Original article

Effect of Moringa Leaf Ethanol Extract on Reduced Levels of Mda, TNF-α and Description of Inflammatory Cells in Rat Sinus Mucosa Model of Acute Rhinosinusitis

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Abstract

Background: Symptoms and signs of acute viral and bacterial rhinosinusitis that are not specific cause difficulties in diagnostic enforcement, causing difficulties in administering therapy, so often doctors provide antibiotic therapy on acute viral rhinosinusitis. Irrelevant antibiotics will cause frequent antibiotic resistance. Adjuvant therapy is required to prevent infections that continue to become chronic, shorten the duration of infection, and avoid unwanted complications. Moringa leaves (M. oleifera)contains antioxidant components that can help rebalance redox metabolism and reduce excess free radicals so as to prevent oxidative stress. ***Method:*** *posttest only control group* research design. Male strain *Sprague Dawley* white rat was maintained and treated at the Nutrition Laboratory of the Center for Food and Nutrition Studies of Gadjah Mada University (UGM). Mice were divided into 4 groups. Group 1: standard feed., group 2: standard feed and *staphylococcus-induced aureus,* group 3: standard feed, *Staphylococcus aureus-induced* given amoxicillin dose of 27 mg/day, group 4: standard feed, *Staphylococcus aureus-induced* was given amoxicillin dose of 27 mg/day and given (EEDK) 200 mg/KgBW/day. Data analysis Of Normality test using *Shapiro-Wilk* Test and homogeneity using *Levene's Test* obtained results p>0.05. Then conducted *One Way Anova*Test obtained p<0.05 and continued with *post-hoc LSD test.* The data is analyzed using SPSS 25.0 *for* Windows. ***Result:*** *MDA test Anova* obtained p value (Sig.) = 0.000 (p< 0.05), *Post Hoc* with LSD all groups (*p-value*) < 0.05, indicated a significant mean difference in average MDA levels among all groups. The lowest mean difference in MDA levels in group K with P3 was 0.92 pg/mL; while P1 with P3 has the largest average MDA rate difference of 7.47 pg/mL**.** TNF-α *Anova* test obtained p value (Sig.) = 0.000 (p< 0.05), *Post Hoc* with LSD of all groups (*p-value*= 0.000) < 0.05, there is a meaningful difference in the average level of TNF-α between all groups. The smallest average difference in TNF-α levels in group K with P3 was 0.31 pg/mL; while P1 with P3 has the largest average TNF-α rate of 5.01 pg/mL**.** Description of inflammation of the mucosa cavum rice and sinus K score 0; P1 score 3; P2 score 2; P3 score 1. ***Conclusion:*** Moringa leaf ethanol extract in this study can be used as adjuvant therapy because it can lower MDA and TNF-α levels in mice induced with *Staphylococcus aureus* along with amoxicillin standard therapy. Extract ethanol moringa leaves in this study can be used as adjuvant therapy because it can lower the score of inflammatory cells better than by only given standard therapy only

Keywords: Extracts of moringa leaf ethanol; acute rhinosinusitis; MDA; TNF-α.

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Introduction

Often doctors prescribe antibiotics in almost all cases of acute rhinosinusitis, antibiotics prescribed in patient visits range from 82-88%, this is due to difficulties in the diagnostic enforcement of acute rhinosinusitis of viruses or bacteria due to similar clinical symptoms[1–3]. Streptococcus pneumoniae (20-45%), Haemophilus influenzae (20-43%), Moraxella

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catarrhalis (14-28%), and Staphylococcus aureus (8-11%) often the cause of acute rhinosinusitis[4] Antibiotics should not be routinely prescribed for therapy in acute rhinosinusitis as they may contribute to an increasing spectrum of antibiotic resistance, so proper diagnosis and proper therapy are expected to prevent overuse of antibiotics and resistance due to unwise use of antibiotics. As cited from Haque 2020, antibiotic resistance conditions will get worse because almost no new antibiotics will soon appear on the market to fight antibiotic resistance.[6] Adequate therapy is required to prevent the onset of complications of acute rhinosinusitis into chronic rhinosinusitis. Where surgery is the only treatment to treat chronic rhinosinusitis and other complications that occur when an acute infection is not treated properly. The cost of surgical therapy is relatively small and does not guarantee 100% of patients not to experience acute re-infection with rhinosinusitis in the future[7]

