Dental Journal
Majalah Kedokteran Gigi

Dental Journal
(Majalah Kedokteran Gigi)
2017 September; 50(3): 121–126

Research Report

FGF-2 expression and the amount of fibroblast in the incised wounds of Rattus norvegicus rats induced with Mauli banana (*Musa acuminata*) stem extract

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ABSTRACT

Background: *Traditional wound treatment using herbal medicine is thought to maintain the health of families and society in general economically, effectively, and efficiently without inducing side effects. One genus of plant that can be used as a traditional medicine is the Mauli banana, indigenous to South Borneo. Mauli banana stem contains bioactive compounds, most of which are tannins along with ascorbic acid, saponin, β-carotene, flavonoids, lycopene, alkaloids, and flavonoids. Tanin has antibacterial and antioxidant effects at low concentrations, as wells as antifungal ones at high concentrations.* ***Purpose:*** *This study aimed to analyze the effects of Mauli banana stem extract at concentrations of 25%, 37.5%, and 50% on the quality of incised wound healing in male Rattus norvegicus rats by assessing FGF-2 expression and fibroblast concentration on days 3 and 7.* ***Methods:*** *This research represented an experimental laboratory-based investigation involving 32 rats of the Rattus norvegicus strain aged 2-2.5 months old. Sampling was performed using a simple random sampling technique since the research population was considered homogeneous and divided into 8 treatment groups (C3, M3-25, M3-37.5, M3-50, C7, M7-25, M7-37.5, M7-50). The rats in each group were anesthetized before their back was incised with length and width of 15x15mm with a depth of 2mm. Gel hydroxy propyl cellulose medium (HPMC) was applied to the incised wound of each rat in the control group, while stem Mauli banana extract was applied to that of each rat in the treatment groups three times a day at an interval of 6-8 hours. On day 3, four rats from each group were sacrificed, while, in the remaining groups, the same procedure was performed until day 7, at which point they (8 groups) were sacrificed for HE examination in order to assess the amount of fibroblast and for IHC examination to examine FGF-2 expression. Data regarding FGF-2 expression and the amount of fibroblast were analysed by means of One-way Anova and HSD.* ***Results:*** *The results showed that the Mauli banana stem extract could significantly improve the expression of FGF-2 and the amount of fibroblast cells compared to C3 and C7 groups. The highest increase in FGF-2 expression and fibroblast amount were found in all groups on days 3 and 7 treated with the Mauli banana stem extract at a concentration of 50%.* ***Conclusion:*** *There was an increase of FGF-2 expression and the amount of fibroblast cells in the incision wound healing process that induced with Mauli banana stem extract.*

Keywords: *FGF-2; fibroblast cells; wound healing; Mauli banana stem*

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INTRODUCTION

The body responds to the presence of a foreign object. When the cells and tissues of the body experience damage due to an invasive agent, it will respond by repairing the tissue. The healing process represents tissue response to the wound which serves to restore the original structure. [1]

Wound healing constitutes a complex process of continuous interaction among cells as well as between cells and matrix summed up in three overlapping phases.

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 32a/E/KPT/2017.

DOI: 10.20473/j.djmkg.v50.i3.p121-126

The three phases of the wound healing process are the inflammatory (0-3 days), proliferative and kult tissue formation (3-14 days), and remodeling phases.[2]

Moreover, optimal wound healing will be achieved if complications in the form of deficiency or redundancy of wound healing components, especially collagen and epithelial cells, do not occur. The deficiency or redundancy of these components can be detected more clearly in the remodeling phase. Another form of wound healing complication is that of excessive wound contraction.[3–5]

Indonesia is rich in natural medicinal herbs that have traditionally been used to treat wounds. The long-standing treatment of wounds with medicinal plants is expected to be utilized by society to maintain the health of families and communities cheaply, effectively, and efficiently with minimal side effects. Nevertheless, advances in knowledge and modern technology are expected to go hand-in-hand with the role of traditional medicine. As a result, in order to achieve those objectives, the government has recently promoted a return to natural treatment.[6]

One type of plant that can be used as in traditional medicine is the Mauli banana, a typical banana from South Kalimantan.[7] Evidence strongly suggests that Mauli banana stems have historically been used as a form of wound healing medicine in the Hulu Sungai Utara area of South Kalimantan province.[8] The Mauli banana stem contains numerous bioactive compounds, the most common of which is tannin. Tanin not only demonstrates antibacterial and antioxidant qualities at low concentrations, but also antifungal ones at high concentrations. Other bioactive compounds contained include ascorbic acid, saponins, β-carotene, flavonoids, lycopene, alkaloids, and flavonoids. Saponin serves as an antibacterial agent and painkiller, as well as stimulating the formation of new skin cells. Meanwhile, flavonoids have antifungal, antioxidant, anti-allergic, anti-inflammatory, anti-carcinogenic antithrombic, and hepatoprotective functions, while also stimulating fibroblast formation.[9,10]

