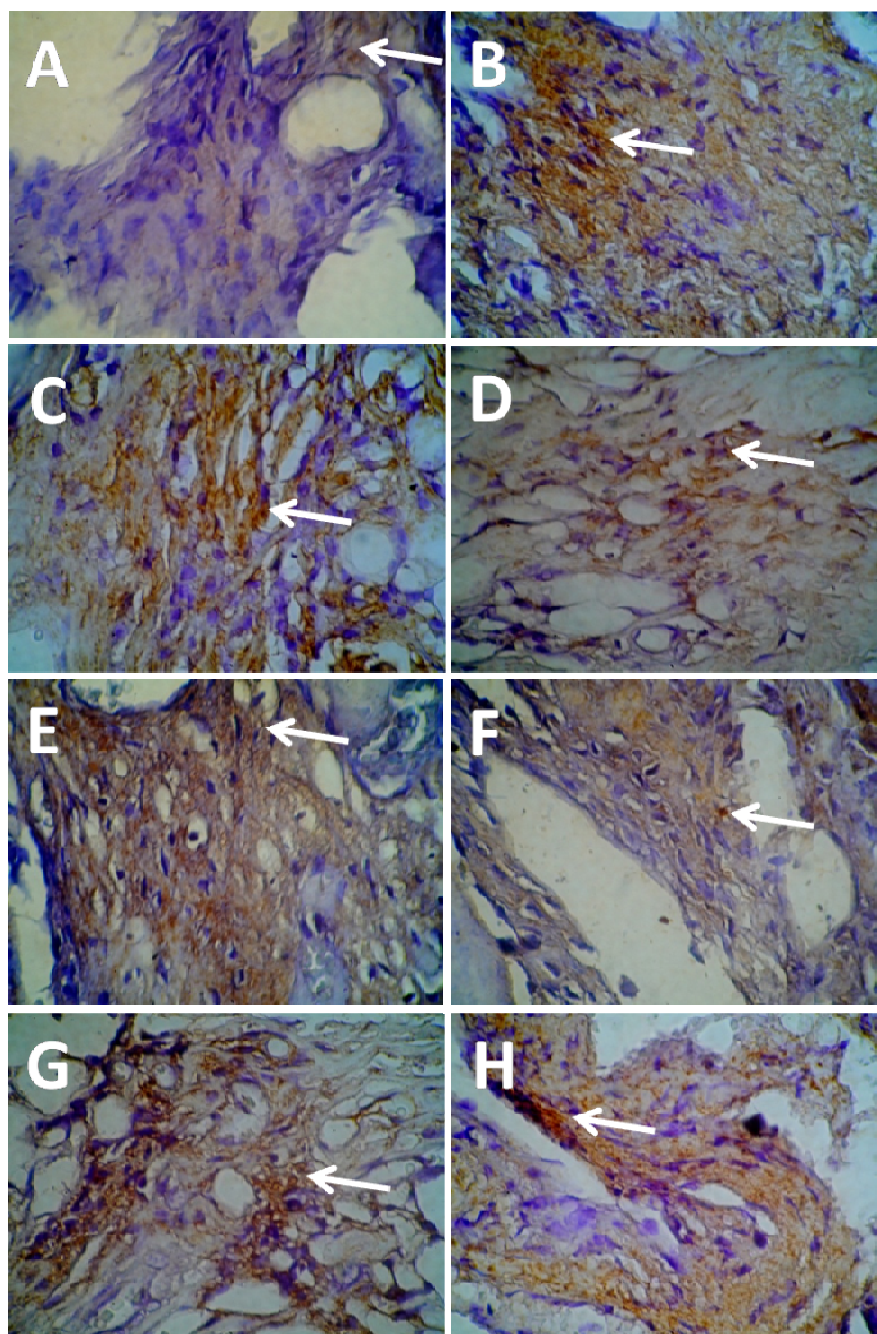
**Figure 1**  
*The expression of HSP-70 in each group*

The expression of HSP-70 was found to increase in group 2 (*curcuminoid*-untreated group) and decreased in *curcuminoid*-treated groups (group 3a, 3b, 4a, 4b, 5a and 5b). In general, a dose of *curcuminoid* 100 mg per day showed statistically significant decreases in the expressions of HSP-70 rather than a dose of *curcuminoid* 50 mg per day (Fig. 1).