The European Position Paper on Rhinosinusitis and Nasal Polyps in 2012 said there was an increase in the number of chronic rhinosinusitis, where 5-15% of the American and British populations suffer from chronic rhinosinusitis. In 2001, more than 35 million American adults suffered from rhinosinusitis and more than 460,000 sinus surgeries were performed annually, making chronic rhinosinusitis one of the most frequent. Based on the definition of EPOS symptoms, the latest data on the prevalence of chronic rhinosinusitis in Brazil is 5.5%, in China 8%, in Europe and Korea 11%, and 12% in the United States as quoted from Dietz de Loos 2019. The main purpose of therapy in acute bacterial rhinosinusitis in order to prevent infections that continue to be chronic, shorten the duration of infection, and avoid unwanted complications. The therapy used for acute bacterial rhinosinusitis is an antibiotic and accompanied by additional therapies in the form of decongestants, mucolytics, antihistamines, nasal irrigation, even antioxidants found to be commonly dense in vitamin C and vitamin E. Moringa leaves (M. oleifera) contain antioxidant components that can help rebalance redox metabolism and reduce excess free radicals so as to prevent oxidative stress[8]. Moringa leaves extracted using methanol, ethyl acetate, dichloromethane, and n-hexane, have been studied in vitro proving that moringa leaves have high antioxidant activity using methods of measuring radical capture by DPPH (1,1-difenil-2-pikrilhidrazil) and methods of removal of cation radicals by ABTS (2,2'-azino-bis-[3-etilbenzotiazolin sulfonate]) [9,10]. Various contents of moringa leaf extract can be proven to prevent the development of acute diseases become chronic with antioxidant agents, anti-inflammatory, hyperlipidemic, hepatoprotector, even antidiabetic and anti-cancer. Phenolic acid content in moringa leaves, such as flavonoids and quercetin can be a potent antioxidant agent in reducing damage caused by oxidative stress in cells[11]

Lin 2018 in previous research stated that moringa leaf extract at a dose of 100mg/kgBW/day was shown to have an antioxidant effect in CCL4-induced rats (Carbon tetrachloride) [12]. The effect of moringa leaf extract at a dose of 80mg/kgBW/day found to be high in antioxidant effects in DMBA-induced rats (Dimethylbenz(α)anthracene) [11]. Free oxygen that is radical is known as Reactive Oxygen Species (ROS) which is produced due to inflammation due to bacteria that cause acute rhinosinusitis if it cannot be controlled by macrophages. ROS is highly reactive and attacks various classes of surrounding biomolecules including proteins, DNA, and lipids such as polyunsaturated fatty acids (PUFA). PUFA arachidonic acid will undergo peroxidation to eventually form malondialdehyde (MDA). The higher the level of free radicals, the higher the level of malondialdehyde in the body, found in previous studies that examined nasal polyps in conjunction with free radicals. Overproduction of ROS will be able to cause oxidative stress, which will stimulate the active transcription factor NF-κB (Nuclear factor kappa B) as a marker of the occurrence of acuteinflammation, an increase in oxidant levels that will express pro-inflammatory agents including TNF-α [12] Acute inflammation that is not treated properly, prolongedly and repeatedly will cause tissue necrosis. Research looked at the histopathological picture of acute rhinosinusitis in humans very less, but Berger *et al,*2000 found inflammatory reactions in lamina propria characterized by edema and infiltration of massive polymorphonuclear cells and mononuclear cells with the formation of micro abscess and necrosis. The purpose of this study was to look at the influence of moringa leaf ethanol extract on MDA, TNF-α levels and histopathology picture of sinus mucosal inflammation in Sprague Dawley white rats that had induced Staphylococcus aureus,as a model of acute rhinosinusitis.

Research design and Methods

Using *posttest only control group* researchdesign. male white rat strain *Sprague Dawley* was used,

maintained and treated at the Nutrition Laboratory of the Center for Food and Nutrition Studies of Gadjah Mada University (UGM). The manufacture of moringa leaf ethanol extract (EEDK) as well as examination of MDA and TNF-α levels with ELISA was conducted at UGM, while histopathology examination of inflammation and necrosis was conducted at the Pa Laboratory of SebelasMaret University (UNS). *Ethical Clearance* obtained from Moewardi Solo Hospital. *Staphylococcus aureus* used with a concentration of 0.5 Mc Farland or equivalent to $1x10^8$ CFU/ml [15], which is induced intra peritoneally and intra nasally.

