International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.12, pp 558-564, 2016

Ambonese banana stem sap (*Musa paradisiaca var. sapientum*) effect on PDGF-BB expressions and fibroblast proliferation in socket wound healing

Budi HS[1]* , Kriswandini IL[1], Sudjarwo SA[2]

[1] Department of Oral Biology, Faculty of Dental Medicine Universitas Airlangga, Surabaya-Indonesia, Jl. Prof Dr Moestopo no.47
[2]Department of Pharmacology, Faculty of Veterinary Medicine Universitas Airlangga, Surabaya-Indonesia, Kampus C Mulyorejo.

Abstract : TGF-β1 and PDGF-BB are potent chemotaxis, mitogen and diffentiation mesenchym cells active in wound healing. Ambonese banana stem sap has been commonly used historically for gastric bleeding, ulcus pepticum and pharyngitis as empirical agents.The aim of this study is to prove PDGF-BBexpression and fibroblast proliferation effect of ambonese banana stem sap (Musa paradiaca var sapientum) on socket wound healing post tooth extraction.The contains of banana stem sap was performed by thin-layer chromatography (TLC) and ultra-violet visible (UV-vis). We have used the post-test only control-group design with 54 male rats.Incisor and mandible teeth were extracted, and then the socket was treated water extract of ambonese banana stem sap 15, 30 and 60 mg dose in 4% hydroxypropylmethylcellulose (HPMC).The socket were observed at 2, 7 and 14 days on immunohistochemistry (IHC) and histology data. Result of this study that the water extract contains saponnins, flavonoids, tannins, anthraquinon and lectin at screening test. The data showed significant difference of PDGF-BB expressions and fibroblast proliferation at p=0,00 and p=0,00 on days 2 and 7after tooth extraction.The conclusion wasthe water extract of ambonese banana stem sap have potential to accelerate socket wound healing post tooth extraction on PDGF-BB expression and fibroblast proliferation.
Keywords: PDGF-BB, fibroblast,ambonese banana.

Introduction

Wound healing is a complex and dynamic process of replacing devitalized and missing cellular structures and tissue layers. Infection, surgical interventions, and drugs can result in a different rate of healing.[1] In tooth extraction, the healing phase must occur through a haemostatic process in pattern of blood clots, leukocyte infiltration, the formation of connective tissue, granulation, epithelialization and remodeling phases. Blood clots are a very determining stage in the wound healing process[2,3,4]. Growth factors secreted by platelets will induce the formation of new fibroblast through miofibroblast proliferation and differentiation, then they synthesize the collagen matrix, capillaries, in which all are seen as granulation tissue[5]. Platelets are the early haemostatic movement, accompanied by the accumulation of growth factors such as TGF-β1 and PDGF[6].

The Ambonese banana (Musa paradisiaca var. sapientum) is a plant that is commonly found in Indonesia, especially in areas with a lot of sunlight. Empirically, Ambonese bananas are widely used to treat

diseases such as uterine bleeding, intestinal ulceration, hemorrhoids, chicken pox, ear and throat swelling, dysentery, colon bleeding and diarrhea[7,8]. The plants with high lectin concentrations can be used for wound healing either through coagulation or blood clot formation[11]. The results of a study on the administration of galectin-3 (which is in the lectin group), showed an increase in Vascular Endothelial Growth Factor (VEGF) and Basic Fibroblast Growth Factor (b-FGF) to angiogenesis respons[12]. The administration of banana stem sap extract showed ulcer healing as indicated by increased levels of hydroxyproline, hexuronic acid, hexosamine, superoxide dismutase and decreased glutathione in granulation tissue and lipid peroxidation, compared to the control group[13]. The result of biocompatibility, anti-inflammatory and analgesic tests showed that banana sap with up to 100% concentration was relatively non-toxic to fibroblast cells, and had efficacy as an anti-inflammatory and analgesic[14,15].

Banana trees contains various compounds that very beneficial for medicine. The high content of allantoin and tannins is often used for treating wounds, laryngitis, bleeding, and urinary tract infections. The roots of this plant are very useful for relieving dental pains, ulcers and intestinal inflammation[16]. Banana sap contains saponins, anthraquinone, and tannins that can serve as antibiotics and pain relief agents. In addition, banana sap also contains lectin, which serves to stimulate skin cell growth. Such compounds can kill bacteria, thus preventing them from infecting the injured area[17]. Lectins are proteins with effects such as antitumor, and may be used as a medication for Human Immunodeficiency Virus (HIV), as well as having antimicrobial and mitogenic properties. In addition, lectins are effective in fighting muscle cancer in mice and lung cancer in humans, with respective dosages of 5 and 50 micrograms, through an important path in mitogen and T cell proliferation[18].

