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Anti-Inflammatory Effect of *Musa acuminata* Stem

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Abstract

Objective This study was designed to assess the anti-inflammatory effect of *Musa acuminata* through the expression of tumor necrosis factor-α (TNF-α) and nuclear factor kappa β (NF-κB) after 3 days of application of *Musa acuminata stem extract* (MASE) gel on oral mucosal wound.

Materials and Methods An experimental study with post-test only control group design was conducted. Twenty male *Rattus norvegicus* (Wistar) were injured on their left buccal mucosa and treated three times a day with MASE gel of varying concentrations: 0% (as control), MASE 25%, MASE 37.5%, and MASE 50%. On day 3, a biopsy was performed on each mucosal wound for later immunohistochemical analysis for the expressions of TNF-α and NF-κB.

Results The highest expression of TNF-α was observed in the control group (13.20 ± 1.79), while the lowest was in the treatment group using 50% MASE (6.40 ± 1.14). Meanwhile the comparison between treatment groups did not highlight any significant difference ($p > 0.05$). The highest expression of NF-κB was observed in the control group (13.20 ± 1.30), whereas the lowest was in the treatment group using MASE 50% (6.40 ± 1.14). NF-κB was significantly lower in the treatment group using MASE 50% when compared with other treatment groups ($p < 0.05$).

Conclusion Application of MASE on mucosal wound reduces the expression of TNF-α and NF-κB at all concentrations. The anti-inflammatory effect of MASE 50% was the strongest one.

Keywords
- *Musa acuminata*
- anti-inflammation
- TNF-α
- NF-κB

Introduction

Indonesia is known as a country that has a wide diversity of biological species. The country also has one of the richest store houses of medicinal plants in the world, despite research and development of these plants remaining extremely limited. Drugs derived from plants, also known as herbal medicines, can be used as alternatives to chemical drugs due to their lower potential for inducing adverse effects. Moreover, many plants and their extracts are also used for wound treatment.[1]

Banana (*Musa* spp.) is one of the most popular fruits in industrialized counties. *Musa* spp. is widely used as a cooking ingredient, especially in desserts. One type of *Musa* widely cultivated in South Borneo, Indonesia, is Mauli banana (*Musa acuminata*), which is very popular due to its sweet and appetizing taste. Interestingly, the stem of *Musa acuminata* contains many bioactive components such as ascorbic acid, β-carotene, lycopene, saponin, alkaloid, flavonoid, and tannin.[2]

Previous studies on *Musa acuminata* revealed that its stem extract (MASE) has antibacterial, antifungal, and antioxidant properties.[3] MASE of 25% concentration exhibited strong antibacterial and antifungal properties against *Streptococcus mutants* and *Candida albicans*.[4] The antioxidant property was achieved through the binding of MASE's bioactive compounds with ferrous iron, hydrogen peroxide, and hydroxyl.

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In addition, MASE was also shown to significantly downregulate malondialdehyde and upregulate super oxide dismutase.[3] A cytotoxicity test of MASE on baby hamster kidney-21 fibroblast cells showed nontoxic properties.[2,3] Moreover, MASE gel of 37.5% concentration can induce epithelium and fibroblast proliferation, as well as vascular endothelial growth factor in mucosal wounds of *Rattus norvegicus*, thus accelerating the wound-healing process.[4,5]

The most abundant bioactive compound in MASE is tannin, which contains polyphenols.[2,3] The polyphenol showed anti-inflammatory effects through the inhibitory mechanism of tumor necrosis factor α (TNF-α) and nuclear factor kappa β (NF-κB) signal.[6] The TNF-α is a pro-inflammatory cytokine produced by activating the NF-κB.[7] The inhibition of NF-κB and TNF-α is the key for the anti-inflammatory effect to increase the oral mucosal wound healing.[8] This study was therefore designed to evaluate the anti-inflammatory effect of *Musa acuminata* through expression of TNF-α and NF-κB after 3 days of application of MASE on oral mucosal wounds.

Materials and Methods

This study was an experimental laboratory research incorporating post-test only control group design. Research procedures were approved by the Ethical Clearance Committee, Faculty of Dentistry, Universitas Lambung Mangkurat; Banjarmasin, South Borneo, Indonesia, with number 039/KEPKG–FKGULM/EC/IX/2017.

