The Effect of *Curcuminoid* to Noise Exposure Viewed from The Expressions of Calcineurin (CaN) and Heat Shock Protein (HSP-70) in Cochlear Fibroblasts of *Rattus Norvegicus*

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**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. Two of them are the increased expressions of Calcineurin (CaN) and Heat Shock Protein (HSP-70) due to noise exposure. This study was conducted to demonstrate *curcuminoid* as the safe and effective phytopharmacy in order to treat the damage of supporting tissues within the cochlear lateral wall which may lead to NIHL viewed from the expressions of CaN and HSP-70. The samples were 24 *Wistar* strain white rats (*Rattus norvegicus*) divided into 4 groups: Group 1 (Control), Group 2 noise (+) for 2 weeks, Group 3 noise (+) 50 mg/day *curcuminoid* (+) for 2 weeks, Group 4 noise (+) 100 mg/day *curcuminoid* (+) for 2 weeks. This study used *curcuminoid* derived from *Curcuma longa* L. (Turmeric) with *curcuminoid* content levels [28.1 ± 1.0]% w/w compared to Standard. All samples were examined for the expressions of CaN and HSP-70 in cochlear fibroblasts by Immunohistochemistry method. The data were processed with ANOVA. The results obtained showed significant differences for the expressions of CaN (p<0.05) in all groups and HSP-70 (p<0.05) in all groups, except in Group 3 and 4. *Curcuminoid* proved to be potentially effective in the treatment for the damage of supporting tissues within the cochlear lateral wall regarding the decreased expressions of CaN and HSP-70.

**Keywords:** *curcuminoid*, calcineurin, HSP-70, NIHL, cochlea
BACKGROUND

Noise is an important environmental issue and is currently a concern and a global problem because of the high prevalence and effects that can affect people of all ages and genders. Continuous noise can lead to serious disturbances and affect the physiological and psychological to the social function and a decrease in productivity of a person (Bashiruddin, 2009).

Hearing loss due to noise / GPAB (Noise-Induced Hearing Loss / NIHL) occurs due to prolonged exposure to noise that causes damage to the hair cells that also occurs gradually, slowly so as not recognized by the sufferer. In the severe stages can interfere with communication, thus affecting social life. NIHL irreversible and incurable. therefore, prevention is very important (Bashiruddin, 2009; Muyassaroh & Habibi, 2011).

World Health Organization (WHO) reported in 2000 there were 250 million (4.2%) experienced NIHL world population. In the United States there are an estimated 5-6 million people are deaf due to noise. While in the Netherlands amounted to 200,000-300,000 people, around 0.2% in England, in Canada and Sweden each about 0.3% of the entire population. In Southeast Asia about 75-140 million (50%), in this case Indonesia ranks fourth in Southeast Asia after Sri Lanka is 4.6% (8.8%), Myanmar (8.4%) and India (6.2%). That number is expected to increase (Amalia & Lanjah, 2012).

According to the National Health and Nutrition Examination Survey in the United States account for 15% of adolescents experience GPAB in 1988. That number jumped to 19.5% in 2000 (Herman, 2011; Supit et al., 2012; Haryuna, 2013).

In order GPAB prevention and treatment have been carried out epidemiological and experimental studies in order to obtain empirical evidence and theory is expected to explain the occurrence of GPAB down to the molecular level. However, until now there has not been found a complete mechanism that can be used as the cornerstone of prevention and treatment, considering the number of the mechanisms underlying the molecular NIHL seen from the changes in the hair cells, fibroblasts cochlear and other structures due to exposure to noise (Minami et al., 2004; Purnami 2009; Haryuna, 2013).

Exposure to noise result in permanent damage to the inner ear to cause interference with hearing, with the occurrence of structural changes, including the
loss of sensory hair cells in the inner ear and stereocilia damage to the intensity and duration of exposure (Minami et al., 2004).

Exposure to noise is also able to cause molecular changes fibroblasts lateral wall of the cochlea as a structural constituent of the extracellular matrix are known to be responsive to excessive noise stimulus in continuous and long-term as a cellular stress. Noise can cause disruption in the cochlea fibroblasts without damage to the sensory hair cells. Based on previous studies found changes in protein expression, where an increase in the expression of HSP-70, TNF-α, IL-6, NFκB, TLR-2, TLR-4, MMP-9 in fibroblasts cochlea due to noise exposure were observed histopathologically. These proteins are found in a pathway that caused the degradation of collagen by fibroblasts GPAB cochlear especially Collagen Type IV (Purnami, 2009; Haryuna, 2013).

Excessive acoustic stimulation increases the concentration of Ca²⁺ in the auditory hair cells and fibroblasts cochlea with increasing cell membrane permeability. This increase implies dysfunction of cochlear hair cells and fibroblasts, both initiating damage after noise exposure (Minami et al., 2004; Purnami, 2009).

There are a number of cell death pathways mediated by Ca²⁺, Ca²⁺ is also involved in the activation of Nitric Oxide Synthase (NOS), phospholipase A2, proteases, and calcineurin (CaN) (Minami et al., 2004).