Mice before being treated, adaptation for 7 days, then randomized and divided into 4 groups. Group 1: got standard feed diet., group 2: got standard feed diet and *staphylococcus aureus*induced, group 3: got standard feed diet, *Staphylococcus aureus* induced as well as given amoxicillin dose of 27 mg/day, group 4: got a standard feed diet, induced *Staphylococcus aureus* as well as given amoxicillin dose of 27 mg/day and given (EEDK) 200 mg/KgBW/day. After rat induced it takes 14 days to become a mouse model of acute rhinosinusitis. Then given therapy for 10 days in each group. Data analysis: Normality test using *Shapiro-Wilk* Test and homogeneity using *Levene's Test* obtained results p>0.05. Then conducted *One Way Anova*Test obtained p<0.05 and continued *post-hoc LSD test.* The data is analyzed using SPSS 25.0 *for* Windows.

Ethical Clearance: This article was approved before submission. 219/ II/ HREC/ 2020

Results

Table 1.1: MDA Level analysis results

Group (N)	Mean ± SD	Min - max	*p-value*
K (8)	1.38 ± 0.31	0.95 – 1.77	
P1(8)	9.77 ± 0.34	9.38 – 10.51	
P2 (8)	5.59 ± 0.36	4.97 – 6.04	0,000
P3 (8)	2.30 ± 0.18	2.02 – 2.58	

The results of the analysis with *Anova* test obtained a value of p (Sig.) = 0.000 (p< 0.05), so H0 was rejected and H1 accepted, meaning "there is an mean difference in MDA levels in all four groups" or "there are at least two groups that have significantly different means of MDA levels". To find out which groups have average/mean differences, the data is then tested with post *hoc* LSD tests.

Post *Hoc* analysis with LSD of all groups obtained a sig value (*p-value*) < 0.05, this means there is a meaningful difference in the average MDA rate between all groups. The smallest mean difference of MDA levels in group K with P3 of 0.92 pg / mL; while P1 with P3 has the largest MDA mean difference of 7.47 pg / mL.

Table 1.2: Analysis of TNF-α Levels

Group (N)	Group	Mean ± SD	Min - max	p-value
K (8)	K	6.19 ± 0.32	5.77 - 6.62	
P1(8)	P1	10.89 ± 0.29	10.55 - 11.40	
P2 (8)	P2	8.72 ± 0.21	8.38 - 9.01	0,000
P3 (8)	P3	5.88 ± 0.16	5.62 - 6.09	

The results of the analysis with *Anova* test obtained a value of p (Sig.) = 0.000 (p< 0.05), so ho was rejected and H1 was accepted, meaning "there aremean difference in TNF-α levels in all four groups" or "there are at least two groups that have significantly different means levels of TNF-α". To find out which groups have mean differences, the data is then tested with post hoc LSD tests.

Post Hoc analysis with LSD of all groups obtained sig values (p-value=0.000) < 0.05, this means there is a meaningful difference in the average TNF-α rate between all groups. The Mean Difference of the smallest difference in TNF-α levels in group K with P3 of 0.31 pg/mL; while P1 with P3 has an average difference (Mean Different) tnf-α and the largest is of 5.01 pg / mL.

Description: K (Shows an overview of normal mucosal tissue in rice cavum and paranasal sinuses. Not visible as inflammatory cells lymphocytes, plasma cells and neutrophils illustrated by score 0); P1 (Showing an overview of the presence of inflammation in rice cavum and paranasal sinuses, lymphocyte inflammatory cells, plasma cells and neutrophils are indicated by yellow arrows. Group P1 illustrated by score 3); P2 (Showing an image of inflammation of the rice cavum and paranasal sinuses, an inflammatory cell of lymphocytes, plasma cells and neutrophils is indicated by a yellow arrow.Group P2 illustrated by score 2); P3(Showing an image of inflammation of the rice cavum and paranasal sinuses, an inflammatory cell of lymphocytes, plasma cells and neutrophils is indicated by a yellow arrow. Group P3 illustrated by score 1).

Discussion

MDA

The results showed that the difference between the treatment group that was only given antibiotics