**Table 1**  
*ANOVA test results in regard of the expressions of HSP-70*

| Contrast                                     | Pr > F |
|--|--------|
| K1 VS K2 & K3a & K3b & K4a & K4b & K5a & K5b | <.0001 |
| K2 VS K3                                     | <.0001 |
| K2 VS K4                                     | <.0001 |
| K2 VS K5                                     | <.0001 |
| K3a VS K3b                                   | 0.0885 |
| K4a VS K4b                                   | 0.0038 |
| K5a VS K5b                                   | 0.0005 |

Data in Table 1 above showed significant differences for the expressions of HSP-70 ( $p < 0.05$ ) in all treatment groups, except in Group 3a and 3b.



**Figure 2**

*The expression of HSP-70 in each group (1000x zoom):*

*(A) group 1; (B) group 2; (C) group 3a; (D) group 3b;*

*(E) group 4a; (F) group 4B; (G) group 5a; (H) group 5b.*

*The white arrow indicates the expression of HSP-70 in cochlear fibroblasts marked by the brown color*

The expression of HSP-70 after being evaluated with Immunohistochemistry method showed an increased expression in group 2 [Fig. 2(B)] compared to other treatment groups. In the *curcuminoid*-treated groups, the expression of HSP-70 seen in the brown color showed lower density [Fig. 2(C), (D), (E), (F), (G), and (H)]. This result showed significant differences in the number of expressed cells among all treatment groups.

## 4. DISCUSSION

This study was conducted on experimental animals, *Wistar* strain white rats (*Rattus norvegicus*), since this species is a laboratory animal commonly used as an essential model in biomedical research in order to explain various diseases in humans. The similarity in structure and ear pathobiology made this animal widely used in genetic research and experiments since it is easily obtainable and rapidly multiplied in order to get homogenous populations required for genetic similarity (Gravel and Ruben, 1996; Canzian, 1997; Steel, 1998). Due to the homologous gene and structural sequence (>70%) with humans, rats possess the potential for developing studies on human genetic hearing loss and proved beneficial in identifying the corresponding gene in humans that plays a role in the development of the auditory system (Purnami, 2009). Moreover, histopathological changes observed from previous studies showed that rats were clinically relevant to humans (Gravel and Ruben, 1996; Canzian, 1997; Steel, 1998). Cellular stress response in receiving extracellular signals in the form of chemical signals such as protein, can affect and even alter the physiological state of a cell, thus influence the protein expression (Stansfield et al., 2006). The expression of HSP-70 in fibroblasts marked by the brown color in the cytoplasm was dominantly expressed in group 2. This increased expression of HSP-70 in fibroblasts complements other studies that also found increased expression of HSP-70 in the cochlear hair cells and stria vascularis as well. The vital role of HSP-70 in cell protection is provided through the facilitation of refolding process or as a molecular *chaperone* to damaged proteins which furthermore can prevent protein aggregation (Fleshner et al., 2007). HSP-70 is present in the cytoplasm and it has a crucial role in intracellular processes and also provides a cytoprotective effect. In the state of stress (noise-exposed), cells spur a striking increase in protein production triggered by the higher activation of HSF-1 than usual. An excessive increase in HSF-1 production provokes the nucleus to regulate activated receptors by activating the transcription of I $\kappa$ B transcripts which serve as inhibitors. The exaggerated increase of HSP-70 in the cytoplasm leads to excessive secretion of HSP-70 in cells called eHSP-70 (Asea, 2005). Intracellular HSP-70 possess an important role in the secretion of HSP-70 out of the cell called eHSP-70 that will be recognized by the receptors of TLR-2 and TLR-4.

