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Original Research Article

Expression of fibroblast growth factor-β and transforming growth factor-β in mauli banana stem (*Musa Acuminate*) extract gel - treated traumatic ulcer

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Abstract

Purpose: *To investigate the effect of mauli banana (Musa acuminate) stem topical gel extract application on the expression of transforming growth factor - β (TGF - β) and fibroblast growth factor-β (FGF-β) during the healing process in traumatic oral ulcers.*
Methods: *The work represented a true experimental study incorporating a post test - only control group design. Four groups of male Wistar rats (Rattus norvegivus) (n = 20) with traumatic oral ulcers were given mauli banana stem extract gel of varying concentrations the negative control group: 0 %; treatment group I: 25 %; treatment group II: 37.5 %; and treatment group III: 50 %. The animals were subsequently sacrificed prior to conducting biopsy on day 5. Immunohistochemical staining was performed in order to analyze the degree of FGF-β and TGF–β expressions.*
Results: *TGF–β was strongly expressed in treatment group II (16.80 ± 1.30). TGF-β expression was significantly different, except between treatment groups II and III (Table 2). FGF-β was strongly expressed in treatment group II (15.60 ± 3.97). There was significant difference in FGF-β expression between all the groups with the exception of treatment groups I and III.*
Conclusion: *Topical application of mauli banana stem extract gel (37.5 % concentration) stimulates FGF-β and TGF-β expression on day 5 of traumatic oral ulcer healing process. Thus, the extract gel has potentials for clinical application for the therapy of traumatic oral ulcers.*

Keywords: *Fibroblast growth factor-β, Mauli banana stem extract gel, Musa acuminate, Transforming growth factor-β, Traumatic oral ulcer*

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INTRODUCTION

Wound healing consists of several phases, namely: homeostasis, inflammation, proliferation and maturation. Macrophages can stimulate the process of angiogenesis and fibroblast formation by means of fibroblast growth factor-β (FGF-β) and transforming growth factor-β (TGF-β) [1-3].

Aloe vera extract gel (a patented drug marketed as Alloclair®) was used to accelerate the traumatic oral ulcer healing process. However,

this drug has the two disadvantages of being difficult to obtain and relatively expensive. In Kalimantan Selatan, Hulu Sungai Utara Regency, communal use is often made of pulverized mauli banana stem (*Musa acuminate*) extract which is applied to skin wounds. Topical application of mauli banana stem extract may potentially accelerate the wound healing process in traumatic oral ulcers since it can increase the number of macrophages, while also producing an immunomodulatory effect [4,6,7]. To date no study has been conducted of the topical application of mauli banana stem extract gel (*Musa acuminate*) as a means of treating traumatic oral ulcers by stimulating the expression of FG β and TGF-β. The purpose of this study was to analyze the effect of mauli banana stem (*Musa acuminate*) extract gel on the expression of FGF-β and TGF-β during traumatic oral ulcer wound healing.

EXPERIMENTAL

This study received ethical clearance, based on international guidelines for animal-based studies, from the Ethics Research Committee of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia (no. 56/KKEPK.FKG/VI/2015) and carried out in accordance with International Guidelines on Animal Model Study for Scientific Laboratory use [8]. The animal study based on previous research reported here [5] represented a true experimental study incorporating post-test only control group design.

The materials used during this experiment comprised 100 grams of mauli banana stems, six liters of 70 % ethanol, carbopol, Hydroxypropyl Cellulose Medium (HPMC), propylenglycol, and aquadest, aluminum foil, hydroxypropyl methylcellulose, banana stems, candy oil, propylene glycol and tween. Mauli banana extract gel was added to 15 % HPMC, 1 % Tween, 80.8 % Propylenglicol, five drops of candy oil and aquades to make up the total weight. The other materials included: chemical substances for immunohistochemistry (xylol, ethanol, PBS, trypsin, alcohol, distilled water, streptavidin biotin, 0.5 % H2O2, substrate and phosphatase buffer), anti - mouse FGF-β monoclonal antibodies (Santa Cruz Biotechnology, Inc) and anti – TGF-β (Santa Cruz Biotechnology, Inc).

