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Ethanol extract of propolis decreases the Interleukin-8 (IL-8) expression and blood Malondialdehyde (MDA) level in otitis media rat model induced by *Pseudomonas aeruginosa*

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ABSTRACT

Background: Pseudomonas aeruginosa is one of the bacteria that cause otitis media, leading to progressive structural damage of the middle ear mucosa due to virulence factor, toxin, and enzyme produced. The excessive inflammatory process can cause oxidative stress, which will aggravate tissue damage, thus difficult to cure. This study aimed to assess the effect of ethanol extract of propolis (EEP) in decreasing IL-8 expression and blood MDA level in the otitis media rat model induced by Pseudomonas aeruginosa.

Methods: A randomized controlled trial was conducted among 42 SDF Sprague-Dawley rats. They were divided into 6 groups: normal control, negative control: Otitis Media (OM), positive control: OM + Ciprofloxacin 20 mg/kg body weight/day, OM + EEP 200 mg/kg body weight/day, OM + Ciprofloxacin 20 mg/kg body weight/day + EEP 100 mg/kg body weight, OM + Ciprofloxacin 20 mg/kg body weight/day + EEP 200 mg/kg body weight/day. Induction of Pseudomonas aeruginosa to obtain the OM model was administered on the first day of the study and waited for 28 days before treatments were given. Data were analyzed with SPSS version 23 for Windows.

Results: After 14 days of treatment, the expression of IL-8 and MDA level in each treatment group (OM + Ciprofloxacin, OM + EEP 200, OM + Ciprofloxacin + EEP 100, OM + Ciprofloxacin + EEP 200) revealed significant decrease compared to OM (negative control group) ($p<0.05$). The reduction of IL-8 expression between the Ciprofloxacin group and EEP 200 was not significantly different ($p>0.05$). There was a significant difference between the Ciprofloxacin group and Ciprofloxacin + EEP 100, and between Ciprofloxacin and Ciprofloxacin + EEP 200 ($p<0.05$).

Conclusion: Ethanol extract of propolis decreased IL-8 expression and MDA level in the OM rat model induced by Pseudomonas aeruginosa.

Keywords: IL-8, MDA, Otitis media, Propolis, *Pseudomonas aeruginosa*

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INTRODUCTION

Otitis media is an inflammation caused by the infiltration of bacteria into the middle ear, which is divided into acute and chronic according to duration. According to the etiology, common bacteria that cause acute otitis media were Streptococcus pneumonia, Staphylococcus aureus, Hemophilus influenza, and Micrococcus catarrhalis. Meanwhile, common bacteria found in chronic suppurative otitis media were Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris, and Klebsiella pneumonia.[1] Between these bacteria that cause chronic suppurative otitis media, several studies showed that the predominant bacteria were Pseudomonas aeruginosa, which accounted for 20-50%,[1] 43.2%,[2] 22-44%.[3]

Pseudomonas aeruginosa is thought to be responsible in causing progressive damage to the middle ear and mastoid structure by their virulence factor, toxin, and enzyme.[4] The immune system, as a response to infection, will undergo an inflammatory process. There are several cellular and biochemical processes that resulted from the tissue damage caused by this infection process. There was a release of inflammatory mediators, such as protein, peptides, glycoprotein, cytokine, arachidonic acid metabolites, nitric oxide, and oxygen-free radical. In otitis media, these substances were produced by the middle ear epithelial cells, endothelial cells, and immune cells' infiltration, such as neutrophils, macrophages, and lymphocytes.[5] These inflammatory mediators were thought to be a double-edged sword, with a role to fight against infection, but also produced damage to their own tissues.[6,7]

Lipopolysaccharides (LPS) on the outer membrane of Pseudomonas aeruginosa have the main role in activating the host's innate and adaptive immune response by producing pro-inflammatory mediators through the NF-κB pathway.[6] IL-8 is an important chemotactic cytokine in the neutrophil function that acts in the accumulation of neutrophils in the middle ear mucosa.[8] Other than that, the primary pro-inflammatory cytokine (IL-1β, IL-6,

