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EFFECT OF PUGUNTANO LEAF EXTRACT (*CURANGAFEL-TERRAE MERR*) ON INTERLEUKIN-1α EXPRESSION OF CHOLESTEATOMA MATRIX CULTURES WITH ATTICOANTRAL CHRONIC SUPURATIVE OTITIS MEDIA

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ABSTRACT Chronic otitis media (COM) with or without cholesteatoma is a major global health problem, especially in developing countries. Until today, there is no other treatment for COM with cholesteatoma (COMch) other than surgery. Puguntano *(Curanganfel-terrae Merr)* is a plant from *Scrophulariaceae* family. It can be found in Asia, especially in China, India, Philippines, Myanmar, Malaysia and Indonesia. This plant has traditionally used as stimulant, antidiuretic, treatment for malaria, hepatoprotective drug, antipyretic, antiherpetic drug, anti-cancer, antihyperglycemic, antioxidant and anti-inflammatory. The objective of this study is to evaluate the effect of ethanol extract from Puguntano leaves on Interleukin-1α expression level in cholesteatoma matrix cultures with atticoantral type chronic supurative otitis media. This study is an *in vitro* experimental research comparing two groups, a keratinocyte cholesteatoma culture with and without Puguntano extract. A group without extract Puguntano was divided into 4 different groups: group with Puguntano extract 1 mg/ml, 2 mg/ml, 4 mg/ml and control group.Interleukin-1α level in cholesteatoma culture is inversely proportional to Puguntano extract. Interleukin-1α expression level in group with Puguntano extract 1 mg/ml is 4,335 pg/ml; 2 mg/ml is 3,83 pg/ml; and 4 mg/ml is 2,74 pg/ml. Those results showed the decreasing level of Interleukin-1α expression compared to control group, which is 8,2889 pg/ml.

KEYWORDS : Puguntano Cholesteatoma Interleukin-1α

INTRODUCTION

The World Health Organization (WHO) stated that CSOM sufferers around the world range from 65 - 330 million people, with 94% of them are in developing countries. 60% among them (39 - 200 million people) could not survive. In general, the prevalence of CSOM in developing countries, such as India, was reported to be higher at 5.2%. The prevalence rate of CSOM in Indonesia was reported as 3.6% (Yarisman et al, 2017).

CSOM is divided into 2 types; tubotimpani type or without cholesteatoma, and aticoantral type or with cholesteatoma (Buchman, Levine and Balkany; 2003).

Until recently, there is no other cholesteatoma treatment except surgery, which is the only treatment for eradication of this disease (Helmi, 2005). The type of surgery for managing cholesteatoma is a collapse wall mastoidectomy which changes the structure of the middle and inner ear (Helmi, 2005).

Cholesteatoma is removed from the temporal bone by surgical resection with both radical and mastoidectomy modifications. The main drawback of this operation is the narrow field of surgery, which is often associated with high rates of cholesteatoma recurrence. (Fathy. A, Ghany. A, 2015) Cholesteatoma perimatriks contain lymphocytes, monocytes, fibroblasts and endothelial cells, which are the sources of proinflammatory cytokines, such as TNF- , IL-1 and IL-6. The expression level of TNF- , IL-1 and IL-6 in cholesteatoma tissue is higher than in granulation tissue (Kuczkowski et al, 2011).

Puguntano (Curangafel-terrae Merr.) is a plant belongs to Scrophulariaceae family that grows in Asian regions such as Indonesia, Malaysia, Philippines, China and India (Harahap et al, 2013). Studies conducted by Harahap, et al. (2013), found that ethanol extracts of Puguntano leaves obtained from percolation and socletation methods have phytochemical contents, such as alkaloids, flavonoids, glycosides, saponins, tannins and steroids/terpenoids (Harahap et al, 2013). One of the classes of chemical compounds responsible for anti-inflammatory effects is steroids/terpenoids, thus it is suspected that this steroid provides the anti-inflammatory effect of the Puguntano leaves.

Based on the description above, the researcher was interested to find out the effect of ethanol extract from Puguntano leaves (Curangafel-terrae Merr.) on IL-1α expression in choleste atoma cultures of hazardous type CSOM patients.

METHOD

This research is an in vitro experimental study conducted in a laboratory. This study compared 4 groups, namely the cholesteatoma keratinocyte culture group which was administered with ethanol extract of Puguntano leaf in several doses and the culture group that was not administered with ethanol extract of Puguntano leaf.

The cholesteatoma tissue was taken during the operation of CSOM patients with cholesteatoma in the Central Surgical Installation at RSUPN CiptoMangunkusumo. The cholesterolemia keratinocyte culture was treated at the Integrated Laboratory of the YARSI University Research Institute and examination of IL-1α levels was conducted in the same laboratory.

