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Research Report

Mauli banana stem extract application increased expression of NF-κB in traumatic ulcer healing

Maharani Laillyza Apriasari,[1] Retno Pudji Rahayu,[2] and Diah Savitri Ernawati[3]
[1]Department of Oral Medicine, Faculty of Dentistry, Universitas Lambung Mangkurat, Banjarmasin - Indonesia
[2]Department of Oral and Maxillofacial Pathology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya - Indonesia
[3]Department of Oral Medicine, Faculty of Dental Medicine, Universitas Airlangga, Surabaya - Indonesia

ABSTRACT

Background: *A traumatic ulcer represents one of the most prevalent disorders affecting the oral cavity. Ulceration of the oral cavity potentially results in secondary infection requiring topical medication which involves the use of antiseptics to accelerate wound healing. Previous research has shown that Mauli banana (Musa acuminata) stem extract (MBSE) contains bioactive material from terpenoid saponin present in Ambon bananas. The terpenoid saponin in Ambon banana stems will be captured by a G protein receptor in the macrophages, subsequently producing a protein kinase C that activates nuclear factor kappa beta (NF-κB). This increases both the activity and number of macrophages.* ***Purpose:*** *To analyze the expression of NF-κB (p50) in traumatic ulcers as an effect of MBSE.* ***Methods:*** *A true experimental design with a post-test only control group. It involved 40 male Rattus norvegicus strain rats as traumatic ulcer models divided into four groups: the negative control group administered gel, and the other treatment groups administered 25%, 37.5% and 50% ethanol extracts of MBSE gel respectively. A biopsy was performed on days 3 and 5. The preparation was produced to analyze the expression of NF-κB (p50) by means of immunohistochemistry examination.* ***Results:*** *There was a significant difference ($p<0.05$) in NF-κB (p50) expression ($p=0.005$) following MBSE gel administration of 37.5% concentration on day 3 compared to day 5.* ***Conclusion:*** *It can be concluded that MBSE gel topical application can increase expression of NF-κB (p50) in traumatic ulcer healing.*

Keywords: *expression; Mauli banana stem extract; NF-κB; traumatic ulcer; wound healing*

Correspondence: Maharani Laillyza Apriasari, Department of Oral Medicine, Faculty of Dentistry, Universitas Lambung Mangkurat, Jl. Veteran 128B, Banjarmasin, Indonesia. E-mail: maharaniroxy@gmail.com

INTRODUCTION

Traumatic ulcers constitute one of the most prevalent disorders affecting the oral cavity resulting from trauma either physical (mechanical, thermal, electrical) or chemical (acid or base substances, spicy foods) in nature. Their prevalence is relatively high as evidenced by several studies showing variations in incidence rates of between 3% and 24% within specific populations and locations.[1,2]

Ulceration of the oral cavity can be potentially subject to secondary infection because of the numerous commensal microorganisms found in the oral cavity. Hence, the need for application of antiseptic topical medication capable of accelerating wound healing to the oral cavity. At the time of writing, individuals suffering from this condition often use patent medicine containing *Aloe vera* leaf extract. One of the antiseptic topical drugs used in the field of dentistry to treat oral ulceration, it is difficult to acquire outside Java.[3–5]

Previous research has shown that Mauli banana stem extract (MBSE) gel at a concentration of 25% produces no significantly different effect compared to other patent medicines containing *Aloe vera* extract in terms of accelerating wound healing. This can be seen from the increased number of macrophage cells on day 3.[5] The bioactive contents of Mauli banana stem consist of 67.59% tannins, 14.49% saponins, 0.34% alkaloids, 0.44% ascorbic acid, 0.25% flavonoids and 0.006% lycopene. MBSE is an antioxidant containing hydrogen peroxide and hydroxyl which stimulates heavy metal binding activity, in addition

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to lowering malondildehyde (MDA) and promoting super oxide dismutase (SOD) activities and catalase in the healing process of rat oral mucosa.[6,7]