TNF-α) can control the expression and secretion of IL-8 during the inflammatory process of the middle ear. Thus, IL-8 can also be considered a secondary cytokine of the middle ear.[9] Therefore, there were more infiltration of neutrophils in the middle ear mucosa, which acted for bacteria clearance.[10]

Oxidative stress is a condition of imbalance between prooxidant and antioxidant, where reactive oxygen species (ROS) as a prooxidant was excessive, which leads to the inability of an antioxidant to neutralize it. This condition will cause cellular damage.[11] There are several pathways of oxidative stress conditions caused by the infection of Pseudomonas aeruginosa. During the inflammatory process, the activation of the pro-inflammatory cytokine, especially IL-8, will increase neutrophil infiltration in the middle ear mucosa. ROS production is the primary effect of the inflammatory process; thus, excessive ROS will cause tissue damage.[12] Furthermore, Pseudomonas aeruginosa also secreted pyocyanin, which can penetrate the epithelial cell membrane barrier and directly activates the nicotinamide adenine dinucleotide phosphate (NADPH) enzyme, which will produce ROS. It can cause cell damage, including through the peroxidation of cell membrane lipids. Peroxidation occurs in a toxic condition that will cause cell death in the form of apoptosis or necrosis. Malondialdehyde (MDA) is the end-product of lipid peroxidation often used as a biomarker to assess oxidative stress.[13]

Propolis is a natural product derived from plant resin collected by honeybees. It has several biological activities, including anti-inflammatory and antioxidant. The anti-inflammatory and antioxidant activities were derived from a flavonoid, quercetin, and caffeic acid phenethyl ester (CAPE).[14] According to Sarsono et al., ethanol extract of propolis of Mount Lawu has been proven to contain $30.24 \pm 3.53 \times 10^{-6}$ g CAPE and $4.42 \pm 0.50 \times 10^{-6}$g quercetin.[15] The inflammatory property of CAPE will inhibit the activation of NF-κB through the inhibition of IκB-α degradation.[16] Meanwhile, quercetin inhibits the release of TNFα, IL-6, and IL-8.[17] The antioxidant property scavenges free radicals and protects the cell membrane from lipid peroxidation.[18]

This study aimed to analyze the effect of ethanol extract of propolis of Mount Lawu on the expression of IL-8 and MDA level in the otitis media rat model induced by Pseudomonas aeruginosa. Therefore, propolis is expected to be developed as adjuvant therapy, combined with antibiotics for the treatment of otitis media.

METHODS

This study was an experimental study that used SDF Sprague-Dawley white rats. The materials were ethanol extract of propolis of Mount Lawu, obtained from the beekeepers in Kerjo, Karanganyar. Ethanol extract of propolis was fabricated by extracting 1 gram of raw propolis powder with 10 ml of 80% ethanol in a shaker at 200 rpm at room temperature for 24 hours. After filtering through filter paper, 25mL of the filtrate was made with 80% ethanol. The dosages used in this study were 100 mg/kg body weight and 200 mg/kg body weight given orally. The experiment was conducted in a laboratory animal breeding unit of PAU UGM Yogyakarta. Induction of otitis media was performed using Pseudomonas aeruginosa FNCC 0063 obtained from the Microbiology Laboratory of PAU Gajah Mada University, Yogyakarta. The bacteria were taken from the reservoir according to the manufacturer's instruction and cultured in blood agar 24 hours at 35-37°C.

After incubation, the bacteria were mixed in a sterile saline solution with a concentration of 1.0×10^6 colony-forming units (CFU)/ml. The induction dose of 0.1 ml was given trans canal to each ear using one cc syringe and a blunt needle until it penetrated the tympanic membrane. Blood MDA level was assessed in the laboratory of PAU UGM Yogyakarta. Meanwhile, immunohistochemistry (IHC) assessment to determine the expression of IL-8 was conducted in the Anatomical Pathology Department of Faculty of Medicine, Sebelas Maret University, and Anatomical Pathology Laboratory of dr. Moewardi Hospital, Surakarta. The ethical clearance was obtained from the ethical committee of dr. Moewardi Hospital, No.1056/IX/HREC/2019.