PDGF-BB and TGF-β1 are play important role in wound healing process reminiscent of tooth extraction and this study to prove the candidate of ambonese banana as herbal medicine have effect on PDGF-BB expression to fibroblast proliferation.

Experimental

The study using post-tes only control group design. Identification and phytochemical screening test to the type of banana stem sap preparations have previously been carried out. Materials used in this study were banana stem sap extract prepared in the Faculty of Pharmacy, Universitas Airlangga.Monoclonal rats antibodies of TGF-β1 and PDGF-BB, 10% EDTA, Paraffin, xilol, ethanol, distilled water, 3% H2O2, PBS, 5% FBS, antibodyGoat anti-mouse IgG biotin labeled, SA-HRP (Strepavidin-Horseradish Peroxidase, DAB (3,3 diamino benzidine tetrahydrochloride), 1% Methyl Green.

Phytochemical screening of Ambonese banana stem sap was conducted using a Thin-layer chromatography (TLC) test with the appearance of a color stain, followed by UV-Visible (Shimitzu) spectrophotometer to saponins, flavonoids, tannins, anthraquinones and lectins.The detection of saponin compound was conducted at a wavelength of 215 nm, flavonoids at a wavelength 226 nm, tannins at a wavelength of 275 nm, anthraquinone at a wavelength of 285 nm and lectins at a wavelength of 228 nm.

The paraffin block preparations[19] is achieved by cleaning the tooth socket tissue specimen and then washing it with PBS 3-5 times to eliminate contaminants. Then it was fixated in 10% formalin for 24 hours, dehydrated using 30%, 50%, 70%, 80%, 96% alcohol gradually and absolute alcohol, each for 60 minutes.Clearing was performed using xilol 2 times, each for 60 minutes. Decalcification was conducted with EDTA.Soft paraffin infiltration was done for 60 minutes at 48^{o}C.Hard paraffin block was made in the molds and allowed to set for a day.It was then put in the holder so that the socket could be cut longitudinally with semi-serial thinness of 4μm by rotary microtome.It was then mounted on an object glass with 5% gelatin.

Immunohistochemical study[19], slides containing the pieces of organs were deparaffinated in xilol I, xilol II and xilol III, respectively for 5 minutes.Slides were rehydrated in graded ethanol (100% I, 100% II, 90%, 80%, 70%, distilled water) each for 5 minutes.Blocking was conducted in 3% H2O2 in PBS incubation for 20 minutes at room temperature to inhibit peroxidase in the tissues. Slides were washed with PBS pH 7.4 and blocked in 5% FBS (Fetal Bovine Serum)/ 1% BSA in PBS for 60 minutes. Slides were labeled with primary antibodies (PDGF-BB, BMP-4 and BMP-7) in 5% Fetal Bovine Serum overnight at 4°C.Slides were then washed with PBS pH 7.4 three times for 5 minutes. Slides were then labeled with secondary antibody of Goat anti mouse IgG biotin label for 1 hour at room temperature. Slides were washed with PBS pH 7.4 three

times for 5 minutes and incubated with SA-HRP (Strepavidin-Horseradish Peroxidase) 1:500 for 40 minutes at room temperature. Slides were washed with PBS pH 7.4 three times for 5 minutes. Slides were then applied withDAB (3,3 diamino benzidine tetrahydrochloride) substrate chromogen for 20 minutes. They were then washed with PBS pH 7.4 three times for 5 minutes followed by dH2 three times each for 5 minutes. Counterstain was conducted with 1% Methyl Green at room temperature. Slides were soaked with tap water for 5 minutes, preparations were cleared up and dried overnight at room temperature.Next step was mounting and slides were observed with a light microscope with 400x magnification.

Histology study [19], washing slides with PBS pH 7.4 for 5 minutes. Staining with hematoxilen for 10 minutes. Submerging into tap water for 10 minutes. Rinsing with dH_20. Performing dehydration with 30% and 50% alcohol gradually respectively for 5 minutes. Then staining with eosin solution for 3 minutes. Rinsing with 30% alcohol. Performing dehydration with 50% graded alcohol to be absolute. Rinsing with xilol 2x, each for 15 minutes. Mounting with entelan and closing it with a cover glass. Examination was conducted using a light microscope with 400X magnification.