MATERIALS AND METHOD

This research constitutes an experimental laboratory study (truly experimental in character) using a post-test only control group design and was approved by the Commission of Health Research Ethics (KKEPK) of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya number: 203/KKEPK.FKG/XI/2015. The 32 samples were male *Rattus norvegicus* rats aged 2-2.5 months old and weighing 250-300 grams, which had never previously been used for research purposes, and were in a healthy condition without any anatomical abnormalities.

The 25%, 37.5%, and 50% Mauli banana stem extract was made into a gel using carbopol material, hydroxy propyl cellulose medium (HPMC) and propylenglycol. The carbopol material was mixed with water and then adjusted before propilenglikol was added. The HPMC was put into the first mixture. Mauli banana stem extract was gradually added to the mixture until it reached a gel type-consistency.

Sampling was performed by means of a simple random sampling technique since the research population was considered to be homogeneous. After one week, certain rats were selected randomly and anesthetized, before their backs were incised using cardboard boxes to a length and width of 15 x 15 mm and a depth of 2 mm.[5] Thereafter, HPMC gel was applied to the incised wound of each rat in the control group on day 3 (C3) and the control group on day 7 (C7), while Mauli banana stem extract gel was applied to the incised wound area of each rat in the treatment groups. The concentrations applied comprised: 25% Mauli banana extract on day 3 (M3-25), 37.5% extract on day 3 (M3-37.5), 50% extract on day 3 (M3-50), 25% extract on day 7 (M7-25), 37.5% extract on day 7 (M7-37.5), 50% extract on day 7 (M7-50) three times a day at intervals of 6-8 hours. These rats were then placed into two cages which had respectively been labeled control group C3, C7 and treatment groups M3-25, M3-37.5, M3-50, M7-25, M7-37.5, M7-50. During the research, all the rats received equal treatment, being exposed to sunlight for 12 hours during the day, but not at night. They were also provided with sufficient food in the form of pellets. On day 3, four rats from each group C3, M3-25, M3-37.5, M3-50 were sacrificed. Meanwhile, in the other treatment groups, C7, M7-25, M7-37.5, M7-50 the same process was performed up to day 7, when these rats were sacrificed. The tissue around the incision area was subsequently cut off, before each specimen was fixed with a formalin buffer. Immuno histo chemistry (IHC) examination was then performed to assess the degree of FGF-2 expression and hematoksilin eosin (HE) examination was conducted to assess the fibroblast count.

RESULTS

Table 1. The mean and standard deviation of FGF-2 expression in the C3, M3-25, M3-37.5, M3-50 and C7, M7-25, M7-37.5, M7-50

Groups	n	Mean ± SD
C3	4	3.25 ± 1.258
C7	4	6.00 ± 2.160
M3-25	4	8.50 ± 1.915
M7-25	4	15.25 ± 2.062
M3-37.5	4	8.50 ± 2.082
M7-37.5	4	15.50 ± 2.082
M3-50	4	14.75 ± 1.708
M7-50	4	18.50 ± 3.109

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 32a/E/KPT/2017.
 Available at http://e-journal.unair.ac.id/index.php/MKG
DOI: 10.20473/j.djmkg.v50.i3.p121-126

Table 2. The significance values of FGF-2 expression between treatment groups on days 3 and 7

Group	C3	C7	M3-25	M7-25	M3-37.5	M7-37.5	M3-50	M7-50
C3	-	0.077	0.002*	0.000*	0.002*	0.000*	0.000*	0.000*
C7	-	-	0.106	0.000*	0.000*	0.000*	0.000*	0.000*
M3-25	-	-	-	0.000*	1.000	0.000*	0.000*	0.000*
M7-25	-	-	-	-	0.000*	0.868	0.740	0.039*
M3-37.5	-	-	-	-	-	0.000*	0.000*	0.000*
M7-37.5	-	-	-	-	-	-	0.619	0.055
M3-50	-	-	-	-	-	-	-	0.019*
M7-50	-	-	-	-	-	-	-	-

