Original Article

Ethanolic Extract of Moringa oleifera Leaves Ameliorates TNF-α and IL-8 Level from Rat Model of *S. Aureus*-Induced Rhinosinusitis

Andriana T. W. Wardani*[1,2], Bambang Purwanto[3,4], Dono Indarto[3,5,8], Brian Wasita[C,F], Risya Cilmiaty AR[C, G]

[1]Doctorate Student of Medical Sciences, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

[2]Department of Anatomic Pathology, Faculty of Medicine, Sultan Agung Islamic University, Semarang, Indonesia

[3]Doctorate Program of Medical Sciences, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

[4]Department of internal medicine, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

[5]Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

[6]Department of Anatomic Pathology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

[7]Department dental and oral disease, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

[8]Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

ABSTRACT

Background: Acute bacterial rhinosinusitis is a common complication that require antibiotic therapy to recover promptly. To avoid the emergence and spread of antibiotic-resistant bacteria, Ethanol extract of moringa leaves (EEMO) contains active ingredients that serve as antioxidants that can help restore redox metabolic balance and can reduce excess free radicals so as to prevent oxidative stress and speed healing.

Materials and Method: Effect of moringa leaves ethanol was evaluated in difference day post treatment, day 7th and day 10th. The level of TNF-a and IL-8 from each treatment group in Staphylococcus aureus rhinosinusitis induced rat was evaluated using ELISA compared to control group. Data were analyzed using SPSS 25.0 for Windows.

Result: The results of the analysis of different tests of paired T-Test can be concluded that there is decrease in the average level of TNF-α and IL-8 on the 7th day with the 10th day in the T2, T3 and T4 groups there is significant difference (p = 0.0001), while the T3 group has the most decrease TNF-α and IL-8 serum level (p = 0.0001).

Conclusion: The combination of Amoxicillin 27 mg/day and EEMO 100 mg/kg is better at decreasing both TNF-α and IL-8 levels during 10 days of administration. EEMO can be used as an adjuvant therapy for acute bacterial rhinosinusitis.

Keywords: Ethanol, moringa leaves, rhinosinusitis, TNF-α, IL8.

Introduction

Acute rhinosinusitis is a disease that is very often experienced by everyone and is widely found in all countries. Diagnosis of acute rhinosinusitis is difficult to establish becauseof nonspecific symptoms between the cause of a virus or bacteria. Difficulty in making a diagnosis will cause difficulties in giving therapy, as a result many viral rhinosinusitis is therapy using antibiotics. Treatment of therapies that

Access this article online	
Quick Response Code:	Website: www.pnrjournal.com
	DOI: 10.47750/pnr.2022.13.01.005

Address for correspondence: Andriana T. W. Wardani, Doctorate Student of Medical Sciences, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia. Phone: +62 271 646994.
E-mail address: andrianawardhani@gmail.com

Submitted: October 15, 2021 **Accepted:** Novtember 23, 2021
Published: December 4, 2021

How to cite this article: Wardani ATW, Purwanto B, Indarto D, Wasita B, Cilmiaty ARR. Ethanolic Extract of Moringa oleifera Leaves Ameliorates TNF- and IL-8 Level from Rat Model of Staphylococcus Aureus-Induced Rhinosinusitis. J Pharm Negative Results 2022;13(1):20–24.

take a long time adds to the amount of antibiotic resistance due to the disobedience of patients who feel better / healthier so they decided to stop taking antibiotics.[1] According to Jorgensen LC *et al,* 2013. Acute rhinosinusitis is one of the most common reasons for visiting a doctor, and the disease imposes huge economic costs on society in terms of direct costs as well as decreased productivity.[2]

Evidence-based clinical practice guidelines from the Infectious Diseases Society of America recommend that when antibiotics are indicated for the treatment of acute bacterial rhinosinusitis, the length of therapy is 5 to 7 days. [3] Improper sinusitis can cause changes in acute sinusitis to chronic sinusitis, and can lead to complications, resulting in greater costs for therapy.[4] The presence of microbes in the paranasal sinus mucosa causes the production of IL-8 from epithelial cells. IL-8 has an exudation effect of neutrophils in the paranasal sinuses. Neutrophils secrete proteases and superoxide causes disturbances in the mucochili then sinus retention occurs. According to existing data, about 29.5% of viral sinusitis patients get antibiotic therapy, causing an increase in the amount of antibiotic resistance due to improper therapy.[1] Moringa leaves (M. oleifera) have pharmacological functions, namely as antimicrobial, antifungal, antihypertensive, antihyperglycemic, antitumor, anticancer, anti-inflammation Moringa leaves extracts contains antioxidants such as flavonoids, carotenoids, phenolic and quercetin.[5] A study found that moringa leaf extract was able to reduce the production of TNF-α, IL-6 and IL-8.[6] Another successfully studied effect of moringa leaf extract is effective in lowering levels of TNF-α and IL-6 in DMBA-induced mice.(*Dimetilbenz(α)antrasen*) using 80mg/kg/day and in induced by aloxan mice at an optimal dose of 100 mg/kg/day.[7] Meanwhile, research on the effects of Moringa leaf extract as an adjuvant therapy on acute bacterial sinusitis were not widely provided yet.

