Application of Mangosteen Peel Extract (Garcinia Mangostana Linn.) to TGF-1, PDGF-B, FGF-2 and VEGF-A Expression on Human Gingival Fibroblast Cell Culture (In Vitro Study)

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Abstract

Wound healing is a complex and dynamic process. Growth factors such as TGF-1, PDGF-B, FGF-2, and VEGF-A, act as chemotactic macrophages in the wound healing process. These proteins increase the proliferation of fibroblasts, which plays an important role in the wound healing process. Mangosteen peel pericarp (MPP) contains tannin, saponin, flavonoid, and xanthone, which function as antioxidants, anti-inflammatories, and antimicrobials increasing the proliferation of fibroblasts and accelerating wound healing.

Analyze the effect of MPP on TGF-β1, PDGF-B, FGF-2, and VEGF-A's expression on human gingival fibroblasts.

This research used a 24 and 48-hour control and a treatment group where MPP was given for periods of 24 and 48 hours. The data was processed by PCR assay with gel electrophoresis. During the 24 and 48-hour treatment period, TGF-β1 and VEGF-A showed a brighter band than the control. After 24 hours of treatment, FGF-2 showed a brighter band than the 24-hour control. There was no increased expression of FGF-2 in the 48-hour treatment period compared to the 48-hour control. PDGF-B in both the 24 and 48-hour control showed a brighter band than the 24 and 48-hour treatment.

MPP increases the expression of TGF-β1, FGF-2, and VEGF-A and decreases the expression of PDGF-B on human gingival fibroblasts.

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Introduction

In the field of dentistry, tooth extraction is a common treatment. This action can cause a wound. Wounds heal easily, but can also cause various kinds of complications that slow the healing process.[1]

Some researchers state that the use of topical drugs after a tooth extraction can reduce the possibility of complications and accelerate the wound healing process.[2] Pharmaceutical technology throughout the world has now focused on ingredients derived from nature because they are safer to use than drugs containing chemical materials.[3] An ingredient that is effective for treatment is mangosteen peel. The peel of the mangosteen can increase the ability of proliferation and differentiation into osteoblasts from MSC.[4] The components are useful in the medical field, namely, xanthones, tannins, flavonoids, and saponins which have pharmacological activities such as anti-inflammatory, anti-histamine, anti-bacterial, anti-fungal can be used even for HIV therapy[5] and potentially effective as a wound healing agent which stimulates proliferation of fibroblast cells.[6]

A sign of good wound healing is an increase in the number of fibroblasts. Fibroblasts begin to appear when there is a decrease in the number of neutrophils and an increase in the number of macrophages. Fibroblasts move from mucosal tissue to the wound area, which begins around 72 hours after injury and multiplies itself

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at the initial stage of the wound healing process.[7,8] The increased number of macrophages stimulates the secretion of more growth factors, so that fibroblast proliferation also increases.[9]

When tissue is inflamed, fibroblasts migrate towards the wound, proliferating and producing collagen matrices stimulated by the platelet-derived growth factor (PDGF) and the fibroblast growth factor (FGF). In addition, the migration of fibroblasts to the wound area is stimulated by the transforming growth factor-1 (TGF-1).[9] Meanwhile, the vascular endothelial growth factor (VEGF) is a potent stimulator in the process of angiogenesis and plays an important role in influencing angiogenesis.[10] By using human gingival fibroblast cells, we can represent the wound healing process in the oral cavity.

Based on this background, this study aimed to examine the expression of TGF-1, PDGF-B, FGF-2, and VEGF-A after being given mangosteen peel extract in human gingival fibroblast cell cultures.

Materials and methods

This research was experimental laboratories with post test control only group design. This study received ethical clearance from the Commission for Health Research Ethics, Faculty of Dental Medicine, Universitas Airlangga (153/KKEPK.FKG/VII/2016).