Treatment of White Rats

This study used 42 white male rats aged 2-3 months with 200-300 mg body weight. Rats with signs of infection in the tympanic membrane and the middle ear during otoscope examination were excluded. They were acclimated for a week. During the study, the rats were given pellets and water ad libitum. The rats were randomly divided into six groups; each consisted of 7 rats. In the normal group (N), the rats were not induced with Pseudomonas aeruginosa and were given 2 ml distilled water with a nasogastric tube. In a negative control group (C-), the rats were induced with Pseudomonas aeruginosa and were given 2 ml distilled water. The positive control group (C+) was induced with Pseudomonas aeruginosa, and after 28 days, each was given 20 mg/kg body weight Ciprofloxacin dissolved in 2 ml distilled water orally for 14 days. The T1 group was induced with Pseudomonas aeruginosa, and after 28 days were given EEP with 200 mg/kg body weight dose per oral with a nasogastric tube for 14 days.

Meanwhile, the T2 group was induced with Pseudomonas aeruginosa, and after 28 days, 20 mg/kg body weight Ciprofloxacin combined with 100 mg/kg body weight EEP orally. The T3 group was treated the same way as the T2 group with 200 mg/kg body weight of EEP. After 14 days, the blood was taken for MDA level assessment. Afterward, the rats were sacrificed for their ear organ (tympanic bulla dissection). The organs were fixated in a formalin buffer for IHC assessment to determine the expression of IL-8.

Examination of IL-8 Expression with IHC

Paraffin blocks were cut at 4-5 microns, placed on a poly-L-lysine slide, and incubated at 37°C overnight. Subsequently, deparaffination was conducted and rinsed with running water for 5 minutes and distilled water for 5 minutes. The specimens were rinsed with PBS 2× for 5 minutes. Antigen retrieval was performed in a microwave oven with Tris EDTA pH 9 at 90°C for 3 minutes and continued at low temperature for 10 minutes. After cooling, the specimens were rinsed with PBS for 2 × 5 minutes. They were given drops of 3% endogen peroxidase methanol H_2O_2 for 20 minutes and rinsed with running water for 5 minutes and given a blocking serum for 10 minutes. Afterward, antibody IL-8 was given (IL-8 polyclonal antibody, Abclonal, USA), and incubated at 4°C for 18 hours. Subsequently, rinsed with PBS 2 × 5 minutes, biotin for 15 minutes, rinsed with PBS, and given drops of streptavidin for 10 minutes and rinsed with PBS. Peroxidase DAB enzyme substrate was given for 5 minutes, rinsed with running water, and given drops of hematoxylin for 4 minutes and rinsed for 10 minutes. The specimens were mounted and covered with a deck glass. IL-8 expression was assessed under a light microscope with 400x magnification. IL-8 expression was measured, where score 0 means negative, one means weak positive (<5%), two means moderate positive (5-75%), and score 3 was strongly positive (>75%). Observation and assessment were conducted using a blind-method by two anatomical pathologists.

MDA Level Assessment

MDA level measurement was conducted using the thiobarbituric acid reacting substances (TBARS) method. The obtained color intensity was measured with a spectrophotometer with 530-540 nm wavelength. The obtained color intensity was comparable to the MDA level obtained in the sample. The unit was represented in nMol/ml.

Statistical Analysis

The expression of IL-8 was analyzed using the Kruskal-Wallis test; the Mann-Whitney test followed any significant differences. The difference of serum MDA level between each group was analyzed using analysis of variance (ANOVA), and the Post-hoc test followed any significant difference. The p-value of < 0.05 was considered statistically significant.