The inflammatory response will cause edema, fluid extravasation, and increased mucus production. The inflammatory cascade involves polarizing type 1 T-helper cytokines associated with tumor-β necrosis factors and interferon-γ. Proinflammatory cytokines such as interleukin (IL)-1β, IL-6, dan IL-8 are potent chemo attractive agent for neutrophils. Mucosal inflammation can cause obstructionof the normal sinus outlet. This obstruction inhibits normal ventilation and normal drainage, leading to a decrease in partial pressure of oxygen, decreased cilia movement, and stasis secretions that can lead to secondary bacterial infections that can develop. The prevalence of bacterial infections in patients with clinically diagnosed ARS is not well defined due to the difficulty of distinguishing viral symptoms and bacterial infections. Clinical overview of ARS viruses and bacteria is similar. There were no clinical findings, including discoloration or the character of the nasal secretions, that predicted whether ARS came from bacteria, adverse effects due to improper antibiotic prescribing.[8]

Currently, acute rhinosinusitis is the fifth most common diagnosis in which antibiotics are prescribed in primary care, more than 80% of patients with symptoms of rhinosinusitis are prescribed antibiotics. Distinguishing the origin of bacteria or viruses from rhinosinusitis is challenging, which makesit difficult to decide whether or not to prescribe antibiotics. This uncertainty leads to antibiotic overprescribing, whichis considered an important reason for the development of bacterial resistance to antibiotics.[2] In airway surface fluid, this has an undeniable effect on inflammatory markers, such as IL-8, IL-4, interferon-gamma and TNF-α.[1] Short-term antibiotics (3-7 days) appear to be as effective as long-term antibiotics (10-14 days) for those clinically diagnosed with bacterial rhinosinusitis without severe disease or other complication factors.[9]

Cited from Matthew E. Falagas 2008, stated that most of the guidelines for the treatment of bacterial acute rhinosinusitis, generally agreed to provide the type of antibiotic recommended for initial treatment. While regarding the duration / duration of appropriate treatment is 10-14 days antibiotic therapy is most often recommended. This recommendation comes primarily from the microbiological efficacy of treatment for 10 days in bacterial acute rhinosinusitis.[10] Although antibiotics for acute rhinosinusitis should be given to patients with a high likelihood of bacterial disease, accurate clinical diagnosis is often difficult to achieve. Short-term antibiotic treatment has an effectiveness comparable to long-term treatment for bacterial acute rhinosinusitis. Shortened treatment, especially for non-severe patients and sting factors, can lead to fewer side effects, better patient adherence, lower levels of resistance progression, and the cost is cheaper.[10] Antibiotics recommended for treatment are amoxicillin/clavulanate which is indicated when the symptoms do not improve.[11]

Materials and Method

Ethics and Study Design

Male white rats aged 8-12 weeks, divided into 5 groups of 7 animals in each groups. The groups were normal control, rats induced by Staphylococcus aureus that received corn oil as vehicle as negative control. Rats that induced by Staphylococcus were divided into several treatments such as amoxicillin, moringa leaf ethanol extract and combination of amoxicillin and moringa leaf ethanol extract). This experiment has been approved by the Ethics Commission Health Research of Dr. Moewardi General Hospital with Ethical Clearance number: 219/ II/ HREC/ 2020.