HSP-70 provides strong contribution together with the expression of TLR-2 and TLR-4 and increased HSP-70 in stressed cells in order to boost the immune system (Asea, 2005; Purnami, 2009). An increase expression of HSP-70 in group 2 triggered by the presence of stress factors (noise) that stimulates Ca<sup>2+</sup> entry into cells excessively then activates protein kinase. This activation provokes the phosphorylation of HSF, leading to the loss of the bond between HSP-70 and HSF. This free HSP-70 lies within the cytoplasm while HSF enters towards the nucleus as a transcription factor that binds to HSE and activates HSP-70 genes in order to get transcribed. This study proved that *curcuminoid* can decrease the expression of HSP-70 in the cochlear fibroblast cytoplasm since *curcuminoid* is able to inhibit the phosphorylation of HSF-1, leading to less translocation of HSF-1 towards the nucleus. Hence, HSF-1 in the nucleus as a transcription factor that binds to HSE become less in amount as well as the activation of HSP-70 gene to produce HSP-70. In group 3, dose differences did not affect the expression of HSP-70 while in group 4 and 5, dose differences did affect the expression of HSP-70, where a dose of *curcuminoid* 100 mg per day showed significant decreases in the expression of HSP compared to a dose of *curcuminoid* 50 mg per day. This is due to that a higher dose of *curcuminoid* can inhibit more phosphorylation of HSF-1. Group 4 showed lower expression of HSP-70 than in group 5 due to the dismissal of noise stimuli for 2 weeks before the termination process, thus during that time, the phosphorylation of HSF occurred minimally.

## 5. CONCLUSION

This study proved that *curcuminoid* was able to prevent the damage of cochlear fibroblasts exposed to noise at a frequency range of 1-10 kHz, 100 dB SPL intensity for 2 hours per day, which was statistically significant, regarding the decreased expression of HSP-70; where a dose of *curcuminoid* 100 mg per day was more preferable than a dose of *curcuminoid* 50 mg per day. As it has already been proven for experimental animals, in further research, *curcuminoid* is hopefully able to prevent and potentially repair the noise-exposed cochlear fibroblasts in humans, so that *curcuminoid* can be effectively and largely used by communities as a phytopharmacy in order to prevent and treat NIHL.

## 6. ACKNOWLEDGEMENT

The authors are deeply indebted to Biochemistry Laboratory, Faculty of Medicine, Universitas Airlangga, Surabaya; Anatomic Pathology

Laboratory, Faculty of Medicine, Dr. Soetomo General Hospital, Surabaya; Biochemistry Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, for providing equipment and scientific apparatus.