Plants extract preparation

Garcinia Mangostana Linn (G. Mangostana L.) was collected from Blitar, East Java, Indonesia plantation and identified by staff of the phytochemistry laboratory in the Faculty of Pharmacy at Universitas Airlangga, Surabaya, East Java, Indonesia. The mangosteen peels were collected, chopped, and kept in a dryer service tunnel. Extraction was performed based on the maceration method using distilled ethanol 70% as the solvent for collecting G. Mangostana L. peel extract (MPE).[11]

Samples and cell culture

Human gingival tissue was extracted from ten permanent third molars of healthy subjects (age range 18–32 years old; five women and five men) at the Dental Hospital of Universitas Airlangga, from January to April 2017 after written consent was obtained from the subjects. Human gingival fibroblasts (HGFs) were isolated and grown at 37ºC in a humidified atmosphere of 5% CO_2.[12] The cells were cultured in 75-cm^2 cell culture flasks containing Dulbecco's modified Eagle's medium (DMEM, Gibco-BRL) supplemented with 10% fetal bovine serum (FBS, Gibsco-BRL, Grand Island, NY), and antibiotics (100 µg/mL streptomycin and 100U/mL penicillin G, Sigma Chemical Co., St.Louis, MO, USA).

The cells were subcultured using 0.25% trypsin and 0.05% EDTA (Invitrogen, Carlsbad, CA) after reaching a 90% confluency. Cells between the third and fifth passages were used for experiments.

MTT Assay

Cell proliferation was determined with a commercial Cell Counting Kit-8 (CCK-8) kit following the manufacturer's instruction. The samples were divided into six groups, Group 1. 200 µg/ml, Group 2. 400 µg/ml, Group 3. 600 µg/ml, Group 4. 800 µg/ml, Group 5. 1000 µg/ml, and Group 6. 1200 µg/ml. Each well contained 5,000 cells and 100 mL DMEM + 10% FBS + 1% penicillin-streptomycin and was incubated in an incubator at a temperature of 37ºC for 24 hours. Cell calculation used the Countess Automated Cell Counter from Invitrogen. After incubation, 10 mL of the reagent kit, Cell Counting 8 (Dojindo Molecular Technologies, Japan) was added to each well and incubated for 2 hours. Absorbance at 450 nm was measured with a microplate reader, and the relative cell viability calculated.

RT-PCR

Total RNA was isolated using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. cDNA was synthesized using ReverTra Ace (TOYOBO, Osaka, Japan). Semiquantitative RT-PCR was performed with 30 cycles of denaturation at 95°C for 30 seconds, annealing for 30 seconds, and an extension at 72°C for 1 min. PCR primers were as follows:

- PDGF-B*forward5'-GATCCGCTCCTTTGATGATC-3'* (385bp)
- PDGF-B*reverse5'-GTCTCACACTTGCATGCCAG-3'* (385bp)
- TGF-β1*forward5'-CGAAATCTATGACAAGTTCAAGCA-3'* (192bp)
- TGF-β1*reverse5'-GAGGTATCGCCAGGAATTGTT-3'* (192bp)
- FGF-2*forward* 5'-CTTCTTCCTGCGCATGCACC-3' (262bp)
- FGF-2*reverse* 5'-CACATACCAACTGGTGTATTT-3' (262bp)

- VEGF*forward* 5'-CTGCTGTCTTGGGTGCATTG-3' (499bp)
- VEGF*reverse* :5'-CTCGGCTTGTCACACATACGC-3' (499bp)
- GAPDH *forward 5'ACCACAGTCCATGCCATCAC-3'* (353bp)
- GAPDH reverse *5'CAGCCCCAGCGTCAAAGGTG-3'* (353bp).

PCR products were separated by electrophoresis in 2% agarose gels (Sigma, Missouri, USA) and visualized by staining with ethidium bromide (Sigma, Missouri, USA). The visualization results were seen with the doc gel machine (Bio-Rad, Mississauga, Canada) and analyzed by comparing the band thickness between the control group and the treatment group using ImageJ (NIOH and LOCI, University of Wisconsin, USA) software.