Description: * significant at $\alpha = 0.05$

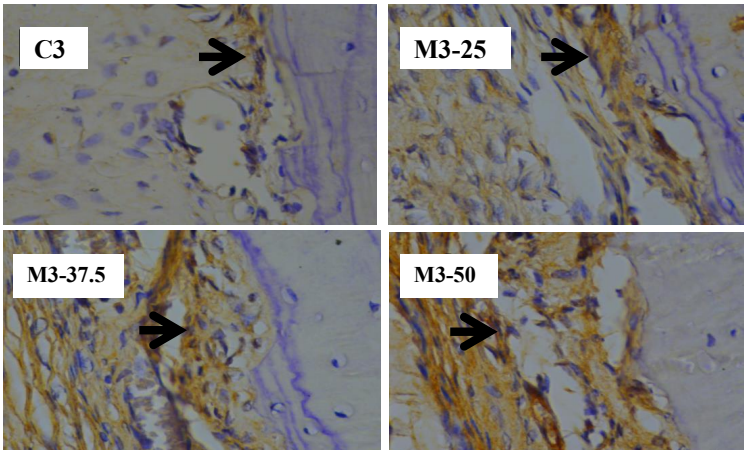

Figure 1. FGF-2 expression in the control and treatment groups on day 3.
Note: C3 : FGF-2 expression in the control group on day 3
M3-25 : FGF-2 expression in the treatment group with 25% Mauli banana stem extract on day 3
M3-37.5: FGF-2 expression in the treatment group with 37.5% Mauli banana stem extract on day 3
M3-50 : FGF-2 expression in the treatment group with 50% Mauli banana stem extract on day 3

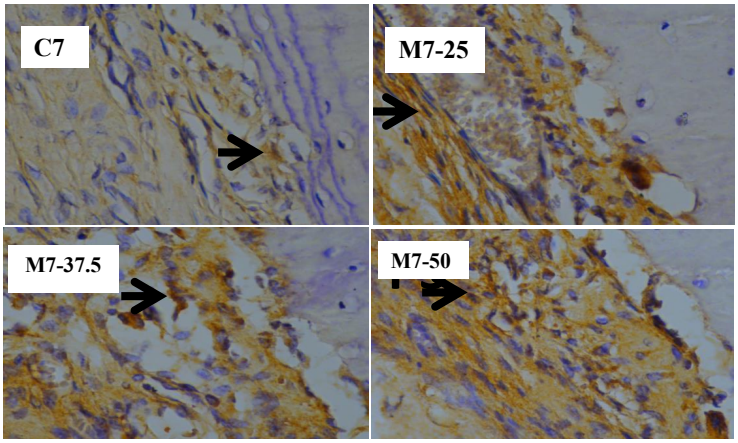

Figure 2. FGF-2 expression in the control and the treatment groups on day 7.
Note: C7 : FGF-2 expression in the control group on day 7
M7-25 : FGF-2 expression in the treatment group with 25% Mauli banana stem extract on day 7
M7-37.5: FGF-2 expression in the treatment group with 37.5% Mauli banana stem extract on day 7
M7-50 : FGF-2 expression in the treatment group with 50% Mauli banana stem extract on day 7

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 32a/E/KPT/2017.

DOI: 10.20473/j.djmkg.v50.i3.p121-126

Table 3. The mean and standard deviation of fibroblast amount in the control and treatment groups on days 3 and 7

Groups	n	Mean ± SD
C3	4	8.00 ± 2.582
C7	4	14.25 ± 2.217
M3-25	4	14.00 ± 2.582
M7-25	4	18.75 ± 1.708
M3-37.5	4	17.50 ± 3.000
M7-37.5	4	24.00 ± 2.944
M3-50	4	23.75 ± 2.217
M7-50	4	30.50 ± 2.082

DISCUSSION

Mauli banana (*Musa acuminata*) stem extract contains the same substances as other bananas, such as terpenoid saponin. The Ambon banana (*Musa paradisiaca* var sapientum), for instance, also contains bioactive triterpenoid saponin which is known to accelerate the wound healing process in skin.[11] Terpenoid saponin contained in Mauli banana stem extract is considered to be an immunostimulator that can increase activity and the number of macrophages. Terpenoid saponins will also be captured by G protein receptors in macrophages. Then, through a process of generating protein kinase C, it will activate nuclear factor kappa beta (NFkβ), thereby increasing the activity and number of macrophages.[12] According to research conducted by Apriasari in 2015, Mauli banana extract gel

Table 4. The significance values of fibroblasts numbers between treatment groups on days 3 and 7