Preparation of MASE

Extraction of *Musa acuminata's* stem (MASE) was performed in several steps. Stems of *Musa acuminata* were washed under running water, cut into small pieces, and dried in an oven at temperatures of 40 to 60°C for 3 days. The dried stems were then smoothed in a blender and soaked in 70% ethanol for 72 hours (maceration method) to produce the extract. The extract was agitated and filtered every day before being evaporated twice in a vacuum rotatory evaporator at temperatures of 40 to 50°C and a water bath until a thick extract was obtained. The resulting ethanol-free extract was then weighed and used to produce 0%, 25%, 37.5%, and 50% gel concentrations by adding 15% hydroxypropyl cellulose medium, 1% tween 80, 8% propylene glycol, 5 drops of candy oil, and distilled water until 100% weight was reached.

In Vivo Study

Twenty male *Rattus norvegicus* (Wistar) rats were randomly divided into four groups. Their left buccal mucosae were injured by means of a punch biopsy with a 6 mm diameter and 1 mm depth after being placed under general anesthesia ether.[5] The wound was ensured to be confined to the epithelium, without damaging the underlying muscle. Each of the four groups was then treated with MASE gel of varying concentrations three times a day (every 6–8 h); 0% (as control), MASE 25%, MASE 37.5%, and MASE 50%.

After 3 days of application, the *Rattus norvegicus* were sacrificed with lethal dose of ether by inhalation and their buccal mucosae biopsied for immunohistochemistry (IHC) analysis. Immunohistochemical staining was conducted using anti-mouse TNF-α monoclonal antibodies (Santa Cruz Biotechnology Inc, TNF-α [M-18]: sc 1348) and NF-κB monoclonal antibodies (Santa Cruz Biotechnology Inc, NF-κB p65 [F-6]: sc 8008). The number of macrophage cells showing the expressions of TNF-α and NF-κB was calculated in three different field locations using a light microscope (Olympus, United States) at 400× magnification, and subsequently averaged.

Statistical Analysis

The results were analyzed using one-way analysis of variance parametric test based on Shapiro—Wilk normality test and Levene's variance homogeneity test. The results showed normal data distribution and homogenous data variances. Consequently, further analysis by means of a Post Hoc Least Significant Difference test was conducted with significance $p < 0.05$.

Result

The anti-inflammatory effect of MASE on mucosal wounds was analyzed using IHC. The results of IHC analysis are shown in ►**Figs. 1** and **2**.

The highest expression of TNF-α was observed in the control group (13.20 ± 1.79), while the lowest was in the treatment group using MASE 50% (6.40 ± 1.14). The highest expression of NF-κB was observed in the control group (13.20 ± 1.30), whereas the lowest was in the treatment group using MASE 50% (6.40 ± 1.14; ►**Table 1**).

The expression of TNF-α in control group is higher compared to MASE 25% ($p = 0.000$), MSE 37.5% ($p = 0.000$), and MASE 50% ($p = 0.000$). The expression of TNF-α comparison between treatment groups did not show any difference ($p > 0.05$; ►**Table 1**).

The expression of NF-κB in control group is higher compared to MASE 25% ($p = 0.000$), MSE 37.5% ($p = 0.000$), and MASE 50% ($p = 0.000$). The expression of NF-κB was lower in MASE 50% compared to MASE 37.5% ($p = 0.045$) and MASE 25% ($p = 0.019$; ►**Table 1**).

Discussion

Inflammation is a protective response to tissue injury and infection characterized by a series of host responses including: vasodilation, and recruitment of immune cells and plasma proteins to the injured tissue.[9] Immune cells play a vital role in wound healing and contribute to the release of lysosomal enzymes and reactive oxygen species. In addition, these cells also clean the damaged area of debris.[10]

Cellular response induces pro-inflammatory mediators produced either from endogenous leukocytes (macrophages, monocytes, dendritic cells, or lymphocytes) and/or from the tissue cells themselves. These mediators are the main keys for inflammatory response, which is initiated by the recruitment of neutrophils.[11] Neutrophils are the main immune cells in

