Ethanol Extraction of Hoya Carnosa Leaves Improved Stroma of Middle Ear Epithelium Infected by Pseudomonas Aeruginosa

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Hoya carnosa leaves or tebel-tebel leaves are known by the Balinese people in the countryside to treat middle ear infections in the medical language, otitis media or “curek” in the Balinese language. The study is true experimental randomized posttest-only control design to evaluate the impact of ethanol extract sample as antibacterial. As antibacterial, the extract has an impact on the thickness of stroma middle ear epithelium with otitis media due to Pseudomonas Aeruginosa. The result showed average mean thickness stroma in the treatment group lower than controls.

Keywords: Ethanol extract, Hoya carnosa, stroma, middle ear, Pseudomonas aeruginosa.

Otitis media is a disease that is still often found in society especially in developing countries like Indonesia. Otitis media that occurs chronically is mainly caused by Pseudomonas aeruginosa. Pseudomonas aeruginosa is a gram-negative bacterium with the ability to produce biofilms which increases the potential for resistance to some antibiotics. Pseudomonas aeruginosa is also a malignant gram-negative bacterium because the enzyme produced is osteolytic which causes deeper damage to the structure of the middle ear. Handling this infection is quite difficult and tends to cost a lot. The main choice of treatment for otitis media due to Pseudomonas aeruginosa is topical. The choice of topical antibiotics is limited and quite expensive. Research conducted by Lee et al. (2012) between 2001 and 2008 showed a tendency to increase resistance to Pseudomonas aeruginosa bacteria and decrease sensitivity to aminoglycoside antibiotics, cephalosporin, penicillin, imipenem and quinolones1. Vlastarakos et al. (2007) also found resistance of Pseudomonas aeruginosa by 18% to the quinolone class2. Jang et al. (2007) found an association between antibiotic resistance and biofilm formation by Pseudomonas aeruginosa in CSOM3.

During otitis media occur transition middle ear epithelium from single layer simple
squamous epithelial cells and a rudimentary stroma to a pseudostratified respiratory epithelium with extensive stroma. Normally middle ear mucosa when otitis media histologically showed significant thickening at day 2 and resolution at day 10.

Indonesia with diverse medicinal plants is very potential to be studied for its effectiveness as an antibacterial in the handling of otitis media due to the *Pseudomonas aeruginosa* bacteria. Balinese people in ancient times empirically used ear drops from the extract of the leaves of *Hoya carnosa*, which is better known as the area “don tebel-tebel” which means the leaves are thick or often also called “don curek” to treat inflammation of the middle ear in language the area of Bali is called “curek”. *H. carnosa* is a species of Asclepiadaceae, included in the list of Chinese medicinal plants which contain phenolic components such as phenolic alkaloids, phenolic acids, flavonoids, tannins, phenolic terpenoids, quinone, stilbenes, volatiles and aliphatic components. Most of the contents of the plant are flavonoids. The results of the experimental antibacterial activity of extract showed that with a concentration of 80% and 100% had a strong inhibitory effect on *Staphylococcus aureus*, *Streptococcus sp* and *Pseudomonas aeruginosa*.

It is necessary to do an in vivo test to find the ability of *tebel-tebel* leaves as anti-bacterial and anti-inflammatory that show resolution of middle ear mucosal stroma after infections by *Pseudomonas aeruginosa*.

**MATERIAL AND METHODS**

This study is true experimental randomized posttest-only control group design to evaluate the impact of ethanol extract sample as antibacterial by improving stroma of middle ear epithelium. This study already have approval from Ethic Committee Udayana University. The sample used in this study were 36 male BalbC mice, having a body weight of 25-30 grams and aged 3-3.5 months, which were maintained during study with standard feed and randomized into treatment and control group. The preparation material for induction of otitis media is *Pseudomonas Aeruginosa* ATCC 9027 from Microbiology Laboratory Udayana University.

**Research procedure**

The action is carried out aseptically. Mice is anesthetized with ketamine 100 mg kg BB and xylazine 0.1 mg / kg intramuscularly. The next step mice placed in a pronation position. The tympanic membrane was identified under the help of SHIN-NIPPON OP-2 otology microscope. The intact tympanic membrane and pearl white images without the shadow of an effusion in the middle ear indicate mice is a suitable candidate as a research sample. Next, 0.1 ml of *Pseudomonas aeruginosa*

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<th>Table 1. Stroma Thickness</th>
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<td><strong>Group</strong></td>
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<td>Stroma Treatment</td>
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<td>Stroma Control</td>
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Fig. 1. Treatment group(A) showed thinner stroma and less infiltration of cell imun than control group(B)
solution (CFU) was applied to the right and left tympanic bullae trans tympanic. Mice middle ear was evaluated with otolaryngology microscope 48 hours after bacterial inoculation to see the presence of otitis media. The presence of changes in opacity and color tympanic membrane and the image of the effusion in the middle ear indicate the presence of otitis media. Furthermore, the extraction of 0.1 ml tebel-tebel leaves was dropped in the right and left tympanic bullae of the treatment group via trans tympanic route. 24 hours after giving the extract, mice euthanized by decapitation technique and the tympanic bullae tissue is taken. Tympanic bullae tissue then processed for Hematoxylin and Eosin staining.

RESULTS AND DISCUSSION

Test of normality data using Saphiro–Wilk. Levene’s test show homogen of data. Independent t- test to check equality of means showed significant differences stromal thickness between treatment and control group.

Histopathological strom alepithelial from treatment group thinner than control. This probably caused by antibacterial activity of the extract on middle ear epithelium that already infected by Pseudomonas Aeruginosa. The component of flavonoids, tannin, phenolic and alkaloid work together as antibacterial at multiple site of bacteria and infected tissue make improvement of middle ear stroma.

ACKNOWLEDGMENT

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REFERENCES

5. Yully, P. 2016. In vitro test of the antibacterial effectiveness of tebel-tebelleaf extract (Hoya carcosa) on gram-positive and gram-negative bacteria causing benign type active CSOM. Udayana University.