Previous research has shown that banana stems contain a condensed tannin bioactive material and terpenoid saponins also found in Ambon banana.[8,9] In addition, MBSE contains condensed tannins and terpenoid saponins common to other banana stems. Terpenoid saponin in Ambon banana stems constitutes an immunomodulator that can increase both the number and activity of macrophages. Captured by the G protein receptor in macrophages, it activates nuclear factor kappa beta/NF-κB (p50) through a protein kinase C-yielding process, thereby increasing the number and activity of macrophages.[10,11]

Based on the above statement, the Mauli banana extract has the potential to accelerate wound healing as an immunomodulator through an increase in the number of macrophage cells by means of enhancing expression of NF-κB (p50). The purpose of this study is to analyze the expression of NF-κB (p50) in traumatic ulcers as an effect of MBSE.

MATERIALS AND METHODS

The materials used in this experiment were 100 gms of Mauli banana stems, six liters of 70% ethanol, carbopol, hydroxypropyl cellulose medium (HPMC), propylenglycol, aquadest, aluminum foil, hydroxypropyl methylcellulose, banana stem, candy oil (CV. Cahaya Kimia), propylene glycol (Brataco) and tween 80 (Brataco). Each gel composition of Mauli banana ethanol extract at concentrations of 25%, 37.5%, and 50% respectively was added to 15% HPMC, 1% Tween 80, 8% Propylenglikol, five drops of candy oil and aquades making up the total weight. The other materials included chemical substances required for immunohistochemistry (xylol, ethanol, PBS, trypsin, alcohol, aquadesilata, streptavidin biotin, 0.5% H2O2, substrate and phosphotase buffer), monocloal anti-mouse antibody to NF-κB (p50) (E-10): sc-8414 (Santa Cruz Biotechnology, Inc.) and staining material of haematoxylin eosin (HE).

Samples of the banana stems to be extracted were washed with running water and cut into small pieces, then dried in the oven at a temperature of 40–60 degrees for three days. When dehydrated, the pieces were smoothed in a blender and weighed again before extraction was carried out using maceration method. This involved soaking the extract in 750 ml of 70% ethanol for a period of 72 hours while occasionally agitating it. The resulting liquid was evaporated with a vacuum rotary evaporator at a temperature of 40–50°C until a thick extract was obtained which was subjected to ethanol-free examination. 25%, 37.5% and 50% concentrations of the extracts were subsequently made by means of hydroxypropyl methylcellulose (HPMC).

This study received ethical clearance for experimentation on animal subjects (No. 56/KKEPK.FKG/VI/2015) from the Ethics Research Committee, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia. The research type constituted a true experimental study incorporating post-test only control group design using male, 300 gm, Wistar strain *Rattus norvegicus* as the models afflicted with a traumatic ulcer. The treatment was initiated with the inhalation of 0.75 ml of diethyl ether for 5–10 minutes to sedate each subject. The right buccal mucosa was then penetrated with a 6 mm diameter biopsy punch to a depth of 1 mm. From a clinical perspective, the traumatic ulcers induced in the subjects' right buccal mucosa could be seen to have extended into the epithelial tissue, but not into the muscles.

This study involved 40, male, Wistar strain *Rattus norvegicus* samples divided into a negative control group (K) given gel without MBSE every 6–8 hours; a treatment group 1 (P1) given MBSE gel of 25% concentration three times a day every 6–8 hours; treatment group 2 (P2) given MBSE gel of 37.5% concentration three times a day every 6-8 hours and treatment group 3 (P3) given MBSE gel of 50% concentration three times a day every 6–8 hours. The traumatic ulcer tissues of the left buccal mucosa of the subjects were removed for preparation, followed by imunohistochemical staining, in order to analyze NF-κB (p50) expression.

RESULTS

Statistical analysis began with a Saphiro Wilk normality test and continued with a homogeneity test, the results of which both proved normal ($p>0.05$) and homogeneous ($p>0.05$). The analytical process continued with ANOVA and post hoc LSD tests whose results are presented in Table 1. There was a significant difference ($p<0.05$) in NF-κB expression ($p=0.005$) in the MBSE gel at 37.5% concentration on day 3 compared to day 5.