RESULTS

Effect of Ethanol Extract of Propolis on IL-8 Expression in the Right Ear

The difference between groups was assessed using the Kruskal-Wallis test and obtained significant results ($p = 0.010$). Afterward, the differences between each group were assessed using the Mann-Whitney test. Table 1 revealed that Pseudomonas aeruginosa induction in the right ear significantly increased the expression of IL-8 ($p = 0.017$). The administration of 200 mg/kg body weight/day of EEP significantly reduced the expression of IL-8 ($p = 0.030$). The decrease of IL-8 expression was insignificant between the administration of 200 mg/kg body weight/day of EEP compared to 20 mg/kg body weight/day of Ciprofloxacin. This means that EEP provided an anti-inflammatory effect by decreasing the expression of IL-8 comparable to Ciprofloxacin. The administration of a combination of Ciprofloxacin 20 mg/kg body weight/day and EEP 100 mg/kg/body weight/day, combination of Ciprofloxacin 20 mg/kg body weight/day and EEP 200 mg/kg body weight/day showed a significant decrease of IL-8 expression. A combination of Ciprofloxacin and 200 mg/kg body weight of EEP reduced more IL-8 expression, albeit insignificant ($p = 0.254$). The administration of a combination of Ciprofloxacin and propolis compared to propolis revealed a significant difference in the combination of propolis with 100 mg/kg body weight dose ($p = 0.037$). Meanwhile, the combination of propolis with 200 mg/kg body weight showed an insignificant difference.

The Effect of Ethanol Extract of Propolis on the Expression of IL-8 in the Left Ear

The difference between groups assessed by the Kruskal-Wallis test showed a significant result ($p = 0.011$). The differences between each group were then assessed with the Mann-Whitney test. Table 2 represented a significant increase in IL-8 expression in the left ear induced by Pseudomonas aeruginosa ($p = 0.017$). The administration of 200 mg/kg body weight/day of EEP showed a significant decrease in IL-8 expression ($p = 0.038$). The decrease of IL-8 expression was insignificant between 200 mg/kg body weight/day of propolis compared to 20 mg/body weight/day of Ciprofloxacin. This means that propolis provided an anti-inflammatory

Table 1 Comparisons of IL-8 expression score in the right ear between groups

Group	Median (Min-Max)	Mann-Whitney Test						p-value
		N	C(-)	C(+)	T1	T2	T3	
N	*1.0 (1-2)*	-	*0.017**	*1.000*	*0.298*	*0.254*	*1.000*	
C(-)	*2.0 (1-3)*		-	*0.010**	*0.030**	*0.002**	*0.010**	
C(+)	*1.0 (1-2)*			-	*0.298*	*0.254*	*1.000*	*0.010**
T1	*2.0 (1-2)*				-	*0.037**	*0.298*	
T2	*1.0 (1-2)*					-	*0.254*	
T3	*1.0 (1-2)*						-	

N: Normal group; C-: Negative control, OM + distilled water, C+: Positive control, OM + Ciprofloxacin 20 mg/kg body weight; T1: OM + EEP 200 mg/kg body weight; T2: OM + Ciprofloxacin 20 mg/kg body weight + EEP 100 mg/kg body weight; T3: OM + Ciprofloxacin 20 mg/kg body weight + EEP 200 mg/kg body weight. ≠ $P < 0.05$ the difference between all groups (Kruskal-Wallis test); * $P < 0.05$ the difference between each group (Mann-Whitney test).

Table 2 Comparisons of IL-8 expression score in the left ear between groups

Group	Median (Min-Max)	Mann-Whitney Test						p-value
		N	C(-)	C(+)	T1	T2	T3	
N	*1.0 (1-2)*	-	*0.017**	*0.710*	*1.000*	*0.383*	*0.710*	
C(-)	*2.0 (1-3)*		-	*0.038**	*0.017**	*0.002**	*0.038**	
C(+)	*1.0 (1-2)*			-	*0.710*	*0.209*	*1.000*	*0.011**
T1	*2.0 (1-2)*				-	*0.254*	*0.606*	
T2	*1.0 (1-2)*					-	*0.107*	
T3	*1.0 (1-2)*						-	

N: Normal group; C-: Negative control, OM + distilled water, C+: Positive control, OM + Ciprofloxacin 20 mg/kg body weight; T1: OM + EEP 200 mg/kg body weight; T2: OM + Ciprofloxacin 20 mg/kg body weight + EEP 100 mg/kg body weight; T3: OM + Ciprofloxacin 20 mg/kg body weight + EEP 200 mg/kg body weight. ≠ $P < 0.05$ the difference between all groups (Kruskal-Wallis test); * $P < 0.05$ the difference between each group (Mann-Whitney test).