Results

Phytochemical screening of Ambonese banana stem sap was conducted using Thin Slope Chromatography (KLT) test with the appearance of a color stain of for saponins, flavonoids, tannins, anthraquinones (figure 1), and followed lectins by UV-Visible (Shimitzu) spectrophotometer (table 1).

Table 1. The compound contents of each type of preparation

Types of preparations	saponins (%)	Flavonoids (%)	Tanin (%)	Anthraquinone (%)	Lectin (%)
Water extract	1.34	0.29	1.52	0.31	0.28

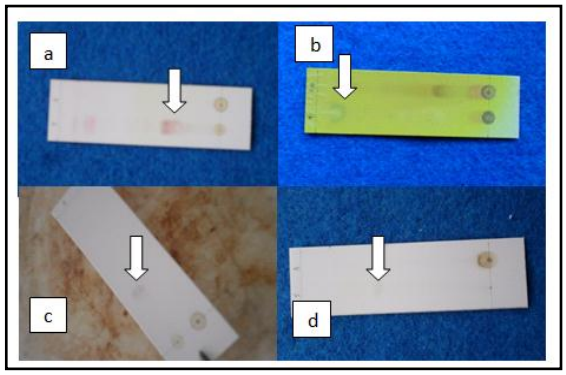

Figure 1. The appearance of a color stain on a. saponins, b. flavonoids, c. tannins and d. antraquinones.

The data showed a significant difference between control and treatment groups at p=0.00, p=0.00 and p=0.00 respectively days, but no significant difference between 30 mg and 60 mg of PDGF-BB expression (figure 2) and fibroblast proliferation (figure 3) at p=0.58 and p=0.12 on day 2, p=0.61 and p=0.70 on day 7. The increasing dose for each preparation showed a significant difference in PDGF-BB expression and fibroblast proliferation, but no significant difference on day 14. (table 2, tabel 3).

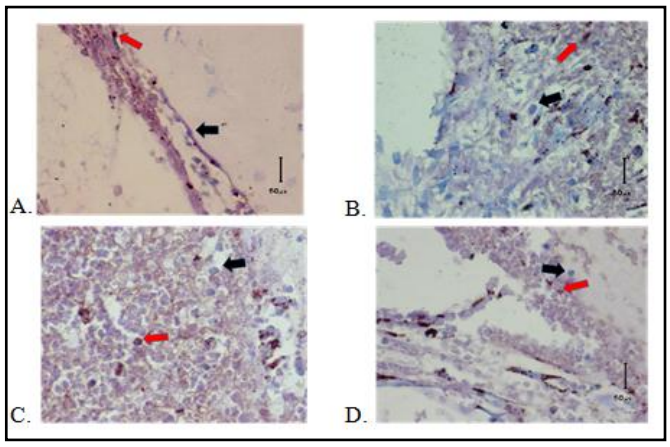

Figure 2. PDGF-BB expression on socket wound healing day 2 with microscope magnifecent 400x. Red arrow is positive, black is negatip. A. control, B. dose 15 mg, C. Dose 30 mg, and D. dose 60 mg.

Table 2. Average of fibroblast cells expressing PDGF-BB in each treatment

Types of preparations	**Dose (mg)**	**Day**		
		2	**7**	**14**
Water extract	15	23.5 ± 1.37^{b}	46.67 ± 1.36^{b}	44.17 ± 1.94^{b}
	30	27.0 ± 2.00^{c}	$58.00 \pm .89^{c}$	55.33 ± 2.25^{b}
	60	31.0 ± 1.26^{cd}	61.17 ± 2.31^{cd}	58.67 ± 3.88^{b}
Control -		6.3 ± 1.86^{a}	5.50 ± 1.76^{a}	10.50 ± 1.76^{a}