On day 3, there was a significant difference in NF-κB (p50) expression between the negative control and all treatments (MBSE concentrations of 25%, 37.5% and 50%). A significant difference also existed in NF-κB (p50) expression between MBSE gel of 25% concentration and all other groups, except with MBSE gel of 37.5% concentration. On the same day, there was a significant difference in NF-κB expression between MBSE gel of 50% and all other groups (see Figure 1).

Following the application of MBSE gel on day 5, there was a significant difference in NF-κB (p50) expression between the negative control group and all treatment groups (MBSE concentrations of 25%, 37.5%, and 50%). On day 5, there was both a significant difference in NF-κB (p50) expression between MBSE gel of 25% concentration and that of all other groups and between NF-κB (p50) expression and MBSE gel concentration of 37.5% in all groups. On the same day there was a significant difference

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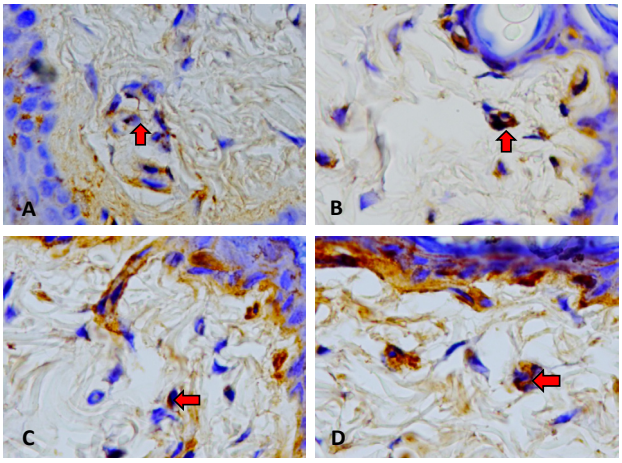

Figure 1. The immunohistochemistry examination results showed NF-κB (p50) expression in oral buccal mucosal tissue (brownish in the nucleus) in each group on day 3.

Notes:
A. NF-κB expression in macrophage cells on administration of negative control
B. NF-κB expression in macrophage cells on administration of MBSE gel concentration of 25%
C. NF-κB expression in macrophage cells on administration of MBSE gel concentration of 37.5%
D. NF-κB expression in macrophage cells on administration of MBSE gel concentration of 50%

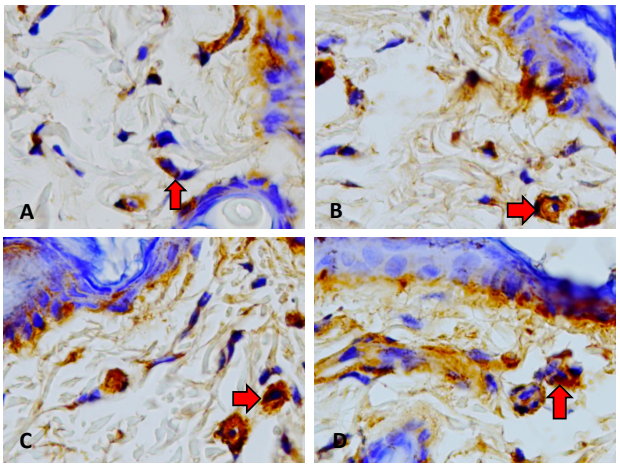

Figure 2. The immunohistochemistry examination results showed NF-κB (p50) expression in oral buccal mucosal tissue (brownish in the nucleus) in each group on day 5.

Notes:
A. NF-κB expression in macrophage cells on administration of negative control
B. NF-κB expression in macrophage cells on administration of MBSE gel concentration of 25%
C. NF-κB expression in macrophage cells on administration of MBSE gel concentration of 37.5%
D. NF-κB expression in macrophage cells on administration of MBSE gel concentration of 50%

Table 1. Means of NF-κB (p50) expression in macrophage cells of traumatic ulcer healing

Group	Day 3	Day 5	p
Negative Control	4.40 ± 1.34^{a}	4.60 ± 1.52^{a}	0.831
MBSE 25%	7.20 ± 1.48^{b}	8.00 ± 0.82^{b}	0.369
MBSE 37.5%	8.60 ± 1.14^{b}	12.00 ± 1.58^{c}	0.005*
MBSE 50%	12.60 ± 1.34^{c}	14.80 ± 1.92^{d}	0.069
p	0.000*	0.000*	0.000*

Notes: MBSE = Mauli banana stem extract
* significant in $\alpha = 0{,}05$.
^{abcd} the same superscripts showed no difference among groups.

between MBSE gel concentration of 50% and that of all groups. This can be seen in Figure 2.