Results

This study used human gingival fibroblasts (HGF). The sample in the treatment group was given mangosteen peel extract with an optimal concentration according to the MTT results equal to 800 µg/ml with the highest number of living cells at 162.6%.

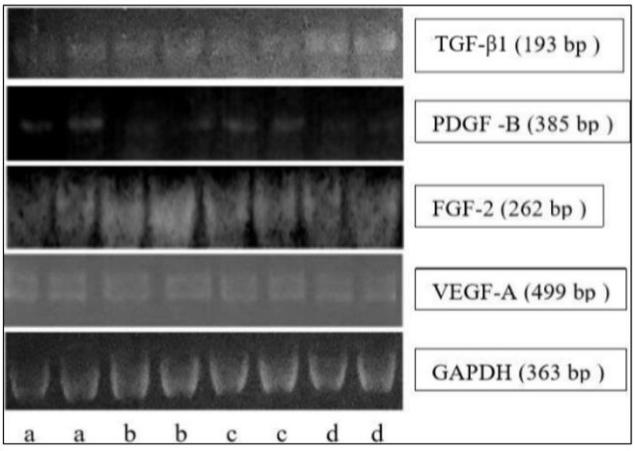

Figure 1. Results of reading TGF-β1, PDGF-B, FGF-2, VEGF-A and GAPDH on electrophoresis gel using gel doc. (a) 24-hour control, (b) 24-hour treatment, (c) 48-hour control, (d) 48-hour treatment.

PCR samples were processed by electrophoresis for groups over 24 hours and 48 hours and then read with gel doc (Figures 1). The results of the luminescent band on the reading were calibrated, and a score produced, so that it can be compared using tables and diagrams. Based on the results of the luminescence (Figure 1), the band showed TGF-β1 expression at 193 bp, PDGF-B expression at 385 bp, FGF-2 expression at 262 bp and VEGF-A expression at 499 bp. Band fluorescence was calibrated using the J image application to produce scoring from each band in both the control and treatment groups. The scores of each calibration result were made in the form of bar graphs.

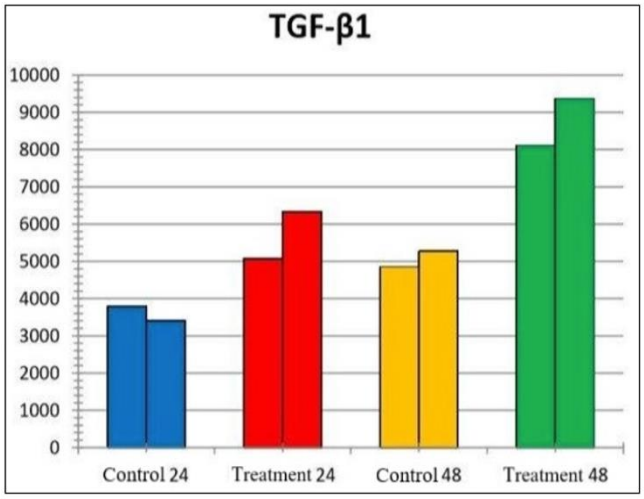

Figure 2. Comparison diagram of TGF-β1 between the 24 and 48-hour control and treatment groups.

The TGF-β1 graph shows an increase in both the 24-hour and 48-hour treatment groups compared with the 24-hour and 48-hour control groups (Figure 2). The PDGF-B graph shows the 24-hour control group expresses higher PDGF-B than the 24-hour treatment group (Figure 3). The 48-hour control group also expressed higher PDGF-B than the 48-hour treatment group. The FGF-2 chart showed an increase in the 24-hour treatment group and a decrease in the 48-hour treatment group compared with the control group (Figure 4). VEGF-A graphics showed an increase in the 24-hour treatment group and the 48-hour treatment group compared to the control group (Figure 5). GAPDH expression was used as a comparison showing that the number of cells used was the same in each group.