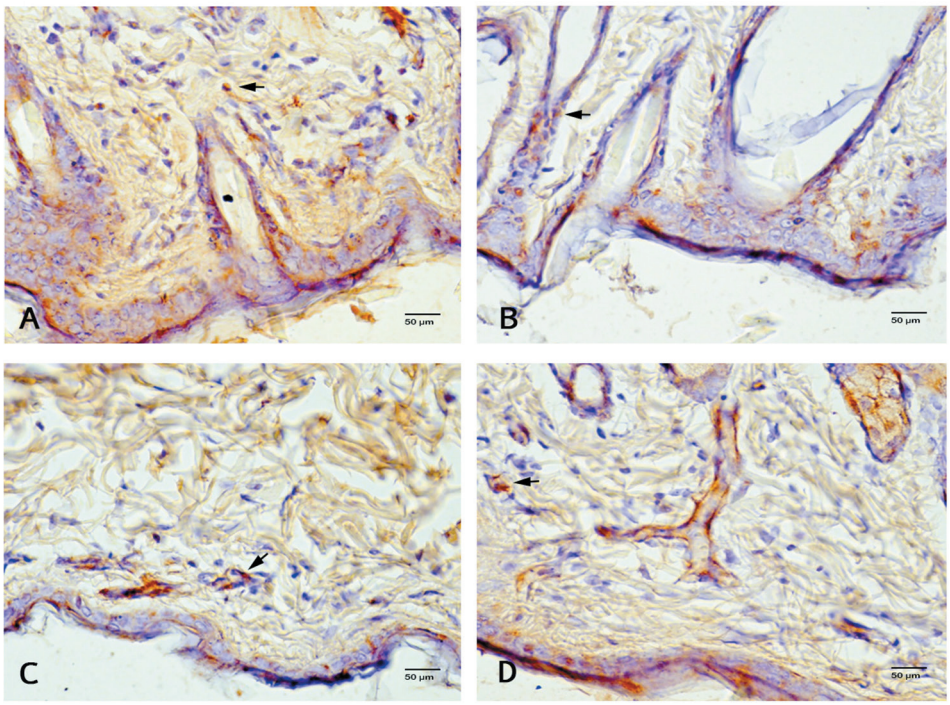

Fig. 1 Macrophage's expression of tumor necrosis factor-α(TNF-α; black arrow) on control group (**A**), *Musa acuminata* stem extract (MASE) concentrations of 25% (**B**), MASE concentrations of 37.5% (**C**), and MASE concentrations of 50% (**D**).

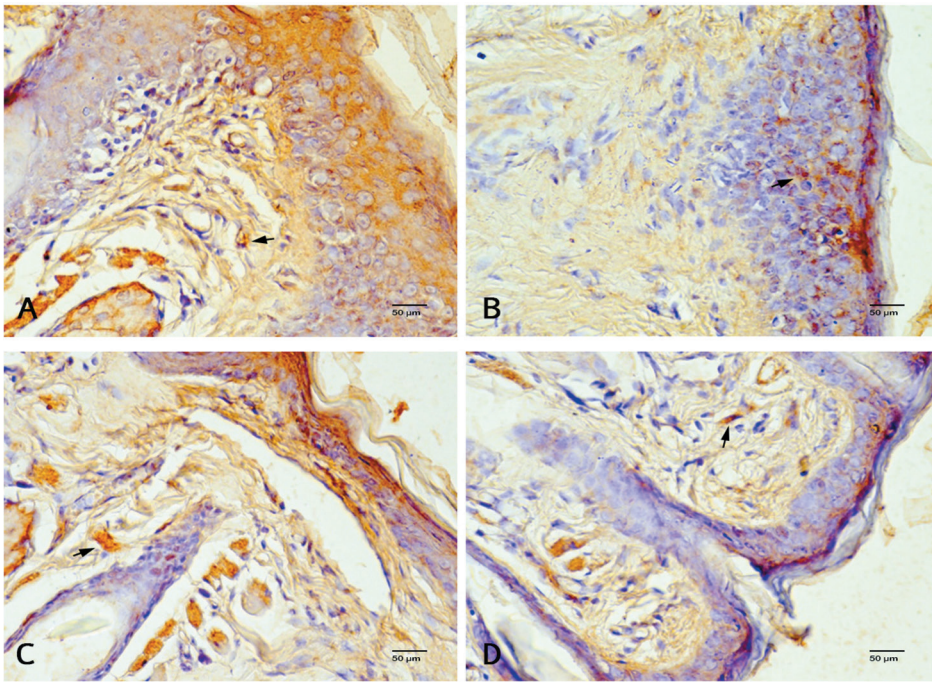

Fig. 2 Macrophage expression of nuclear factor kappa β(NF-κB; black arrow) in control group (**A**), *Musa acuminata* stem extract (MASE) concentrations of 25% (**B**), MASE concentrations of 37.5% (**C**), and MASE concentrations of 50% (**D**).

the first inflammation phase (48 h after injury) and begin to wane after 24 to 36 hours due to apoptosis at the time the circulating monocytes enter the wound and mature into tissue macrophages that play a crucial role in wound healing.[12] Macrophages are capable of inducing apoptosis of neutrophils with the help of membrane-bound NF-α, β3-integrins, and CD36.[13]

Following neutrophils, monocytes will migrate to the wound site and become macrophage, which play a central role in both the inflammatory phase and all stages of repair.[12] These cells are also responsible for regulating cellular components of the wound by inducing apoptosis or phagocytosing certain cells.[13] Meanwhile, macrophages phagocytose debris and bacteria, and produce and orchestrate inflammatory