Remarks: superscript difference shows significant differences

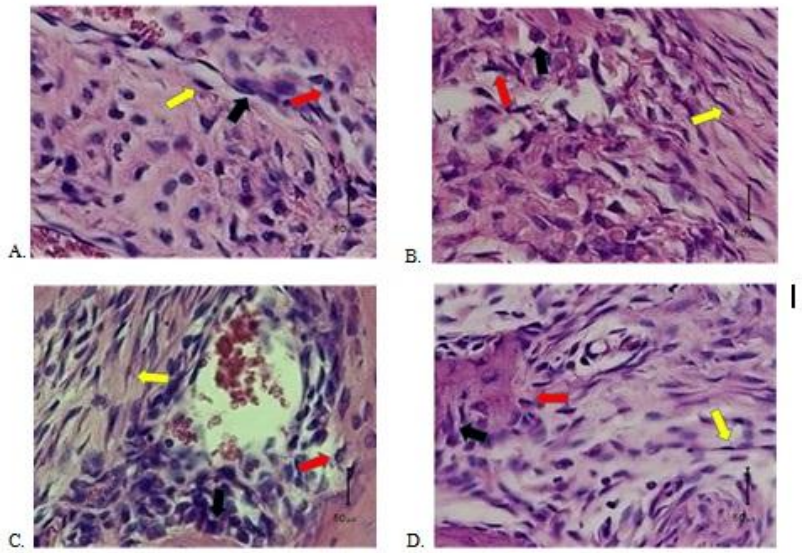

Figure 3. Fibroblast, osteoblast and osteoclaston socket day 2 with microscope magnificent 400x. A. control, B. dose 15 mg, C. dose 30 mg, and D. Dose 60 mg. yellow arrow : fibroblast, red : osteoblast, green : osteoclast.

Table 3. Average fibroblast proliferation in each treatment

Types of preparations	Dose (mg)	Day		
		2	**7**	**14**
Water extract	15	30.8 ± 3.60^{b}	40.6 ± 6.28^{b}	52.3 ± 5.00^{b}
	30	33.3 ± 4.03^{c}	51.1 ± 2.56^{c}	57.6 ± 2.50^{b}
	60	37.3 ± 1.04^{d}	37.8 ± 2.13^{d}	56.0 ± 1.26^{b}
Control -		13.0 ± 2.60^{a}	20.6 ± 2.73^{a}	26.8 ± 1.47^{a}

Remarks: superscript difference shows significant differences

Discussion

The wound healing process is basically the same between oral and skin mucosa. Following tooth extraction, the healing phase must actuate through a haemostatic process with the formation of blood clots, inflammation with leukocyte infiltration, proliferation with the formation of connective tissue, granulation and epithelialization and remodeling phases[3,4,20]. The blood clot is a very determining crucial stage in the wound healing process. Growth factors secreted by platelets will induce the formation of new fibroblast through miofibroblast proliferation and differentiation, then synthesize the collagen matrix, capillaries, in which all are seen as granulation tissue. Platelet is the onset of haemostatic, accompanied by the accumulation of growth factors such as Transforming Growth Factor-β1 (TGF-β1) and Platelet DerivedGrowth Factor PDGF.TGF-β1 will stimulate cell proliferation and differentiation, the accumulation of inflammatory cells, fibroblasts, wound closure and angiogenesis[21].In vitro, PDGF will stimulate Deoxyribonucleid Acid (DNA) synthesis, chemotaxis of fibroblasts and smooth muscle cells, collagen stimulation, glycosaminoglycans, and collagenase production.TGF-β1 and PDGF secretion cause chemotactics and proliferation of fibroblasts, and then an increase in extracellular matrix formation[22]. The result of phytochemical screening has revealed that banana stem sap contains saponins, flavonoids, tannins, anthraquinones and lectins. These components play important roles in the wound healing process, through the formation of stain colors reacting with chemical compounds given to Ambonese banana stem sap using a CTL test and UV-Visible (Shimitzu) spectrophotometer.

A process for getting banana stem sap easily and cheaply in large quantities is necessary because bananas are not herbal medicine but are rather cultivated for public consumption. Therefore, the plants chosen were the ones aged approximately 8 months and have borne fruit. The study used sap derived from banana stems, obtained by making fresh, water extract and ethanol extract preparations. Total sap weight obtained after being dried using freeze dry indicated that the ethanol extract preparation obtained a larger number than fresh preparation and water extract preparation of 5.98 grams per 200 ml of fluid. The result of phytochemical screening has showed that banana stem sap contains saponins, flavonoids, tannins, anthraquinones and lectins. These components play important roles in the wound healing process, through the formation of stain colors reacting with chemical compounds given to Ambonese banana stem sap using TLC and UV-Visible (Shimitzu)spectrophotometer. Saponins, flavonoids, tannins, anthraquinones and lectins contents were found in a considerable amount in the ethanol extract preparation, and then followed by a water extract preparation and last was the fresh preparation. This means that ethanol is a good solvent for those compounds[23].