Ca²⁺ excess intra-cell binds to Calmodulin (CaM) to activate CaN. Ties are both significantly dependent on Ca²⁺, where the presence of Ca²⁺ CaM binding has led to a high affinity to CaN (Perrino and Soderling, 1998).

HSP is a protein (in the form of molecular chaperones) resulting from the Heat Shock Response (HSR). HSR is a genetically based response to induce genes that encode molecular chaperones, proteases and proteins that are important in defense mechanisms and the recovery of cellular injury associated with the occurrence of misfolded proteins (protein folding which is unusual). HSR is a response of cells to a variety of disorders, both physiological as well as from the environment (Henderson, Hu & Bielefeld, 2008).

In the physiological state, HSP function as molecular chaperones and assist the synthesis, folding, assembly and intracellular transport of various proteins. HSP expression increases under conditions of full stress such attacks hyperthermia (heat) and other stress stimuli, including ischemic, viral infections, exposure to noise, toxic materials and others (Wijdaja, Santoso & Waspadji, 2009).
HSP in particular ear cochlear cells is induced after exposure to excessive noise from the environment. When induced by noise with a certain level of HSP showed a protective effect against the cochlea by maintaining the integrity of the cell membrane protein structure and protein function of other cells so that the cells are not damaged cochlea after noise exposure (Widjaja, Santoso & Waspadji, 2009).

At the level of the organ of Corti, the ears do the heat shock response by activating the expression of cellular defense factors, resulting in a heat shock protein, HSP-70 in particular (Yang, et al., 2006).

Turmeric (Curcuma domestica Val.) with synonyms Curcuma longa L. including herbal plants originating from Asia, especially Southeast Asia. In Asia, turmeric has been used as a medicine since 2000 BC. The use of turmeric in medicine increased rapidly after the discovery of phenolic compounds commonly called curcuminoid. Curcuminoid is a yellow pigment extracted from rhizomes, which can also be derived from Curcuma Roxb xanthorrhiza (ginger). Turmeric contains three main active curcuminoid is curcumin, bisdemethoxycurcumin and demethoxycurcumin. Curcuminoid compounds are widely studied because they have many pharmacological and therapeutic activities. Among them as an anti-oxidant, anti-microbial, anti-viral, anti-carcinogenic, anti-inflammatory, chemo sensitization, radio sensitization and wound healing (Shim et al., 2001; Surh et al., 2001; Wright, 2002; Hong et al., 2004 ; Lao et al., 2006; Mehrotra et al., 2013).

This experimental study using mice as experimental animals. This is done with respect to the treatment procedure and final examination are fatal. Rats also has some similarities with the structure of the ear in humans and has been used as animal models to study human diseases and genetic deafness proved useful in helping to identify the corresponding gene in humans that plays a role in the development of the auditory system. Through genetic identification and the sequence, say rodents homologous (> 70%) to human (Gillespie et al., 2006).

The treatment is given in the form of exposure to noise at a frequency of 1 s / d 10 kHz with an intensity of 100 dB SPL 2 hours per day for 2 weeks. Frequency noise with a range of 1 s / d 10 kHz was selected based on consideration of noise characteristics that are influenced by a range of auditory sensitivity catching ability of human and mouse. Noise exposure dose of 100 dB SPL for 2 hours used based on previous studies that obtain significant protein expression differences for HSP-70, TNF-α, IL-6, NFκB, TLR-2, TLR-4, MMP-9, Collagen Type II and type IV Collagen.
These proteins are found in other pathways that underlie the occurrence of structural damage fibroblasts GPAB through cochlear Rattus norvegicus (Bashiruddin and Soetirto, 2007; Heffner, 2007; Purnami 2009; Haryuna, 2013).

This study used curcuminoid derived from Curcuma longa L. as expected curcuminoid can treat and even prevent damage to the cochlea as fibroblast cells undergoing stress due to noise exposure given. Curcuminoid preparations given in the form of powder at a dose of 50 mg and 100 mg per day / rat because based on previous studies, the dose may decrease the expression of NFkB, TLR-2, TLR-4, and MMP-9. These proteins are found in other pathways underlying GPAB, through the degradation of type IV Collagen in the cochlea Rattus norvegicus fibroblasts (Haryuna, 2013).

The results of this study are expected to be useful in efforts to find a preventive measure and handling NIHL in molecular biology that ultimately aimed to reduce the prevalence of clinically NIHL.

MATERIAL & METHOD

The type of research laboratory experimental ex vivo. The design of this study used a randomized design post-test only control group experimental design laboratory to determine the effects of exposure to noise curcuminoid in experimental units with variable measure that is only performed after the treatment.

The samples were 24 Wistar strain white rats (Rattus norvegicus) divided into 4 groups: Group 1 (Control), Group 2 noise (+) for 2 weeks, Group 3 noise (+) 50 mg/day curcuminoid (+) for 2 weeks, Group 4 noise (+) 100 mg/day curcuminoid (+) for 2 weeks.