## 7. REFERENCES

1. Alexzander Asea. Stress proteins and initiation of immune response: chaperone activity of hsp72. *Exerc Immunol Rev.* 2005;11:34-35
2. Clifford Johnson B and Antonia Mattia N. Curcumin. 2006 (Cited 2012 April 7). Available from <http://www.inchem.org/documents/jecfa/jecmono.htm>
3. Collen Le Prell G, David Dolan F, Jochen Schacht, Josef Miller M, Margaret Lomax I and Richard Altschuler A. Pathways for protection from noise induced hearing loss. *Noise and Health.* 2003;5(20):1-17.
4. Deepa Thaloor, Kristy Miller J, Jonathan Gephart, Patrick Mitchell O and Grace Pavlath K. Systemic administration of the NF- $\kappa$ B inhibitor curcumin stimulates muscle regeneration after traumatic injury. *Am J Physiol.* 1999;277(2 Pt 1):320-329.
5. Donald Henderson, Eric Bielefeld E, Kelly Harris C and Bo Hu H. The role of oxidative stress in noise-induced hearing loss. *Ear and Hearing.* 2006;27(1):1-19.
6. Federico Canzian. Phylogenetics of the laboratory rat *rattus norvegicus*. *Genome Res.* 1997;7(3):262-267.
7. Henry Heffner E and Rickye Heffner S. Hearing ranges of laboratory animals. *JAALAS.* 2007;46(1):11-13.
8. James Wright S. Predicting the antioxidant activity of curcumin and curcuminoids. *J Mol Struct (Theochem)* 2002;59:207-217.
9. Jenny Bashiruddin and Indro Soetirto. Gangguan pendengaran akibat bising (noise-induced hearing loss). 4th ed. Jakarta: Balai Penerbit FK UI; c2007. Buku Ajar Ilmu Kesehatan Telinga Hidung Tenggorokan Kepala dan Leher; p.49-52.
10. Judith Gravel S and Robert Ruben J. Auditory deprivation and its consequences: from animal models to humans. New York: Springer-Verlag; c1996. *Clinical Aspects of Hearing*; p.86-109.
11. Jungil Hong, Mousumi Bose, Jihyeung Ju, Jae Ryu H, Xiaoxin Chen, Shengmin Sang, Mao Lee J and Chung Yang S. Modulation of arachidonic acid metabolism by curcumin and related beta-diketone derivatives: effects on cytosolic phospholipase A(2), cyclooxygenases and 5-lipoxygenase. *Carcinogenesis.* 2004;25(9):1671-1679.
12. Karen Steel P. A new era in the genetics of deafness. *New Engl J Med.* 1998;339(21):1545-1547.
13. Keiko Hirose and Michael Liberman C. Lateral wall histopathology and endocochlear potential in the noise damaged mouse cochlea. *J Assoc Res Otolaryngol.* 2003;4(3):339-352.
14. Monika Fleshner, Craig Sharkey M, Molly Nickerson and John Johnson D. Bi-directional effects of stress on immune function: possible explanations for salubrious as well as harmful effects. 4th ed. New York: Elsevier Academic Press; c2007. *Psychoneuroimmunology*; p.1013-1028.
15. Natalie Cappaert LM, Sjaak Klis FL, Hans Muijser, Beverly Kulig M and Guido Smoorenburg F. Noise-induced hearing loss in rats. *Noise and Health.* 2000;3(9):23-32.
16. Nyilo Purnami. Efek pajanan bising terhadap peningkatan HSP 70, TNF $\alpha$ , TLR-2 dan TLR-4 pada fibroblast koklea *Rattus norvegicus*. PhD diss. 2009. Universitas Airlangga: Surabaya.
17. Richard Altschuler A, Damon Fairfield A, Younsook Cho, Elena Leonova, Ivor Benjamin J, Josef Miller M and Margaret Lomax I. Stress pathways in the rat cochlea and potential for protection from acquired deafness. *Audiol Neurotol.* 2002;7(3):152-156.
18. Sharon Kujawa G and Michael Liberman C. Acceleration of age-related hearing loss by early noise exposure: evidence of a missed youth. *J Neurosci.* 2006;26(7):2115-2123.
19. Stacey Wyke M, Stephen Russel T and Michael Tisdale J. Induction of proteasome

- expresion in skeletal muscle is attenuated by inhibitor of NF- $\kappa$ B activation. *Br J Cancer*. 2004;91(9):1742-1750.
20. Stuart Calderwood K, Salamatu Mambula S, Phillip Gray J and Jimmy Theriault R. Extracellular heat shock proteins in cell signaling. *FEBS Lett*. 2007;581:3689-3694.
21. William Stansfield, Jaime Colome and Raul Cano. *Biologi Molekuler dan Sel*. Schaum's Easy Outlines. Jakarta: Penerbit Erlangga. 2006:101.
22. World Health Organization. Primary ear and hearing care training resource advance level. 2006. (Cited 2012 April 7). Available from <http://www.who.int/pbd/deafness/activities/hearingcare/advance.pdf>.
23. Yong Wang, Keiko Hirose and Michael Liberman C. Dynamics of noise-induced cellular injury and repair in the mouse cochlea. *J Assoc Res Otolaryngol*. 2002;3(3):248-268.
24. Young Surh J, Kyung Chun S, Hyun Cha H, Seong Han S, Young Keum S, Kwang Park K and Sang Lee S. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: Down-regulation of COX-2 and iNOS through suppression of NF- $\kappa$ B activation. *Mutat Res*. 2001;480-481:243-268.