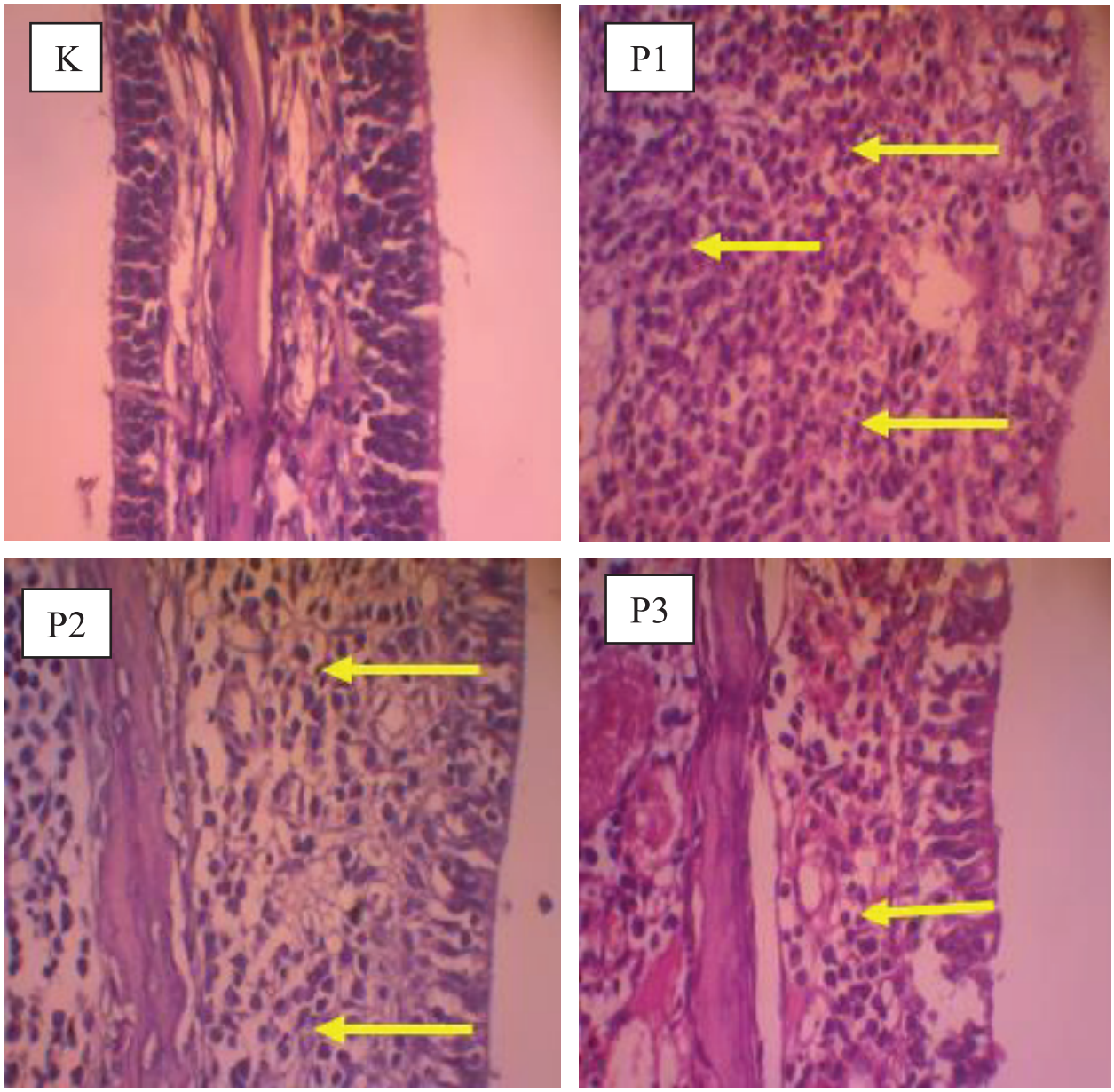

Fig: Illustration of inflammatory cell histopathology:

and the antibiotic treatment group and EEDK was statistically significant because it had a $p <$ of 0.05. This explains that administering *EEDK* therapy 40 mg / 200 grBW has the effect of lowering the MDA levels of rats modeled on acute bacterial rhinosinusitis induced *Staphylococcus aureus.* The results were obtained that the group of mice who got antibiotics and *EEDK* 40 mg / 200 grBW had MDA levels of 2.30 pg / mL. While the group of mice that only get antibiotics alone has an MDA level of 5.58 pg / mL. From these results, it was seen that mice that did not get EEDK *adjuvant* therapy had higher MDA levels, which is a difference of 3.28 pg/mL. The results of this study are in line with research conducted by Sumandjar (2020) in LPS-induced mice and given a fraction of acetyl acetate moringa leaves at doses of 10, 20, and 40 mg / kgBW, obtained statistically significant results to reduce MDA.