Prior to extraction, the mauli banana stems were washed and rinsed in water before being minced and subsequently dried in an oven at 40 - 60 ^{o}C for three days. On completion of the drying process, the stems were weighed and then smoothed in a mixer. The extraction process was completed by means of maceration involving immersion of the mauli banana stems in 750 ml of 70 % ethanol for 72 h. At that point, the stem extract was filtered and evaporated twice; first, in a vacuum rotary evaporator at 40 – 50 ºC and then in a waterbath to produce a viscous extract. Applying the research methods of Apriasari *et al* [5], the ethanol - free gel extract produced was then divided into samples with respective concentrations of 2 %, 5 % , 37.5 % and 50 %.

The research methods applied incorporated the previously described stages [5]. The study used male Wistar rats (*Rattus norvegicus)* weighing 250 - 300 grams as the models suffering from traumatic oral ulcers. Twenty samples were divided into four groups: a control group given EBPM gel (0 % concentration) three times a day every 6 - 8 h, treatment group 1 given 25 % EBPM gel three times a day every 6 - 8 h, treatment group 2 given 37.5 % EBPM gel three times a day every 6 - 8 h and treatment group 3 given 50 % EBPM gel three times a day every 6 - 8 h. The treatment was initiated with the inhalation of ether anesthesia before the left buccal mucosa was punctured to a depth of 1 mm with a biopsy punch 6 mm in diameter. A scalpel was then used to remove tissue. A traumatic oral ulcer was induced in the left buccal mucosa of sufficient depth to reach epithelial tissue, but not muscle. Each group was observed on a daily basis and sacrificed on Day 5. Traumatic ulcer tissue from the left buccal mucosa of male Wistar rats (*Rattus norvegicus)* was removed by biopsy for immunohistochemical (IHC) staining examination in order to assess the expression of FGF-β and TGF-β on Day 5.

Statistical analysis

All data was analyzed using the Statistical Package for the Social Sciences (SPSS) 21.0 version (IBM Corporation, Illinois, Chicago, United State) A One-way ANOVA parametric test *(*$p < 0.05$) was performed based on a normality test ($p > 0.\ 05$) prior to a test for data homogeneity ($p > 0.05$). The results confirmed normal data distribution and homogenous data variances which were subjected to a post - hoc Least Significant Difference (LSD) test ($p < 0.05$).

RESULTS

Mauli banana stem topical gel extract application on Day 5 indicated that treatment group II presented the highest TGF - β and FGF - β expression (Table 1). TGF - β expression, except that between treatment II and treatment III (Table 2), had a significant difference. There was a

significant difference in FGF - β expression between groups except between treatment groups I and III (Table 3). Treatment group II showed strong and increased expression of TGF - β and FGF - β expression analyzed by means of IHC (Figures 1 and 2)**.**

Table 1: Means of expression of TGF-β and FGF-β after application of mauli banana stem topical gel extract application

Group	Mean ± SD	
	TGF-β	***FGF-β***
Control	7.40 ± 1.14	6.20 ± 1.79
Treatment I	10.25 ± 3.09	11.50 ± 2.08
Treatment II	16.80 ± 1.30	15.60 ± 3.97
Treatment III	16.20 ± 1.92	12.20 ± 1.92

Table 2: ANOVA and post-hoc LSD test data for TGF-β expression

Group	Control	Treatment I	Treatment II	Treatment III
Control	-	0.050 *	0.000 *	0.000 *
Treatment I	-	-	0.000 *	0.000 *
Treatment II	-	-	-	0.652
Treatment III	-	-	-	-

*information: Significant $p < 0.05$

Table 3: ANOVA and post-hoc LSD test data for FGF-β expression

Group	Control	Treatment I	Treatment II	Treatment III
Control	-	0.001 *	0.000 *	0.000 *
Treatment I	-	-	0.010 *	0.641
Treatment II	-	-	-	0.021 *
Treatment III	-	-	-	-

*Significant at $p < 0.05$.