Table 3 Comparisons of MDA levels between groups after 14 days of treatment

Group	Mean (± SD)	Post-hoc Test						p-value
		N	C(-)	C(+)	T1	T2	T3	
N	1.076(± 0.233)	-	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	
C(-)	9.431(± 0.285)		-	*0.000*	*0.000*	*0.000*	*0.000*	
C(+)	4.033(± 0.264)			-	*0.757**	*0.000*	*0.000*	*0.000**
T1	3.980 (±0.456)				-	*0.000*	*0.000*	
T2	3.165(± 0.187)					-	*0.000*	
T3	2.453(± 0.395)						-	

N: Normal group; C-: Negative control, OM + distilled water, C+: Positive control, OM + Ciprofloxacin 20 mg/kg body weight; T1: OM + EEP 200 mg/kg body weight; T2: OM + Ciprofloxacin 20 mg/kg body weight + EEP 100 mg/kg body weight; T3: OM + Ciprofloxacin 20 mg/kg body weight + EEP 200 mg/kg body weight. ≠ $P < 0.01$ the difference between all groups (ANOVA test); * $P > 0.05$ the difference between each group (Post-hoc test).

effect by decreasing IL-8 expression as effective as Ciprofloxacin. The administration of the combination of 20 mg/kg body weight/day of Ciprofloxacin and 100 mg/kg body weight per day of EEP, and the combination of 20 mg/kg body weight/day of Ciprofloxacin and 200 mg/kg body weight/day of EEP has been proven to significantly decrease the expression of IL-8, with higher result in the combination of Ciprofloxacin and 200 mg/kg body weight of EEP, albeit insignificant ($p = 0.107$). The administration of the combination of Ciprofloxacin and 100 mg/kg body weight of EEP and the combination with 200 mg/kg body weight EEP showed an insignificant difference. There was an insignificant difference in IL-8 expression median between the right and left ears ($p = 0.778$).

The Effect of Ethanol Extract of Propolis on the blood MDA level

The resulting data of MDA level assessment were analyzed for data normality using the Shapiro-Wilk test with a result of normal distribution ($p >$

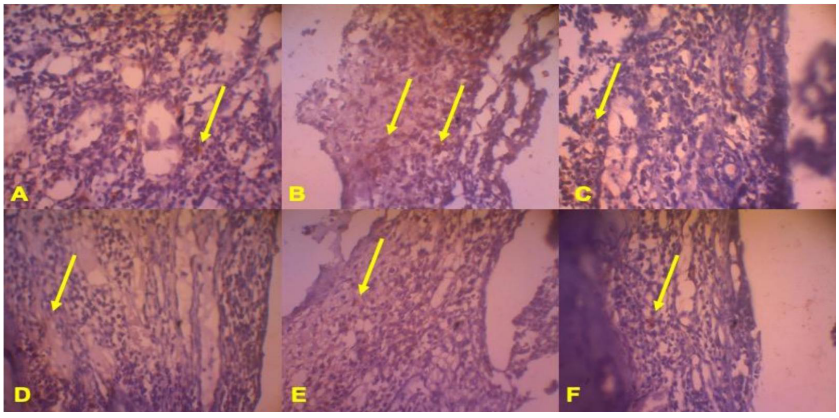

Figure 1 Image of IL-8 expression (arrow) in the middle ear mucosa of white rats. (A) N: Normal group; (B) C-: Negative control, OM + distilled water; (C) C+: Positive control; OM + Ciprofloxacin 20 mg/kg body weight; (D) T1: OM + EEP 200mg/kg body weight; (E) T2: OM + Ciprofloxacin 20 mg/kg body weight + EEP 100 mg/kg body weight; (F) T3: OM + Ciprofloxacin 20 mg/kg body weight + EEP 200 mg/kg body weight; (400x magnification)