The content of banana stem sap, such as saponins, anthraquinone, and tannins can serve as antibiotics and pain relief agents. In addition, banana sap also contains lectin that serves to stimulate skin cell growth. Those contents can kill bacteria, thus preventing them from entering the injured area[17]. Administering water extract preparations of banana stem sap in the wound healing process after rats tooth extraction has shown increasing in PDGF-BB expression and fibroblast proliferation compared to the control group. In accordance with the previous study, fibroblasts begin to migrate into the wound area on day 2 and 3 after injury and reach a maximum at day 7[24]. Fibroblasts will form mucopolysaccharides, aminoglisin acid and proline which are the basic components of collagen. The wound will load up with inflammatory cells, fibroblasts and collagen, forming granulation tissue consisting of small blood vessels. Fibroblasts are responsible for the formation of extracellular matrix components during the wound healing process. If granulation tissue has matured, the number of inflammatory cells will decrease so that the number of fibroblasts will also decrease.

Lectins play a role in the adhesion to damaged endothelium as a result of tooth revocation, which will enable the GPIb, that is a glycoprotein serving in blood clotting. Platelets will easily adhere to endotheliums because of GPIb on the surface, and cause platelet activation resulting in platelet aggregation. Platelets are in fact very active components in the haemostatic phase. Platelets have several receptors, mainly, glycoprotein Ib-IX-V. Glycoprotein on the endothelial surface is essential in adhesion reactions and platelet aggregation.Platelet adhesion to the damaged endothelium causes platelet activation and releases the granule contents such as adenosine 5-diphosphate (ADP), thromboxane (TXA-2), serotonin and growth factors. The secretion of granule contents causes platelets to move closer to each other to form platelet aggregation through receptor GPIIb binding fibrinogen. Platelet binding with fibrinogen will strengthen the existing platelet plug[25]. At the time of degranulation, a number of cytokines will be released by platelets, in the form of PDGF (Platelet Derived Growth Factors). PDGF is cytokine having a potential as a neutrophil chemoattractant, a dominant cell in the inflammation phase[26]. This phase is the initial phase in minimizing the very real risk of infection in the next phase[27]. The presence of other components in Ambonese banana stem sap also plays a role in the initial process of wound healing. Tannins will play a role in the blood clotting process, so that the blood clot will soon accumulate fibroblast tissue and prevent infection through a bacteria coagulation process around the wound. However, the local administration in the socket area in gel form will be released slowly, so that the effects will be visible on observations day 2, 7 and 14. The administration of a water extract at a dose of 30 mg showed the same effect as with dose of 60 mg. This means that a dose of 30 is the optimum dose to obtain a medicinal effect.

Conclusion

Ambonese banana stem sap plays role in accelerating the wound healing process after tooth extraction in rats by increasing PDGF-BB expression with fibroblasts proliferation. The optimal dose of Ambonese banana stem sap as wound healing after tooth extraction in Wistar white rats is a 30 mg dose with a water extract preparation.

References

1. Molnar J. Nutrition and chronic wounds. Adv wound care., 2014, Vol 3(11): 663-681.
2. Petersen JK, Krogsgaarda J, Nielsena KM, Nørgaarda, EB.A comparison between 2 absorbable hemostatic agents:gelatin sponge (Spongostan®) and oxidized regenerated cellulose (Surgicel®).International Journal of Oral Surgery., 2008,Vol. 13(5): 406-10.
3. Bowe DC, Rogers S, Stassen LF.The management of dry socket/alveolar osteitis. J Ir dent Assoc., 2011, 61: 305-310.
4. Lin Chen, Zarema H Arbieva, Shujuan Guo, Phillip T Marucha, Thomas A Mustoe, Luisa A Dipietro. Positional differences in the wound transcriptome of skin and oral mucosa. BMC Genomics., 2010, 11: 471, doi: 10.1186/1471-2164-11-471.
5. Hans Peter Mueller. Periodontology. Thieme, Stutgart German, 2015, p 12-23.
6. Antonio Albaniese, maria E Licata, Bianca Polizzi, and Giuseppina Campisi. Platelet-rich plasma (PRP) in dental and oral surgery : from the wound healing to bone regeneration. Immunity and Ageing., 2013, 10: 23.
7. Prihatman K. Pisang :budidaya tanaman pertanian. Kantor Deputi Menegristek Bidang Pendayagunaan dan Pemasyarakatan Ilmu Pengetahuan dan Teknologi, Jakarta, 2000, hal. 1-13.
8. DJ Deshphande. Handbook of medicinal herbs. Narayana Verlag GmBH, Kanden, Germany, 2006, p. 54-55.
9. Sudirga SK.Pemanfaatan tumbuhan sebagai obat tradisional di desa trunyan kecamatan kintamani kabupaten bangli. e-jurnal Bumi Lestari Udayana, 2000, Vol.12: 7-18.
10. OGTR.The biology of musa.The Office of the Gene Technolgy Regulator. Vol 1. Canberra. Australia. Available online on http://www.ogtr.gov.au. Access on 11 November 2008.
11. Coopoosamy RM. In-vitro studies on lectin derivatives of aloe excelsa (Berger).Journal of Medicinal Plants Research, 2010, Vol. 4(17): 1738-42.
12. Anna MI, Fu-Tong L, Noorjahan P. Galectin-3 is an important mediator of VEGF- and bFGF-mediated angiogenic response.The Journal of experimental medicine, 2010, Vol. 207(9): 1981-93.