The results of this study in the group of mice that received adjuvant EEDK therapy had lower MDA levels than in the group of mice that did not get EEDK adjuvant therapy this is in line with el-Sayed 2018 study that stated *M.Oleifera* or moringa leaves significantly reduced levels of NO and MDA in mice infected with *Cryptosporidiu*[17]. The results of other studies in line with the study were shown in a study conducted by Aekthammarat (2019), which aims to determine the antioxidant effect of moringa leaf extract by looking at MDA levels in hypertensive model mice. The result of the study was the

administration of moringa extract effective to lower plasma and aortic MDA levels of hypertensive rats [18] also able to prevent the occurrence of elevated levels of malondialdehyde (MDA) in hypercholesterolemia model mice, where the higher the dose of moringa leaf extract given the lower levels of malondialdehyde (MDA)[19]. In ulfah*et al,* 2019 study, the administration of a combination extract derived from androgynous sauropus leaves and moringa leaves, can decrease the concentration of MDA by 71.19% in ration-induced rats into anemia. This may be due to the correlation between the antioxidant components of Androgynous Sauropus extract and moringa leaf extract.[20]

In this study it can be concluded that acute bacterial rhinosinusitis, antibiotic therapy as the main therapy coupled with EEDK as adjuvant therapy effectively accelerates the healing process in acute inflammation characterized by a decrease in MDA levels, as a compound that is a marker of the occurrence of oxidative stress, where MDA is an oxidation product of unsaturated fatty acids by free radicals as well as metabolites of cell components produced by free radicals. Seeing high MDA levels indicates the oxidation process in cell membranes, when high antioxidants will usually be followed by a decrease in MDA levels[21] In this study the decrease in MDA levels is due to the presence of antioxidant content in EEDK that can reduce oxidative stress at the time of acute inflammation. The difference in MDA levels can be seen from the group given the main antibiotic therapy alone compared to the group of antibiotic therapy given additional adjuvant therapy EEDK.

TNF-α

The results showed that the difference between the treatment group that was only given antibiotics and the treatment group that got antibiotics and EEDK was statistically significant because it had a *p* value of < 0.05. This explains that administering *EEDK* therapy 40 mg / 200 grBW has the effect of lowering levels of TNF-αratsmodel acute bacterial rhinosinusitis induced *Staphylococcus aureus.* The results were obtained that the group of mice who received antibiotic therapy and *EEDK* 40 mg / 200 grBW had a tnf-α level of 5.88 pg / mL. While the group of mice that only get antibiotic therapy alone has a tnf-α level of 8.72 pg / mL. From these results, it was seen that mice that did not get EEDK *adjuvant* therapy had higher levels of TNF-α, which is a difference of 2.84 pg/mL.

In this study it can be concluded that acute bacterial rhinosinusitis, antibiotic therapy as the main therapy coupled with EEDK as adjuvant therapy effectively accelerates the acute inflammatory healing process characterized by a decrease in levels of TNF-α. The decrease in TNF-α levels is caused by antioxidant content in EEDK which can reduce oxidative stress during the occurrence of acute inflammation. The difference in TNF-α levels can be seen from the group given the main antibiotic therapy alone compared to the group of antibiotic therapy given additional adjuvant therapy EEDK.

Inflammatory Cell Overview

To see the expansion of inflammatory cells is carried out assessment using the following inflammatory scores.

0 found inflammatory cells (lymphocytes, plasma cells and neutrophil) < 1% area of nasal cavity and paranasal sinuses

1 found inflammatory cells in 1-25% area of nasal cavity and paranasal sinuses

2 found inflammatory cells in 26-50% area of nasal cavity and paranasal sinuses

3 found inflammatory cells in 51-75% area of rice cavum and paranasal sinus

4 found inflammatory cells in 76-100% area of nasal cavity and paranasal sinus

The results of the analysis using non-parametric statistical test Kruskall Wallis, obtained p value (sig)=0.002 <0.05, there were significant differences in inflammatory cells in the four groups or there were at least two significant different groups". To find out the differences between the groups followed by the Mann-Whitney test. The results of the Mann-Whitnet test could be seen that there were significant differences between the control group mice and the treatment group. Experimental animal models histologically revealed inflammatory reactions in the lamina propria, with massive infiltration of white blood cells and edema. There is a massive inflammatory reaction with the recruitment of many polymorphonuclear cells in the form and mononuclear.[22]. While in the group P2 with P3 obtained p-value= 0.445 ≥0.05 which means there is no meaningful difference between P2 and P3. However, when viewed from the expansion of inflammatory cells, the P3 group scores better than P2, so it can be concluded that the administration of EEDK as adjuvant therapy provides improvements in the expansion of inflammatory cells.

Conclusions that can be taken as follows: Moringa leaf ethanol extract in this study can be used as adjuvant therapy can lower MDA and TNF-α levels in rats induced with Staphylococcus a*ureus*along with standard therapy. *amoxicillin* Ethanol extract of moringa leaves in this study can be used as adjuvant therapy can lower the inflammatory cell scores better than by only standard therapy administration only.

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The authors declared no conflicts of interests

Contribution of Authors:

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Editing and approval of final draft: Andriana Tjitria Widi Wardani

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