0.05). Therefore, ANOVA can be performed for analysis. The difference between groups analyzed by ANOVA revealed a significant result ($p = 0.000$). Subsequently, Post-hoc test was performed to determine the differences between each group. Table 3 showed that the induction of Pseudomonas aeruginosa could significantly increase blood MDA levels ($p < 0.001$). The administration of 200 mg/kg body weight/day of EEP significantly decreases serum MDA level, as well as the administration of 20 mg/kg body weight/day of Ciprofloxacin ($p < 0.001$). However, the difference between these ingredients was insignificant ($p = 0.757$). This means that the antioxidant property of EEP was as effective as Ciprofloxacin. The administration of Ciprofloxacin combined with 100 mg and 200 mg EEP was proven to significantly decrease blood MDA levels. Two hundred milligrams of propolis reduced the MDA level more significantly than 100 mg of EEP ($p < 0.001$). The administration of ciprofloxacin combined with 100 mg or 200 mg EEP reduced the MDA level more significantly than the administration of only 200 mg of EEP.

DISCUSSION

At a molecular level, otitis media is defined as an inflammatory response of the middle ear as a result of pro-inflammatory transcription factor activation, followed by the production and release of pro-inflammatory cytokines, mucosa hyperplasia, leukocyte infiltration and mucous fluid secretion in the middle ear mucosa. These activities were efforts to clearance bacteria.[5]

In this study, the induction of Pseudomonas aeruginosa has significantly increased the IL-8 expression in the middle ear. Several pathways can cause increased IL-8 expression due to Pseudomonas aeruginosa induction. The first response of Pseudomonas aeruginosa on macrophages consisted of the bond of LPS in the membrane of Pseudomonas aeruginosa with TLR4, which will activate the expression of pro-inflammatory cytokine gene expression, including the expression of IL-8 through NF-κB. This cytokine acts in the recruitment of neutrophils to the location of the inflammation. Other than that, the IL-1β pro-inflammatory cytokine bonded with IL1-R expressed in the middle ear mucosal epithelial cells will respond by producing chemokines such as IL-8, and rapidly attract neutrophils to the middle ear mucosa, which will increase antimicrobial activity through ROS increase.[6] Another path showed that pyocyanin produced by Pseudomonas aeruginosa would increase the release of IL-8 in epithelial cells, both in vivo and in vitro. Furthermore, pyocyanin can induce monocyte to produce IL-8 by activating the signaling pathway of MPAKs and NF-κB.[19]

Propolis, with its flavonoid content of quercetin and CAPE, was reported in several studies to have an anti-inflammatory effect. In this study, 200 mg/kg body weight/day of EEP has been proven to significantly decrease the expression of IL-8 due to the induction of Pseudomonas aeruginosa. NF-κB is one of the most important transcription factors which regulate several gene expressions, including inflammation. The inhibition of NF-κB will produce a decrease in several pro-inflammatory cytokines. Inactive NF-κB is in the cytoplasm because it binds with IκB-α protein. An inflammation stimulus will cause phosphorylation and degradation of IκB-α, which leads to the translocation of NF-κB to the nucleus. Therefore, the inhibition of IκB-α degradation will inhibit the translocation of NF-κB, and the inflammatory effect of EEP is known to work at this level.[20] This was in accordance to Song *et al.*, who showed that CAPE inhibited the expression of TNF-α, which was induced by LPS and the production of IL-8 in the middle ear epithelial cells and also inhibited the degradation of IκB-α.[17] In another study, quercetin was proven to decrease protein and expression of IL-8 gene in ARPE-19 cell, which was stimulated by IL-1β.[21] Quercetin has also been proven to decrease IL-8 inflammatory cytokine production in gingival fibroblasts induced by LPS. The anti-inflammatory mechanism was through the activation of PPAR-γ, which will inhibit NF-κB.[22]

In this study, there was an insignificant difference between the administration of 200 mg/kg body weight/day of EEP compared to 20 mg/kg body weight/day of Ciprofloxacin on IL-8 expression. This means that the anti-inflammatory effect of EEP was as effective as Ciprofloxacin.

The administration of 20 mg/kg body weight/day of Ciprofloxacin combined with EEP 200 mg/kg body weight per day showed an insignificant difference in the IL-8 expression decrease compared to Ciprofloxacin only, which means that the combination of Ciprofloxacin and EEP had a synergic anti-inflammatory effect, albeit insignificant.