Table 1 The expression of TNF-α and NF-κB following 3 days of MASE application

Group	Mean ± SD	
	Expression of TNF-α	Expression of NF-κB
Control	13.20 ± 1.79[a,b,c]	13.20 ± 1.30[a,b,c]
MASE 25%	6.60 ± 1.81[a]	9.00 ± 1.22[a,d]
MASE 37.5%	7.20 ± 0.83[b]	8.60 ± 1.51[b,e]
MASE 50%	6.40 ± 1.14[c]	6.40 ± 1.14[c,d,e]

Abbreviations: MASE, *Musa acuminata* stem extract; NF-KB, nuclear factor kappa β; SD, standard deviation; TNF-α, tumor necrosis factor-α.
Note: The same superscript character in each variable shows the differences for each group ($p < 0.05$).
a, b, c and d in TNF-α expression has value $p = 0.000$
a, b, c in NFkB expression has value $p = 0.000$
d in NF-κB expression has value $p = 0.019$
e in NFkB expression has value $p = 0.045$

cytokines (including growth factors) such as TNF-α, interleukin (IL)-6, IL-1, and basic fibroblast growth factor.[12]

TNF-α, also known as cachectin, is an inflammatory cytokine that plays a key role in the inflammatory response. TNF acts on several different signaling pathways through two cell surface receptors, TNFR1 and TNFR2, to regulate apoptotic pathways, NF-κB activation of inflammation, and activate stress-activated protein kinases.[14] Moreover, TNF-α represent the archetypal pro-inflammatory cytokines that are rapidly released following tissue injury or infection. TNFα leads the activation of RelA- or cRel-containing complexes in NF-κB.[15]

In this study, the anti-inflammatory effect of MASE gel on mucosal wounds through TNF-α and NF-κB expressions is evaluated. A study by Ritsu et al[16] using a mouse model had previously shown that TNF-α production was detected at a peak concentration on day 3 after the inflicting of the wound. In keeping with this finding, the biopsy and IHC analysis contained in this study were also performed within the same time frame.[16] The results of the study showed that the expression of TNF-α decreased with MASE-dose-dependent application. The MASE 50% showed the lowest expression of TNF-α.

TNF-α represents the pro-inflammatory cytokines that are immediately released after injury to tissues. The NF-κB pathway has been considered as the prototypical pro-inflammatory signaling pathway largely based on the activation of NF-κB by pro-inflammatory cytokines such as TNF-α.[7] TNF-α can induce the degradation of NF-κB inhibitor (IκB) via a specific cascade pathway. IκB sequesters NF-κB in the cytoplasmic compartment by binding to its inhibitory subunit. The breakdown of IκB therefore promotes the release of NF-κB that can translocate into the nucleus and activate specific inflammatory genes. Inflammation is a complex physiological process and the role of NF-κB in the inflammatory response cannot be extrapolated from *in vitro* studies.[15-17]

In the onset of inflammation, NF-κB promotes pro-inflammatory gene induction and also anti-inflammatory genes. In the nucleus, NF-κB binds to specific DNA targets to form what are known as kβ sites. They are present in gene target, assimilate with the basal transcriptional machinery, and associate with other transcription factors, including AP-1 and chromatin remodeling proteins such as CREB binding protein and p300. An active NF-κB nuclear translocation can execute the role of a pro- and anti-inflammatory mediator.[18] Prolonged inflammatory response can be resolved by the inhibition of NF-κB.[16,19]

This study showed that the expression of TNF-α decreased at all MASE concentrations compared with the control. Inhibition of TNF-α also decreased NF-κB expression in the macrophage cells involved in the mucosal wound-healing process. MASE gel contains polyphenol-possessed anti-inflammatory effect. The polyphenol can mediate the effect of TNF-α expression in macrophage cells by inhibiting p300/CREB-specific acetyl transferase and lead to the repression of the acetylation of histone/nonhistone proteins and histone acetyl transferase-dependent chromatin transcription. It may subsequently down-modulate the activation of NF-κB.[12]

MASE 50% has anti-inflammatory effects stronger than MASE 25% and MASE 37.5%. The application of MASE gel three times a day every 6 to 8 hours for 3 days can prevent the overexpression of TNF-α and NF-κB. The application of MASE 50% can accelerate wound healing.

Conclusion

It can be concluded that during the wound-healing process, the highest anti-inflammatory effect is obtained after the application of MASE 50% for 3 days on the oral mucosal wound.

Conflict of Interest
None declared.

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