This study showed that MBSE gel at 50% concentration can increase the highest NF-κB (p50) expression in traumatic ulcers on days 3 and 5. However, MBSE gel at 37.5% concentration provoked the highest NF-κB (p50) expression in traumatic ulcers from day 3 compared to day 5, as can be seen in Table 1.

DISCUSSION

The results of this study confirmed an increase in the expression of NF-κB (p50) as the effect of MBSE gel administration at all concentrations (25%, 37.5% and 50%) compared to the negative control group on day 3 and day 5. The MBSE gel containing terpenoid saponin is absorbed by G protein receptors which then penetrate the cell membranes. The concentration level will depend on how many receptors bind to saponins. The number of receptors, the type of receptor bonds with the ligand and the receptor binding strength of the ligand also determine the concentration of drug required and the subsequent effect produced. The application of low concentrations of drugs will have a limited pharmacological effect. An increase in their concentration will strengthen the pharmacological effects until a maximum level is reached beyond which no further enhancement is possible.[9,10]

The increase in NF-κB expression will augment cells' ability to avoid extermination through activation of c-Jun N-terminal kinase (JNK). Normally, signals from the JNK and ERK MAP kinase path will initiate cell growth. JNK activity plays an important role in the migration of fibroblast cells within the wound healing process.[11,12]

NF-κB constitutes a family of transcription factors including regulators in the proinflammatory process and transcription of the antiapoptotic gene. This plays a role in the homoeostasis governing the host immune response. NF-κB is mediated by a transcription as the end of a series of reaction complexes initiated by several stimuli from cellular stresses in receptor involvement which mediate

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innate and adaptive immunity.[13]

NF-κB in *eukaryotic* microorganisms represents a family of transcription factors governing the expression of a large variety of genes involved in several processes such as inflammatory, immune, growth and cellular responses. NF-κB transcription factor is activated in response to various signals, including: cytokines, wounds and other stressful conditions. In a stimulated cell, NF-κB is bound to the IκB inhibition protein. The NF-κB and IκB compounds in the cytoplasm will prevent NF-κB from binding to DNA. The activation of signaling on NF-κB is initiated by extracellular stimuli.[14,15] In this research, MBSE gel application was shown to influence the increase of signaling in NF-κB.

In previous studies, *Astragalus* plants containing terpenoid saponin were shown to increase NF-κB expression along with that of mRNA from IL-1κ and TNF-κ cytokines that produce effects as immunostimulators.[16] Furthermore, this will increase the number and activity of macrophages as in MBSE gel application, a fact showing that MBSE gel is also an immunostimulator.

Macrophages play an important role in wound healing because they produce growth factors and initiate angiogenesis and fibrogenesis. The macrophages released will exterminate bacteria prior to cleaning the tissue debris. In the transition from an inflammatory process to one of wound healing, macrophages stimulate cell migration, proliferation and tissue matrix formation. The growth factors promoting angiogenesis comprise TGFκ, VEGF and FGF-2.[17,18]

In a prevailing condition of macrophage deficiency, inhibition of wound healing then ensues. A balance between the number of neutrophils and macrophages in the wound healing process is required. In contrast to a chronic inflammatory state that inhibits wound healing, an excessive increase in neutrophil leads to a surplus of macrophages. This, in turn, causes severe tissue damage and long-term hypoxic conditions.[19]

Previous research has shown that plants containing antioxidants possess the potential to become immunomodulators which have immunostimulator and immunosuppressant effects on conditions influenced by the amount of extract concentration.[16] MBSE gel has antioxidant and immunostimulator properties at concentrations of 25%, 37.5% and 50%. It can be concluded, that MBSE gel topical application can increase the expression of NF-κB (p50) in traumatic ulcer healing.