In this study, the induction of Pseudomonas aeruginosa has been proven to increase ROS in the middle ear mucosa, shown by the significant increase of the MDA level. During the infection process, Pseudomonas aeruginosa induced ROS production in the middle ear mucosa in several ways. Pyocyanin secreted to the microenvironment will penetrate the epithelial cell membrane, which directly oxidizes NADPH that produces superoxide and ROS.[23] The LPS of Pseudomonas aeruginosa identified by the epithelial cells will produce ROS through protein kinase C (PKC) – NADPH oxidase signaling pathway in the epithelial cells.[24] Other potential ROS production sources from active epithelial cells occur through the induction of mitochondria electron transport chain, cytochrome p450, and xanthine oxidase. In the inflammatory process, where neutrophils were recruited to fight bacteria, the respiratory burst will occur, leading to ROS production. ROS from this inflammatory process is derived from the oxidation of NADPH, electron transport of mitochondria, uncoupled NOS and xanthine-oxidase.[25]

In this study, the administration of 200 mg/kg body weight of EEP has been proven to decrease serum MDA levels significantly. Propolis, known with its rich content, was proven to be a potent antioxidant. Its main component of CAPE and quercetin has been proven to inhibit ROS in several mechanisms.[26] Propolis inhibits lipid peroxidation by the ROS scavenging mechanism, reducing the formation of free radicals and metal ion chelate.[27] In another study, propolis was shown to inhibit the oxidation of NADPH and increase NOS expression,[28] increase antioxidant capacity in animals and humans, thus decreasing lipid peroxidase, inhibit DNA damage by inhibiting hydrogen peroxide.[29]

The administration of 20 mg/kg body weight/day of Ciprofloxacin can significantly decrease IL-8 expression and MDA levels. Ciprofloxacin is a broad-spectrum antibiotic used for bacterial infection and is included in the list of essential medicines by the World Health Organization (WHO).[30] Its sensitivity to Pseudomonas aeruginosa has been proven in vitro and in vivo studies, especially planktonic microorganisms. It works by inhibiting the helicase DNA of bacteria, which prevents bacteria from replicating.[31] With this bactericidal effect, the administration of Ciprofloxacin will reduce the production and secretion of pro-inflammatory cytokines, which lead to decreased ROS production. This was proven by the results of this study, in which IL-8 expression and MDA levels were reduced.

In this study, the administration of 20 mg/kg body weight of Ciprofloxacin showed an insignificant difference in decreasing the MDA level compared to EEP 200 mg/kg body weight. Therefore, the antioxidant effect of EEP was comparable to Ciprofloxacin. However, the administration of Ciprofloxacin combined with 100 mg or 200 mg EEP was shown to be more effective in decreasing the MDA level significantly compared to a single dose of Ciprofloxacin or EEP 200 mg. Thus, the antioxidant effect of EEP was synergistic if given in combination with Ciprofloxacin. The combination of Ciprofloxacin and EEP 200 mg significantly decreased MDA level compared to Ciprofloxacin combined with EEP 100 mg. Therefore, the administration of Ciprofloxacin combined with EEP 200 mg provided the highest antioxidant effect.

CONCLUSION

Based on this study, 200 mg/kg body weight/day of extract ethanol of propolis could significantly decrease IL-8 expression and MDA level in the otitis media rat model induced by Pseudomonas aeruginosa. The combination of 20 mg/kg body weight of Ciprofloxacin and 100 mg/kg body weight or 200 mg/kg body weight of EEP has been proven to significantly decrease MDA level compared to 200 mg/kg body weight of EEP only.

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CONFLICT OF INTEREST

The authors declare that there is no competing interest regarding the manuscript.

ETHICAL CONSIDERATION

The ethical clearance was obtained from the ethical committee of dr. Moewardi Hospital, No.1056/IX/ HREC/2019.

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AUTHOR CONTRIBUTION

All of the authors are equally contributed to the study from the conceptual framework, data gathering, data analysis, until interpreting the results of the study on publication.

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