The ethanol extraction of puguntano leaves will also be conducted in the YARSI laboratory. The puguntano ethanol extract was made using maceration technique.

The comparison parameters were the amount of IL-1α of cholesteatoma keratocyte culture which were administered with puguntano ethanol extract and the control group. The data obtained were processed and analyzed using the ANOVA test.

Table 1. The levels of IL-1α in the sample before treatment

Sample	Measurement1	Measurement2	Average (pg/ml)	SD
Kontrol	8,5577	8,0150	8,2889	0,38

RESULT

After receiving treatment, which is the administration of ethanol extract from puguntano leaves each with the composition of 1 mg/ml, 2 mg/ml and 4 mg/ml respectively, the levels of IL-1α in cholesteatoma are presented in table 2.

Table 2. The levels of IL-1α in samples after treatment

Sample	Measurement1	Measurement2	Average (pg/ml)	SD
PG 1 mg/ml	4,3366	4,3266	4,3350	0,007
PG 2 mg/ml	3,9648	3,7035	3,83	0,18
PG 4 mg/ml	2,8442	2,6381	2,74	0,14

Referring to Table 2, it appears that there is a decrease in IL-1α levels in line with the increased levels of ethanol extract of puguntano leaves administration.

The statistical test results on the administration of ethanol extract of puguntano leaves on IL-1α levels in the cholesteatoma matrix are presented in table 3. below.

Table 3. The ANOVA Test Results of the treatments on the control group

Test of control	F	P value	Conclusion
1 mg/ml	212,340	0,005	Shows statistical differences
2 mg/ml	218,545	0,005	Shows statistical differences
4 mg/ml	364,976	0,003	Shows statistical differences

DISCUSSION

Chronic suppurative otitis media (CSOM) is the result of an episode of acute otitis media, the symptom is a continuous discharge of fluid from the middle part of the ear through the tympanic membrane perforation, which is an important cause of hearing loss (WHO, 2004). CSOM is divided into 2 types; tubotimpani type or without cholesteatoma, and aticoantral type or with cholesteatoma (Buchman, Levine and Balkany; 2003). Until recently, there is no cholesteatoma treatment except surgery, which is the only treatment for eradication of this disease (Helmi, 2005).

Cholesteatoma contains cytokines, such as IL-1, IL-6, TNF-alpha and TGF, thus it is hyperproliferative, destructive, and capable of doing angiogenesis. The cholesteatoma compresses and forces the surrounding organs and causes bone necrosis. The condition is exacerbated by acid formation from bacterial decay. This pathology process facilitates the emergence of complications and affects the process of delivery and sound amplification, thus worsening the hearing loss (Samosir et al, 2018). This finding is in accordance with the research by Kuczkowski et al. (2011). His research, using the immunoblast method, showed an increase of IL-1 and IL-6 expression in cholesteatoma compared to granulation tissue and in the skin.

The results of phytochemical screening by Harahap. et al (2015) showed that the ethanol extract of poguntano leaf water contained flavonoid, glycoside, saponin, tannin, steroid, and terpenoid compounds. The anti-inflammatory steroid compounds/terpenoids have the ability to inhibit the enzymatic action of phospholipase, causing it to prevent the release of mediators of inflammatory processes, namely arachidonic acid and its metabolites such as prostaglandins (PG), leukotrienes (LT), thromboxane and prostacyclin. Therefore, the occurrence of further inflammation can be avoided (Lindarto et al, 2016). Through various studies of flovanoidini compounds contained in puguntano leaves, it has been proven that the compounds contain anti-inflammatory effects, both in vitro and in vivo, and it works through various mechanisms to prevent and weaken the inflammatory response (Pan, et al, 2010; Hassan, et al. , 2012). Based on the results of this study in Table 2, it appears that there was a decrease in IL-1 levels in line with the increased levels of ethanol extract of puguntano leaves administration. This finding is consistent with the study by Lindarto et al, 2016, that ethanol extract of puguntano leaf can reduce levels of C-Reactive Protein (CRP) in patients with type 2 DM, where this CRP is one of the acute phase proteins that arise in the same inflammatory process as IL-1 . A research on puguntano leaf extract as an anti-inflammatory was also conducted by Shi (2016) by extracting Picfeltarraenin ethanol IA from this plant. Shi proved that Picfeltarraenin IA was able to inhibit LPS-induced COX-2 expression, as well as IL-8 and PGE2 production in A549 epithelial cells of the human lung. Therefore, this research is expected to be the cornerstone for further studies to find alternative therapies in aticoantral type CSOM patients, and to bring a new hope for aticoantral type CSOM patients.

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