Animal Experimental

The groups were The treatment group were described as follows: Control (CN): Male white rats that received a standard feed diet; Treatment Group 1 (T1): Male white rats induced *Staphylococcus aureus* and received a standard feed diet; Treatment Group 2 (T2): Male white mice induced *Staphylococcus aureus,* and given the antibiotic amoxicillin 27 mg/day and standard feed; ; Treatment Group 3 (T3): Male white rats induced *Staphylococcus aureus,* given antibiotic amoxicillin 27 mg/day and ethanol extract of

moringa leaves 100 mg/kg and received feed diet; Treatment Group 4 (T4): Male white mice induced Staphylococcus aureus, given an extract 100 mg/Kg of moringa leaf ethanol rack and received standard feed diet. Treatment was conducted in total 10 days. After following treatment, rats were terminated and the serum was removed for analysis. Tumor tissue was processed and embedded in paraffin blocks for ELISA testing.

Enzyme-linked immunosorbent assay of TNF-α and IL-8 Levels

Result

The result of TNF-α showed on day 7 all treatment groups are normally distributed ($p > 0.05$), but not homogeneous ($p < 0.05$). The Oneway Anova test result of $p = 0.0001$ ($p < 0.05$) indicated the data were significantly different. The results of the IL-8 normality and homogeneity test on day 7 showed all treatment groups were normal ($p > 0.05$), and homogeneous ($p > 0.05$). From Oneway Anova the p-value = 0.0001 ($p < 0.05$) showed a significant difference.

The normality and homogeneity test showed that TNF-α levels on day 10 of all treatment group data were normally distributed ($p > 0.05$) and homogeneous ($p > 0.05$). Oneway Anova test showed that the data were significantly different ($p = 0.0001$). The results of the normality and homogeneity test of IL-8 levels on day 10 were normally distributed (p value > 0.005) and not homogeneous (p value < 0.05). The Oneway Anova test showed that the data were significantly different ($p = 0.0001$)

Based on Figure 1, the analysis of paired T-Test can be concluded the decrease in average levels of TNF-α on the 7th day with the 10th day in the T2, T3 and T4 groups there are significant difference. ($p = 0{,}0001$). T3 groups showed the highest decline rate of TNF- α serum level.

Based on Figure 2 illustrated the analysis of paired T-test and can be concluded that the mean decrease in IL-8 levels on the 7th day with the 10th day in the T2, T3, T4 groups were significantly different ($p = 0.0001$). T2 groups showed the highest decline rate of IL-8 serum level.

Table 1 : Test of normality and homogeneity of TNF-α and IL-8 levels in day-7 post treatment

VARIABLE	Group					p-value
	CN	T1	T2	T3	T4	
	Mean ±SD	Mean ±SD	Mean±SD	Mean ±SD	Mean ±SD	
Rate TNF-α (pg/mL)	5.88±0.38	10.58±0.30	8.93±0.52	7.36±0.18	6.921±0.14	
Shapiro wilk	0.353*	0.243*	0.591*	0.979*	0.722*	
Levene's test						0.034
One way Anova						0.0001**
Rate IL-8 (pg/mL)	4.24±0.3	11.48±0.43	9.58±0.13	8.90±0.28	5.49±0.61	
Shapiro wilk	0.658*	0.807*	0.285*	0.673*	0.708*	
Levene's test						0.092*
One way Anova						0.0001**

Description:

* Significant>0,05

** Significant <0,05

Table 2 : Test of normality and homogeneity of TNF-α and IL-8 levels in day-10 post treatment

VARIABLE	Group					p-value
	CN	T1	T2	T3	T4	
	Mean ±SD	Mean ±SD	Mean±SD	Mean ±SD	Mean ±SD	
Rate TNF-α (pg/mL)	6.21±0.34	10.90±0.31	8.77±0.18	6.86±0.23	6.46±0.14	
Shapiro wilk	0.405*	0.565*	0.877*	0.991*	0.333*	
Levene's test						0,124*
One way Anova						0.0001**
Rate IL-8 (pg/mL)	4.78±0.34	12.11±0.37	7.61±0.55	7.51±0.25	5.10±0.55	
Shapiro wilk	0.729*	0.996*	0.445*	0.957*	0.381*	
Levene's test						0,031
One way Anova						0.0001**

Description:

* Significant>0,05

** Significant <0,05

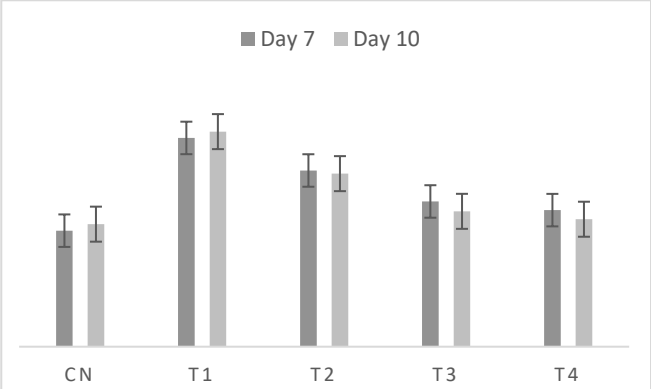

Figure 1 : - TNF- α Serum level on day 7 and 10 Post Treatment

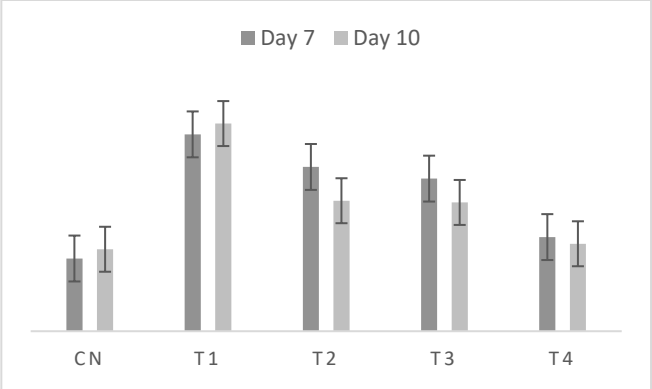

Figure 2 : IL-8 Serum level on day 7 and 10 Post Treatment

Discussion

The results of studies that have been conducted showed that in the treatment group of male white rats induced Staphylococcus aureus and given EEMO and amoxicillin, can decrease the inflammatory response seen from levels of TNF-α and IL-8 on the 7th and 10th days. Antibiotics are used because it is an empirical treatment of acute bacterial sinusitis. In this study given amoxicillin with an adjusted dose of 27 mg/day and which is recommended as a first-line therapy in bacterial rhinosinusitis.[12] EEMO dose based on previous research that stated that a dose of 100 mg/kg/day provides a more optimal reduction effect as an anti-inflammatory and high antioxidant effect.[13] Extract water and ethanol moringa seeds (Moringa oleifera) and soursop fruit pods (Annona muricata) have antibacterial effects against Staphylococcus aureus, Vibrio cholerae, Escherichia coli and Salmonella Enteritidis.[14]

These results are in line with previous studies that have been conducted by using the same dose.[13] According to previous research, the administration of moringa extract is effectively used as an anti-inflammatory agent because it has been shown to be effective in lowering IL-8 levels, lowering the production of TNF-α in response to lipopolysaccharides and cigarettes.[15] Article by Tiloke (2018) explained that moringa leaf extract has not only anti-inflammatory effect , but also as an anti-cancer because it is able to inhibit the increase in IL-8 levels.[16] In mice the model induced into hyperuricemia, then given moringa leaf extract, was shown to lower levels of the inflammatory response studied, namely TNF-α hyperuricemia.[17] Other evidence was presented in model mice with dyslipidemia, where the disease is known to have an increased inflammatory response. After being given moringa leaf extract can lower the levels of acute inflammatory response of TNF-α.

Decreased levels of TNF-α in the treatment of the T2, T3 T4 between day 7 and day 10 there is a significant difference, the most decrease in IL-8 and TNF- α levels was found in the T2 and T3, respectively. The best decrease was found on day 10 compared to day 7.

It can be concluded that the combination of amoxicillin 27 mg/day and EEMO 100 mg/kg is better in decreasing both TNF-α and IL-8 levels during 10 days of administration. EEMO can be used as an adjuvant therapy in acute bacterial rhinosinusitis. Average levels of the inflammatory responses TNF-α and IL-8 in Staphylococcus aureus-induced mice were optimally decreased after being given amoxicillin and EEMO.

Acknowledgments

Source of Funding: Faculty of Medicine Sultan Agung Islamic University, Semarang, Indonesia

References

[1] Mainz JG, Jaudszus A, Pletz MW. Development of a clinical decision rule for diagnosing sinus infections–to reduce unnecessary antibiotic prescribing. Expert Rev Clin Pharmacol 2018;11:923–5. doi:10.1080/17512433.2018.1524753.

[2] Jorgensen LC, Friis Christensen S, Cordoba Currea G, Llor C, Bjerrum L. Antibiotic prescribing in patients with acute rhinosinusitis is not in agreement with European recommendations. Scand J Prim Health Care 2013;31:101–5. doi:10.3109/02813432.2013.788270.