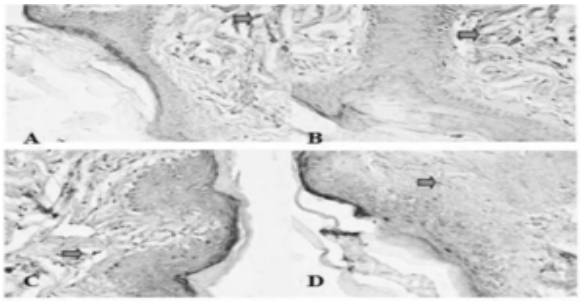

Figure 1: IHC micrograph of TGF-β expression in fibroblast cells; positive expression in the cytoplasm (arrow) on Day 5; A: Control Group; B: Treatment Group I, C: Treatment Group II, D: Treatment Group III. The results confirm that Treatment Group II had the highest expression of TGF-β

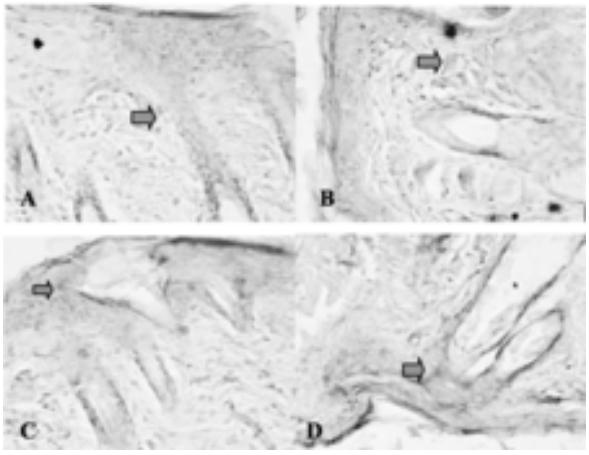

Figure 2: IHC micrograph of FGF - β expression in the fibroblast cells, positive expression appears brown in the cytoplasm (arrow) on Day 5; A: Control Group; B: Treatment Group I, C: Treatment Group II, D: Treatment Group III.

DISCUSSION

The topical application of mauli banana stem gel extract (37.5 % concentration) on Day 5 (Treatment III) produced the highest TGF-β and FGF-β expression. Mauli banana stem gel extract contains bioactive components of 67.59 % condensed tannin and 14.49 % saponin terpenoid. A previous study showed that mauli banana stem extract increases the number of macrophages during wound regeneration [4,5]. Macrophages can stimulate cell migration, proliferation and tissue matrix formation. The growth factors involved are TGF-β, VEGF and FGF-β for angiogenesis [2,9].

Condensed tannin is a polymer flavonoid compound containing carbon bindings which can increase insulin receptor signaling. Previous studies have showed that insulin receptors can stimulate autophosphorylation in the Tyrosin Kinase Domain (TKD). Tyrosine kinase receptor constitutes the key regulator of the cellular process in cell proliferation, differentiation, survival, metabolism, migration and cycle control [10,11]. Previous studies have confirmed that herbal plants which contain active components such as saponin and terpenoid can stimulate FGF-β and TGF-β [12].

The proliferative phase progression will involve TGF - β which plays a role in controlling fibroblast proliferation. TGF-β can also increase the production of matrix protein by increasing the gene transcription of collagen, proteoglycans and fibronectin [13]. TGF-β is produced in an inactive form and prepared for activation when bonded with their receptors. These TGF-β play an important role during wound regeneration

consisting of the inflammation process and angiogenesis stimulation. The three receptors will bind and produce the signaling pathway through two TGF receptors; TβRI and TβRII. TGF-β expression will be stable during the normal wound healing process and decrease during the remodeling phase [14].