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REFERENCES

1. Cavalcante GM, de Paula RJS, de Souza LP, Sousa FB, Mota MRL, Alves APNN. Experimental model of traumatic ulcer in the cheek mucosa of rats. Acta Cirúrgica Bras. 2011; 26(3): 227–34.
2. Regezi JA, Sciubba JJ, Jordan RCK. Oral pathology: clinical pathologic correlations. 7th ed. St. Louis: Saunders; 2016. p. 23–6.
3. Majewska I, Gendaszewska-Darmach E. Proangiogenic activity of plant extracts in accelerating wound healing - a new face of old phytomedicines. Acta Biochim Pol. 2011; 58(4): 449–60.
4. Yadav KCH, Kumar JR, Basha SI, Deshmukh GR, Gujjala R, Santhamma B. Wound healing activity of topical application of aloe vera gel in experimental animal models. Int J Pharma Bio Sci. 2012; 3(2): 63–72.
5. Puspitasari D, Apriasari ML. Analysis of traumatic ulcer healing time under the treatment of the mauli banana (musa acuminata) 25% stem extract gel. Padjadjaran J Dent. 2017; 29: 21–5.
6. Apriasari ML, Dachlan YP, Ernawati DS. Effect of musa acuminata stem by immunohistochemistry test in ulcer. Asian J Biochem. 2016; 11(3): 135–41.
7. Apriasari ML, Iskandar I, Suhartono E. Bioactive compound and antioxidant activity of methanol extract mauli bananas (Musa sp) stem. Int J Biosci Biochem Bioinforma. 2014; 4(2): 110–5.
8. Noora WF, Apriantia N, Saputra SR, Afifah B, Apriasari ML, Suhartono E. Oxidative stress on Buccal Mucosa wound in rats and rule of topical application of ethanolic extracts of mauli banana (Musa acuminata) stem. J Trop Life Sci. 2015; 5(2): 84–7.
9. Prasetyo BF, Wientarsih I, Priosoeryanto BP. Aktivitas sediaan salep ekstrak batang pohon pisang Ambon (Musa Paradisiaca Var Sapientum) dalam proses persembuhan luka pada mencit (Mus Musculus Albinus). Majalah Obat Tradisional. 2010; 15(3): 121–37.
10. Ghag SB, Shekhawat UKS, Ganapathi TR. Silencing of MusaANR1 gene reduces proanthocyanidin content in transgenic banana plants. Plant Cell Tissue Organ Cult. 2015; 121(3): 693–702.
11. Besung INK. Pegagan (Centella asiatica) sebagai alternatif pencegahan penyakit infeksi pada ternak. Buletin Veteriner Udayana. 2009; 1(2): 61–7.
12. Basori A, Zakaria S, Purwaningsih S, Rochmanti M, Setiawati Y, Indiastuti DN, Qotib F, D'Arqom A, Purba AKR. Interaksi antar obat. Surabaya: Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Airlangga; 2014. p. 46–9.
13. Morgan MJ, Liu Z. Crosstalk of reactive oxygen species and NF-κB signaling. Cell Res. 2011; 21(1): 103–15.
14. Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S, Wu P, Wiesmann C, Baker R, Boone DL, Ma A, Koonin E V, Dixit VM. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-κB signalling. Nature. 2004; 430: 694–9.
15. Courtois G, Gilmore TD. Mutations in the NF-κB signaling pathway: implications for human disease. Oncogene. 2006; 25(51): 6831–43.
16. Napetschnig J, Wu H. Molecular basis of NF-κB signaling. Annu Rev Biophys. 2013; 42: 443–68.
17. Mukherjee PK, Nema NK, Bhadra S, Mukherjee D, Braga FC, Matsabisa MG. Immunomodulatory leads from medicinal plants. Indian J Tradit Knowl. 2014; 13(2): 235–56.
18. Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res. 2010; 89(3): 219–29.
19. Soni H, Singhai AK. A recent update of botanicals for wound healing activity. Int Res J Pharm. 2012; 3(7): 1–7.

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