Group	C3	C7	M3-25	M7-25	M3-37.5	M7-37.5	M3-50	M7-50
C3	-	0.001*	0.002*	0.000*	0.000*	0.000*	0.000*	0.000*
C7	-	-	0.887	0.016*	0.073*	0.000*	0.000*	0.000*
M3-25	-	-	-	0.011*	0.055*	0.000*	0.000*	0.000*
M7-25	-	-	-	-	0.478*	0.006*	0.008*	0.000*
M3-37.5	-	-	-	-	-	0.001*	0.001*	0.000*
M7-37.5	-	-	-	-	-	-	0.887	0.001*
M3-50	-	-	-	-	-	-	-	0.001*
M7-50	-	-	-	-	-	-	-	-

Description: * significant at α = 0.05

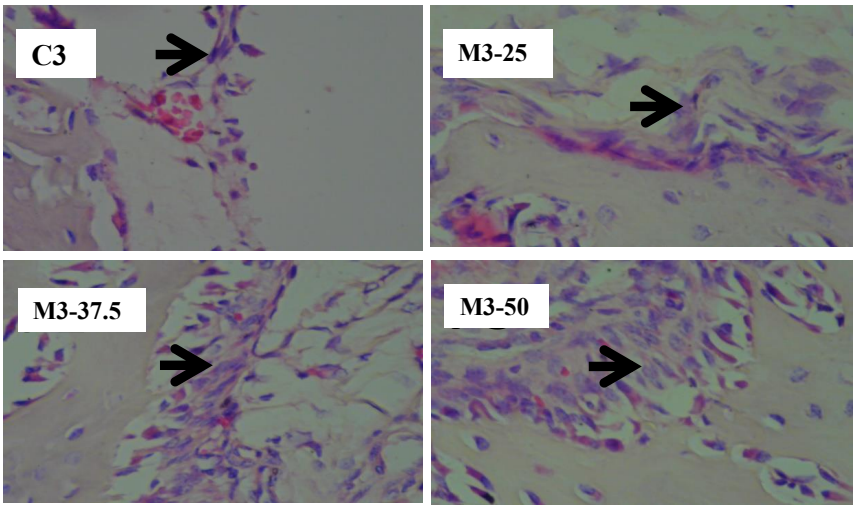

Figure 3. Photographs of the HE examination of the number of fibroblasts on day 3 (400x magnification).
Note: C3 : Amount of fibroblasts cells in the control group on day 3.
M3-25 : Amount of fibroblasts cells in the treatment group with 25% Mauli banana stem extract on day 3.
M3-37.5: Amount of fibroblasts cells in the treatment group with 37.5% Mauli banana stem extract on day 3.
M3-50 : Amount of fibroblasts cells in the treatment group with 50% Mauli banana stem extract on day 3.

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 32a/E/KPT/2017.

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at a concentration of 25% can accelerate the healing process in incised wounds on the oral mucosa of rats by increasing the number of macrophages on day 3, and subsequently decreasing them on the fifth day.[13]

Macrophages play an important role in the wound healing process since they produce growth factors as well as trigger angiogenesis and fibro genesis. The excreted macrophages can even phagocytize bacteria and clean out tissue debris. During the transition from inflammatory processes to wound repair, macrophages can stimulate cell migration, proliferation, and tissue matrix formation. Growth factors involved in angiogenesis are transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), and fibroblast growth factors-2 (FGF-2).[14,15]

Based on the contents of Table 1, the highest mean number of FGF-2 expressions was found in the Mauli banana stem extract of M3-50, namely 14.75 on the 3rd day and 18.5 on the 7th day. Meanwhile, the lowest mean FGF-2 expressions were found in the control group, namely 3.25 on day 3 and 6.0 on day 7. According to the results of the research that was conducted on the third day, there was a significant increase in FGF-2 expression between the M3-25, M3-37.5, M3-50 groups treated with Mauli banana stems compared to that of the C3 group.

The result of the research conducted on the seventh day confirmed the increase in FGF-2 between the groups given M7-25, M7-37.5% and M7-50% extracts compared to that of C7 group. Group extract of Mauli banana M7-25 compared with group of extract of Mauli banana M7-37.5 happened almost the same increase of FGF-2 whereas with group of extract of Mauli banana M7-50 happened increase of FGF-2. Group extract of Mauli banana M7-37.5 showed result of increase of FGF-2 which almost equal to concentration of Mauli banana M7-50 banana extract. For all treatment groups on the seven days that were compared there was an increase in FGF-2 compared to the control group.