TGF-β is a polypeptide found in large numbers which affects tissue differentiation, development, immunologic response control and the wound healing process. TGF-β signaling is established through autocrine, paracrine and endocrine pathways. The increased expression of TGF-β polypeptide induces somatic cell differentiation and proliferation. This, in turn, stimulates an inflammatory response thus increasing ECM production during the wound healing process [15].

Macrophages normally release FGF - β during the acute stage that can induce granulation tissue formation and re-epithelization during wound regeneration. Granulation tissue formation involves various extracellular matrix components which are synthesized and deposited in the damaged area by macrophages through FGF-β signaling regulation. Macrophages also simultaneously induce re-epithelization and accelerate wound regeneration by increasing the motility of keratinocytes, promoting the migration of fibroblast and stimulating its production of collagenase [16].

Fibroblasts are common cells found in connective tissue which, when disruption to tissue integrity occurs, can increase their numbers through proliferation to promote wound healing. The proliferation itself is stimulated by FGF-β which possesses mitogen properties and exerts its mitogen effect via signaling pathways through the mediation of phosphoinositide-3 kinase/protein kinase B (PI3K/Akt) and mitogen activated protein kinase/extracellular signal-regulated kinase (MEK/ERK) [17]. FGF-β ligands demonstrate their interaction with FGF Receptors (FGFRs) by binding and activating the receptors through a dependent relationship in heparan sulfate proteoglycan (HSPG). After the occurrence of receptor - ligand interaction, receptor dimerization and transphosphorylation will be induced. These processes will stimulate the activation of Ras / MAPK and PI3K / Akt pathways as downstream signaling in tissue repair [18].

There are several conditions associated with the proliferation phase of the wound healing process. Fibroblast activity, new blood vessel formation, extracellular matrix components synthesis and proliferation are common activities which can be observed in the proliferation phase. Meanwhile, the closure of the surface wound can be achieved after endothelial cell migration to and proliferation in the granulated tissue [19].

FGF-β and TGF-β are both very important growth factors reputed to interact with extracellular matrix components in order to prevent the degradation of growth factors and promote the concentration of certain growth factors important in the signaling of cell migration. Understanding growth factor-ECM component interaction leads to an appreciation of ECM as a means of growth factor storage. Growth factors can be stored by binding ECM components and released into tissues by diffusion until they connect with their cognate growth factor receptor. Growth factors are known as soluble mediators which can be conducted to their receptors by ECM in order to promote cell activation and signaling. Growth factors can be affected through cellular responses which are regulated after ECM binding which is specifically required for the activation of FGF-β signaling. It involves FGF-β and FGFRs which interact directly with extracellular HSPGs on the surface of the cells. A single HSPGs molecule is capable of binding multiple numbers of FGF-β proteins and FGFR molecules. It acts as a receptor for various macromolecules by facilitating the binding of FGF-β to FGFR and assisting the dimerization of two FGFT molecules [20,21]. Despite the important role of ECM binding in FGF-β signaling activation, under certain circumstances it can also inhibit the activity of growth factors. The binding of TGF-β to ECM components such as proteoglycans decorin, betaglycan, and biglycan is reported to restrict its activity [21].

Several bioactive elements in traditional plants play specific roles including immunomodulator, immunoregulator, anti-inflammatory agent and antioxidant. The active biocomponent can also be found in mauli banana stem gel extract that is capable of accelerating the wound healing process as an immunomodulatory agent through fibroblast proliferation and ECM synthesis. This process is also promoted by the increased expression of FGF-β and TGF-β on day 5.

CONCLUSION

Topical application of mauli banana stem extract (37.5 % concentration) stimulates FGF-β and TGF-β expression in traumatic oral ulcer healing on day 5. This constitutes the critical concentration in terms of increased FGF-β and TGF-β expression for healing traumatic oral

ulcers. Thus, the extract gel has potentials for clinical application for the therapy of traumatic oral ulcers.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was undertaken by the author(s) named in this article and all liabilities pertaining to claims relating to its contents will be borne by said individuals. All the authors made substantial contributions to this study and/or manuscript, approving the final draft of the paper prior to its submission.

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