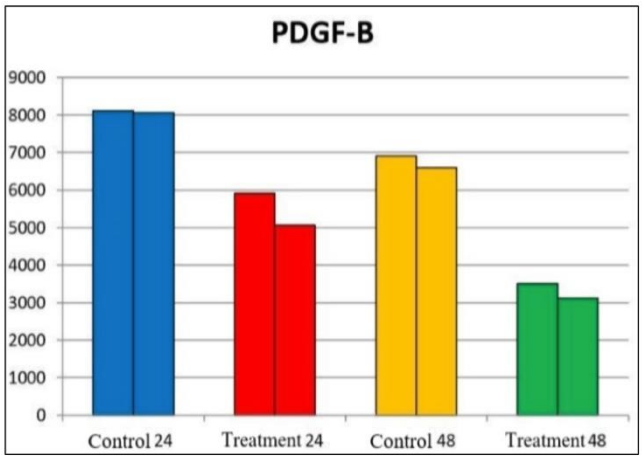

Figure 3. Comparison diagram of PDGF-B between the 24 and 48-hour control and treatment groups.

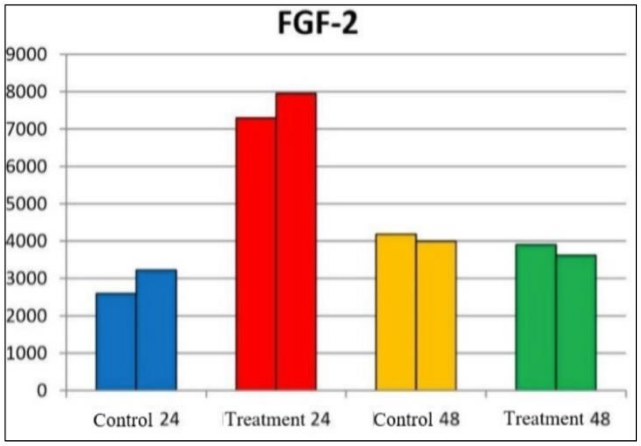

Figure 4. Comparison diagram of FGF-2 between the 24 and 48-hour control and treatment groups.

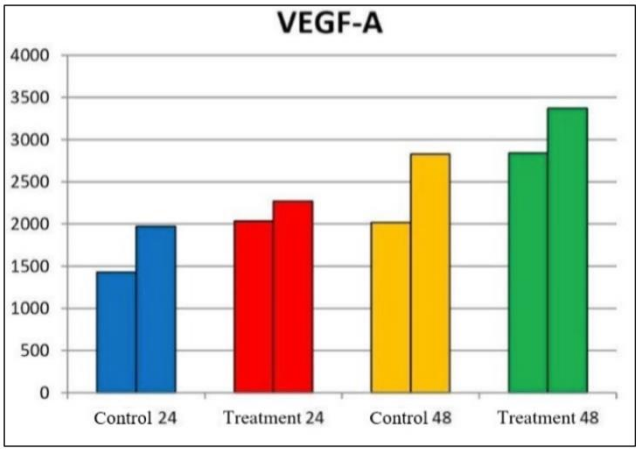

Figure 5. Comparison diagram of VEGF-A between the 24 and 48-hour control and treatment groups.

Discussion

Mangosteen peel extract has xanthones and flavonoids, which have an antioxidizing effect. Antioxidants can neutralize free radicals by releasing electrons to free radicals that play a role in reducing Reactive Oxygen Species (ROS) so that the stimulation of macrophages is not inhibited.[13] Macrophages will secrete growth factors such as FGF, PDGF, TGFβ, and VEGF, which can attract more fibroblasts to the wound area and synthesize collagen and increase capillary blood vessel proliferation.[14,15] This can explain the increase in TGF-β1 in the treatment group. The difference in the TGF-β1 luminescence band in the 24-hour treatment group was not significant when compared with the 48-hour group because the inflammatory phase starts on the second day. In the inflammatory phase, cells release growth factors such as PDGF and TGF ß, granulocyte colony-stimulating factor (G-CSF), C5a, TNFα, IL-1 and IL-8.[16]