It can be said that giving Mauli banana stem gelatin extract can increase FGF-2 expression compared with the control group on both the third and seventh days. The immunohistochemical results also showed that the highest increase in FGF-2 expressions was found in the treatment group administered Mauli banana stem extract at a concentration of 50% since the higher the concentration of Mauli banana stem extract, the greater the amount of tannin substances with anti-inflammatory properties contained. These include: saponin, alkaloids, flavonoids, lycopene, ascorbic acid, and β-carotene that work synergistically to suppress or decrease pro-inflammatory cytokines, thereby accelerating the wound healing process.

The Mauli banana stem extract containing terpenoid saponin has the same mechanism as the Astragalus plant. The Mauli banana is able to increase the proliferation and migration of fibroblasts and, similar to Asiaticoside plants containing terpenoid saponins, can enhance collagen synthesis, granulation tissue formation, and wound contraction.[16] Previous research has also demonstrated that Mauli banana extract gel has the potential to form high quality wound contractions in the oral mucosal of rats.[9]

The results of the research conducted on the third day confirmed an increase in the amount of fibroblast cells between the extracted groups M3-25, M3-37.5 and M3-50 compared to the C3 group. Group of Mauli banana extract M3-25 compared with Mauli banana extract group M3-37.5 happened increase of amount of cells of fibroblas which almost same but still low when compared with Mauli banana

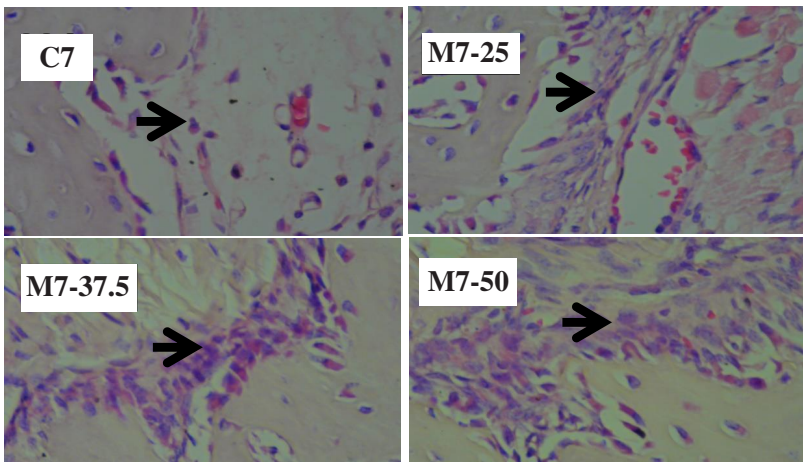

Figure 4. Photographs of the HE examination of the number of fibroblasts on day 7 (400x magnification).

Note: C7 : Amount of fibroblasts cells in the control group on day 7
M7-25 : Amount of fibroblasts cells in the treatment group with 25% Mauli banana stem extract on day 7
M7-37.5 : Amount of fibroblasts cells in the treatment group with 37.5% Mauli banana stem extract on day 7
M7-50 : Amount of fibroblasts cells in the treatment group with 50% Mauli banana stem extract on day 7

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extract M3-50. Similar increase between concentration of M2-25 and M3-37.5 due on the third day is still not expected to show optimization of Mauli banana extract

The results of the research carried out on the seventh day confirmed an increase of the number of fibroblast cells between the extracted group M7-25, M7-37.5 and M7-50 compared to the control group. With regard to the M7-25 Mauli banana extracts group, there was an increase in the amount of fibroblast cells which was similar to the M7-37.5 Mauli banana extract, but still lower when compared with the M7-50 Mauli banana extract group.

The administration of Mauli banana stem extract gel to the M7-25, M7-37.5, and M7-50 could significantly improve the amount of fibroblasts compared to that of C7. The results of this study indicated that the highest level of fibroblast occurred in the treatment group treated with Mauli banana stem extract at a concentration of M7-50. Previous research has even shown that plants containing anti-oxidants potentially play a role as immunomodulators, which produce immunostimulator and immunosuppressant effects triggered by the concentration level.[17] In addition, the results of banana leaf extract study with HE examination revealed that the highest amount of fibroblast occurred in the treatment group treated with Mauli banana stem extract at a concentration of M7-50. In conclusion, there was an increase in FGF-2 expression and the number of fibroblast cells in the incision wound healing process that induced with Mauli banana stem extract.

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Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 32a/E/KPT/2017.

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