[3] King LM, Sanchez G V., Bartoces M, Hicks LA, Fleming-Dutra KE. Antibiotic therapy duration in US adults with sinusitis. JAMA Intern Med 2018;178:992–4. doi:10.1001/jamainternmed.2018.0407.

[4] Rosenfeld RM, Piccirillo JF, Chandrasekhar SS, Brook I, Kumar KA, Kramper M, et al. Clinical Practice Guideline (Update): Adult Sinusitis. Otolaryngol Neck Surg 2S) 2015;152(2S):S1–S39. doi:10.1177/0194599815572097.

[5] Aminah S, Ramdhan T, Yanis M. Kandungan Nutrisi dan Sifat Fungsional Tanaman Kelor (Moringa oleifera). Bul Pertan Perkota 2015;5:35–44.

[6] Kooltheat N, Pankla Sranujit R, Chumark P, Potup P, Laytragoon-Lewin N, Usuwanthim K. An ethyl acetate fraction of Moringa oleifera Lam. inhibits human macrophage cytokine production induced by cigarette smoke. Nutrients 2014;6:697–710. doi:10.3390/nu6020697.

[7] Endang T, Sukma D. Ekstrak Metanol Daun Kelor Menurunkan Kadar TNF- α dan IL-6 Serum , serta MDA Kolon Tikus yang Diinduksi DMBA Methanolic Extract of Moringa oleifera Reduces Serum TNF-α, IL-6, and Colonic Tissue MDA Levels in DMBA-induced Wistar Rats. J Kedokt Brawijaya 2016;29:25–31.

[8] Smith SS, Ference EH, Charlesnika T, Tan BK, Kern RC, Chandra RK. The prevalence of bacterial infection in acute rhinosinusitis: A systematic review and meta-analysis. Laryngoscope 2015;125:57–9.

[9] Chow AW, Benninger MS, Brook I, Brozek JL, Goldstein EJC, Hicks LA, et al. IDSA Clinical Practice Guideline for Acute Bacterial Rhinosinusitis in Children and Adults. Clin Infect Dis 2012;54:1041–5. doi:10.1093/cid/cir1043.

[10] Falagas ME, Karageorgopoulos DE, Grammatikos AP, Matthaiou DK. Effectiveness and safety of short vs. long duration of antibiotic therapy for acute bacterial sinusitis: A meta-analysis of randomized trials. Br J Clin Pharmacol 2009;67:161–71. doi:10.1111/j.1365-2125.2008.03306.x.

[11] Mustafa M, Iftikhar M, Choudhury Shimmi S. Acute and Chronic Rhinosinusitis, Pathophysiology and Treatment Both project View project Multi Drug View project Acute and Chronic Rhinosinusitis, Pathophysiology and Treatment. Int J Pharm Sci Invent ISSN 2015;4:30–6.

[12] Zara M. Patel and Peter H. Hwang. Acute Bacterial Rhinosinusitis. Infect Ears, Nose, Throat, Sinuses 2018:1–393. doi:10.1007/978-3-319-74835-1.

[13] Lin M, Zhang J, Chen X. Bioactive flavonoids in Moringa oleifera and their health-promoting properties. J Funct Foods 2018;47:469–79. doi:10.1016/j.jff.2018.06.011.

[14] Vieira GHF, Mourão JA, Ângelo ÂM, Costa RA, Vieira RHS dos F. Antibacterial effect (in vitro) of moringa oleifera and annona muricata against gram positive and gram negative bacteria. Rev Inst Med Trop Sao Paulo 2010;52:129–32. doi:10.1590/S0036-46652010000300003.

[15] Engsuwan J, Waranuch N, Limpeanchob N, Ingkaninan K. Anti-inflammatory effect of moringa oleifera lam. Leaf extract on uvb-irradiated human keratinocytes. Songklanakarin J Sci Technol 2021;43:774–80. doi:10.14456/sjst-psu.2021.102.

[16] Tiloke C, Anand K, Gengan RM, Chuturgoon AA. Moringa oleifera and their phytonanoparticles: Potential antiproliferative agents against cancer. Biomed Pharmacother 2018;108:457–66. doi:10.1016/j.biopha.2018.09.060.

[17]. Wahyu Pribadi F, Widiartini C. The Effect of Kelor Leaves (Moringa oleifera) Ethanol Extract on Serum Uric Acid and Tumor Necrosis Factor-α of Hyperuricemic White Rats (Rattus norvegicus). IOP Conf Ser Earth Environ Sci 2019;406. doi:10.1088/1755-1315/406/1/012006.