Xanthones can be associated with increased expression of the VEGF-A treatment group because xanthones can bind to an element of radical superoxide (O2-) and release an element-free Nitric Oxide (NO).[17] In small amounts, NO can increase VEGF expression through the PI3K (Phosphoinositide 3-kinases) - Akt pathway, which transduces intracellular signals so that the HIF-1α gene will be activated. Hypoxia-inducible factor (HIF-1) is a key transcription regulator for several angiogenic factors. The signal will affect the nucleus in which there is a VEGF gene. The nucleus expresses VEGF in the cell and is released in the form of a VEGF protein, which later affects the increase in angiogenesis and affects the acceleration of wound healing. The increase in VEGF expression in the PCR results indicated the possibility that the amount of NO in this study was small, and that it was associated with optimal mangosteen peel extract levels of 800 µg/ml. Conversely, if the mangosteen peel extract level is more than 800 µg/ml and the amount of NO is large, the VEGF expression can decrease.[18]

This study observed normal cell conditions that did not experience inflammation and were expected to show increased VEGF expression after administering the mangosteen peel extract. VEGF is one of the factors in wound healing and

is indeed more potent in mitogen activity, which is limited to vascular endothelial cells. The increase in VEGF expression in the 24-hour and the 48-hour treatment groups has shown that the application of mangosteen peel extract results in better fibroblast proliferation activity.

The decrease in PDGF-B in the treatment group can be explained by the content of mangosteen peel extract, namely flavonoids, that are known to inhibit the PDGFR-β receptor, which is a receptor from PDGF-B.[19] Flavonoids break the bonds of PDGFR-β in molecular level signal transduction, namely Ras GAP, SHP-2 (Phosphotyrosine Phosphatase), PI3K, PLCγ (Phospholipase Cγ1). Flavonoids also inhibit Erk activation and initial gene induction and proliferation and migration. A decrease in the 48-hour PDGF-B control group caused PDGF to appear at the beginning of the wound-healing phase, namely the hemostasis phase. Then it declined and reappeared 72 hours later in the chronic inflammatory phase.

In FGF-2, flavonoids can increase the release of Nitric Oxide (NO) from Vascular Smooth Muscle Cells (VSMC), resulting in an increase in FGF-2. It was found that high levels of NO released by VSMC resulted in the release of FGF-2, which stimulated endothelial cell proliferation. NO released from VSMC is an important signal for cellular interactions between endothelial cells and VSMC. This cellular interaction signal involves the transcription of early growth response-1 (EGR-1) through the mitogen-activated protein kinase (MAPK) mechanism. Activation of the MAPK pathway activates Endothelin-3 (ET-3), which is then transcribed by EGR-1, this transcription affects the nucleus in which there is FGF-2. The nucleus then expresses FGF-2 in the cell which later affects the increase in FGF-2 expression.[20,21]

Another content of mangosteen peel extract is saponin, which can increase monocyte proliferation so that it can increase the number of macrophages that stimulate growth factor secretions. Saponin will activate the function of TGF-β1. TGF-β1 has several functions such as extracellular matrix synthesis, cell migration, cell differentiation, and as immunosuppressors.[14]

There are several mechanisms of TGFβ activation by saponins; first, saponins stimulate synthesis, secretion and activation of TGF-β1 in fibroblast cells, secondly, saponins alter the expression of TGFβ receptors, and thirdly saponins modify the suction transduction system at TGFβ post-receptors. Changing the expression of the TGFβ receptor is important because of the acceleration of fibronectin synthesis from fibroblasts caused by saponins.[15]

Conclusions

From this study, we can conclude that mangosteen peel extract can increase TGF-β1 expression, decrease PDGF-B expression, increase FGF-2 expression, and increase VEGF-A expression in human gingival fibroblast cell culture.

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Declaration of Interest

The authors report no conflict of interest.

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