13. Agarwal PK, Singh A, Gaurav K, Goel S, Khanna HD, and Goel RK. Evaluation of wound healing activity of extracts of plantain banana (Musa sapientum var. paradisiaca) in rats.Indian Journal of Experimental Biology, 2009, Vol. 47: 32-40.
14. Budi HS, Yuliastutik WS, Yuliati, Triranti A. Efek antiinflamasi getah pisang ambon (Musa Paradisiaca Var.Sapientum L.) pada Tikus Putih (Rattus Norvegicus) Galur Wistar.E-journal Departemen Biologi Oral Fakultas Kedokteran Gigi Universitas Airlangga, 2010,Vol 2(2): 1-8.
15. Budi HS, Yuliastutik WS, Yuliati, Mulyaningtyas R. Efek analgesik getah pisang ambon (Musa Paradisiaca Var. Sapientum L.) pada Mencit (Mus Musculus). E-journal Departemen Biologi Oral Fakultas Kedokteran Gigi Universitas Airlangga, 2010,Vol 2(2): 9-19.
16. Pelletier and Kenneth R. The best alternative medicine, part I:western herbal medicine.New York:Simon & Schuster, 2002, p 57-60.
17. Priosoeryanto BP, Putriyanda N, Listyanti AR, Juniantito V, Wientarsih I, Prasetyo BF dan Tiuria R. The effect of ambon banana stem sap on the acceleration of wound healing process in mice (Mus musculus albinus).J. Agric and Rural Devp., 2007, p. 35-49.
18. Peumans WJ, Zhang W, Barre A, Astoul CH, Balint-Kurti PJ, Rovira P, Rouge P, May GD, Van Leuven F, Truffa-Bachi P, Van Damme EJM.Fruit-specific lectins from banana and plantain. Planta., 2000, Vol. 211: 546-54.
19. Boenisch T. Immunochemical Staining Methods.6th ed., California:DAKO corp., 2012, p. 26-32.
20. Kumar V, Abbas AK, Fausto N. Robbin and Cohran:Pathologic basic and disease.9th International ed.Philadelphia:Elsevier, 2015, p. 47-119
21. Kim YJ, Carvalho FC, Souza JA, Goncalves PC, Ngueira AV, Spolidorio LC, Roque barreira MC, Cirelli JA. Topical application of lectin artin M acclerates wound healing in rat oral mucosa by enhancing TGF-β and VEGF production. Wound Repair Regen., 2013, Vol 21(3): 456-463.
22. Antonio Nanci. Ten cate's oral histology : development, structure and function. Elsevier., 2014, p. 195-219.
23. Torre JDL and Sholar A, 2006. Wound healing, chronic wounds. e-Medicine from WebMD (serial online). accesed (cited 2009 July 11);1(477) Available from URL:http:// www.emedicine.com /plastic/topic477.htm
24. Hock, Franz J. Drug Discovery and Evaluation, Pharmacological assays. 4nd ed., Berlin:Springer., 2015, p. 280 – 282; 306.
25. White MM and Jeninngs LK. Platelet protocols:research and clinical laboratory procedures.San Antonio:Academic Press, 2006, p. 6-17.
26. Carlo Enrique Marcucci, Patrick Schoettker. Perioperative hemostasis in Surgery:Scientific Principal and Practice.Springer Berlin Heidenberg., 2015, p. 65-70.
27. Anitua E, Andia I, Ardanza B, Nurden P, Nurde AT. Autologous platelet as a source of protein for healing and tissue regeneration. J. Tromb Haemost., 2004, 91:4-15.

*****