Pictures examination with HE staining was used as a comparison with the above Immunohistochemistry staining. Cross section of the lateral cochlear wall painting mice with HE 40x magnification.
RESULTS

Expression of CaN after an evaluation by Immunohistochemistry increased in group 2 (Figure B) compared to other treatment groups. In group 1 (control) (Figure A) visible expression of CaN in the cytoplasm of fibroblasts with brown color density is less than the other treatment groups.

Figure 1. Expression of CaN images in each treatment group.
Remarks: A: Group 1, B: Group 2, C: Group 3, D: Group 4
The arrow indicates the CaN expression in fibroblasts cochlear marked with brown.

Expression of HSP-70 after an evaluation by Immunohistochemistry increased in group 2 (Figure B) compared to other treatment groups. In group 1 (control) (Figure A) visible expression of HSP-70 in the cytoplasm of fibroblasts with brown color density is less than the other treatment groups.
Figure 2. Expression of HSP-70 images in each treatment group.

Remarks: A: Group 1, B: Group 2, C: Group 3, D: Group 4

The arrow indicates the HSP-70 expression in fibroblasts cochlear marked with brown.

Figure 3. Expression relative / Field of view

From the bar chart shows that the curcuminoid able to decrease the expression of CaN and HSP-70.
Table 1. Anova Test Results

<table>
<thead>
<tr>
<th>Group</th>
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<tbody>
<tr>
<td>CaN</td>
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</tr>
<tr>
<td>2</td>
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</tr>
<tr>
<td>4</td>
<td>20,33</td>
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<tr>
<td>3</td>
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<td>C</td>
</tr>
<tr>
<td>1</td>
<td>2,83</td>
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<th>Group</th>
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<td>HSP-70</td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>19,20</td>
<td>A</td>
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<td>4</td>
<td>12,20</td>
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<td>B*</td>
</tr>
<tr>
<td>1</td>
<td>2,80</td>
<td>C</td>
</tr>
</tbody>
</table>

*Figures followed by the same letter are not significantly different according to LSD (least significant different).

Results of statistical analysis showed that there were significant differences for each treatment group, the same was found in HSP-70 except in groups 3 and 4.

DISCUSSION

Increased HSP-70 in group 2 caused by the presence of stress factors (noise) which causes Ca\(^{2+}\) entry into the cell in excess and will activate protein kinases. Activation of protein kinase will lead to phosphorylation of HSF. Phosphorylation of HSF resulted in the loss of the bond between HSP-70 and HSF. HSP-70 will be loose in the cytoplasm while the HSF will fit into the cell nucleus as a transcription factor and binds to the HSE that HSP-70 activates genes for transcripted.

In this study proved that the curcuminoid can decrease the expression of HSP-70 in the cytoplasm in fibroblasts cochlea due curcuminoid fosforilisasi able to inhibit HSF-1 so that HSF-1 into the nucleus becomes less, as a result of HSF-1 in the nucleus as a transcription factor that binds with the HSE being a bit so that the activation of the HSP-70 gene to produce HSP-70 is also the less.

CaN is part of a family of Ca\(^{2+}\) / calmodulin-dependent protein phosphatase, Phospatase Protein 2B (PP2B). CaN is the only protein phosphatase regulation by second messengers Ca\(^{2+}\) (Minami et al., 2004).
Noise exposure caused Ca\(^{2+}\) entry into cells of the cochlea fibroblasts in excess amount. This excess Ca\(^{2+}\) binds to CaM (Calmodulin) to be able to activate CaN to its active form. CaM binding to its own domain in the subunit structure of CNA on CaN. Ties are both significantly dependent on the presence of Ca\(^{2+}\), Where the presence of Ca\(^{2+}\) causes CaM binding has a high affinity for CaN (Perrino and Soderling, 1998; Minami et al., 2004).

In this study, curcumin inhibits the alleged regulation and expression of CaN, seen from a decrease in the expression of CaN in groups 3 and 4.

CaN is activated by HSP 90 through CaM-independent mechanism, and can be activated by HSP 70 through CaM-dependent mechanism. These results suggest that cAMP-dependent protein kinase provides on / off switch for the regulation of CaN by HSP 70 (Lakshmikuttyamma, Selvakumar and Sharma, 2006).

HSP 70 interacts directly with CaN via cAMP-dependent protein kinase which HSP 70 was able to stimulate and activate CaN in the presence of CaM (Seomeren et al., 1999; Lakshmikuttyamma, Selvakumar and Sharma, 2006).

This suggests that the increase in HSP 70 was also followed by an increase in CaN in group 2, and a decrease in HSP 70 as granting curcuminoid also followed by a decrease in CaN in groups 3 and 4.

CONCLUSION

Curcuminoid proved to be potentially effective in the treatment for the damage of supporting tissues within the cochlear lateral wall regarding the decreased expressions of CaN and HSP-70.
REFERENCES


CERTIFICATE

This is to certify that
Tengku Siti Hajar Haryuna

as

Oral Presenter

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