NONI EXTRACT GEL (MORINDA CITRIFOLIA) INCREASED THE NUMBER OF MACROPHAGE AND ANGIOGENESIS IN MANDIBULAR SOCKET AFTER TOOTH EXTRACTION (IN VIVO TEST)

by Mochammad Taha Ma'ruf

Submission date: 07-Mar-2023 07:22PM (UTC+0700)
Submission ID: 2031135307
File name: mengkudu_english.pdf (971.83K)
Word count: 2792
Character count: 15232

NONI EXTRACT GEL (MORINDA CITRIFOLIA) INCREASED THE NUMBER OF MACROPHAGE AND ANGIOGENESIS IN MANDIBULAR SOCKET AFTER TOOTH EXTRACTION (IN VIVO TEST)

MOCHAMMAD TAHA MA'RUF, PUTU SULISTIAWATI DEWI, RIA KOESOEMAWATI, DEWA MADE WEDAGAMA

Department of Oral & Maxillofacial Surgery, Mahasaraswati University, Indonesia
Department of Biomedical Science, Mahasaraswati University, Indonesia

Email: tahamaaruf@gmail.com

ABSTRACT

Objective: The objective of this research was to evaluate the effectiveness of noni extract gel (Morinda Citrifolia) increased the number of macrophage and angiogenesis in mandibular socket after tooth extraction.

Methods: in vivo experimental research consisted of 32 guinea pigs which were divided into 4 unpaired groups, where one control group was given gel CMC-Na 2%, three treatment groups each was given noni extract gel of 10%, 25% and 40%. Tooth extraction was carried out on animals study which then followed by gel application for 1 minute, 2 times in the morning and in the afternoon according to the group. The animals study were being decaputated on the 5th day, where post extraction socket with the surrounding bone removed, fixed and examined histologically. The number of macrophage and angiogenesis was observed and calculated using binocular microscope.

Results: Analytic result shows a significant difference on the number of macrophage and angiogenesis within the treatment group ($p<0.05$). Noni extract gel with concentration of 10% can increase the number of macrophage and angiogenesis more compared to the concentration of 25%, 40% and the controlled group.

Conclusion: It can be concluded that noni extract gel of 10% can accelerate the healing process post tooth extraction in animal study, with an indication of an increased amount of macrophage and angiogenesis. An active compound found in noni, which is flavonoid can increase the number of macrophage as an imunostimulant through an activation of T-limphocytes. Saponin content causes an accelerated angiogenesis by altering the balance of plasminogen inhibitor and activator which affects the stimulation of angiogenesis.

Keywords: noni extract, macrophage, angiogenesis and wound healing

INTRODUCTION

Post tooth extraction wound is one of the medium that can allow pathogenic microbes to breed and infect the wound. Human body has biochemical and cellular ability to repair the integrity and functional tissue capacity caused by wounds, which is called wound healing [1]. Post extraction wound could heal easily as physiological response but can also develop some complications. There is a need of a procedure or treatment which can accelerate would healing post tooth extraction, especially for patients seeking immediate protheses, braces or dental implant. Incorrect consumption of oral antibiotics to prevent

infections could cause an increase of bacterial resistance against antibiotics. For that reason, we are willing to develop topical herbs medicine as healing material for post tooth extraction.

One of medicine trees commonly used by the society and known as a powerful tree is noni (Morinda Citrifolia). Noni contains several compunds from alkaloid, flavonoid, glycoside, saponin and triterpenoid group [2]. Noni fruit also contains several primary active compound which are anthraquinon, terpenoid compound, antibacterial substane, damnacanthal (anti cancer substance), polysaccharide, scopoletin, acsorbic acid, beta carotene, I-arginin, xeronin, and proxeronase enzyme from alkaloid pro-xeronin [3].

One of inflamatory cells which plays an important role in wound healing is macrophage. This cell is able to phagocyte more bacteria compared to other polymorphonuclear such as neutrophils, before neutrophil cells become inactive and die. Macrophage has the ability to phagocyte particles which are larger than its size. Macrophage also lives for many months even for years within the tissue compared to other cell components [4].

The formation of new blood vessels (angiogenesis) is one of the indicators of would healing [5]. The tissue requires nutrition and oxygen supply to allow good proliferation facilitated by angiogenesis. Angiogenesis occurs in proliferation stage of wound healing which is about 2 days up to 3 weeks after the injury. This is an important natural process, required in wound healing to restore blood supply to the tissue after injury took place. The new tissue will receive enough nutrition supply to proliferate [1,6].

There are not many tests or researches being carried out on noni as a substance in wound healing of post tooth extraction. Research [7] showed that noni extract can increase the amount of collagen fibers post tooth extraction in Dawley rats. Another research [8] showed that noni extract can increase the amount of fibroblast post tooth extraction in Dawley rats. In this research, we would like to determine further the effectivity of noni extract gel in increasing the amount of macrophage and angiogenesis in mandibular socket of post tooth extraction.

MATERIALS AND METHODS

Materials

Materials used in this research were noni extract gel, CMC-Na 2%, Guinea pigs (Cavia Cobaya), ketamine, chloroform, alkohol 70%, Harris Hematoxylin-Eosin (HE) stain, formalin buffer liquid of 10%, butcner funnel, Erlenmeyer tube, hemostat and elevator, syringe, pinset, vacuum rotary evaporator, water bath, binocular microscope (Olympus type CX31).

Methods

Noni extract preparation

First, the noni fruits are cleaned, washed, cut thinly and dried in room temperature. Dried noni was then grinded with blender to achieve powder consistency. Noni powder was then macerated with ethanol 95%

for 3 days. The outcome was then filtered three times with butcner funnel which was covered with filter paper and put in Erlenmeyer tubes. The filtrate achieved was steamed with vacuum rotary evaporator and then heated with water bath at a temperatur of 40°C to produce rough extract of noni fruit [9].

Noni extract gel preparation

To produce a gel form, we mixed CMC-Na2% then added with sterile aquadest until gel consistency is met, then noni extract is added depends on the desired concentration [10].

In vivo test

Based on the research method, research sample used was 32 guenia pigs animals study (male, 3 months old, 250-300 gram body weight, healthy). The animals were divided into 4 groups, one controlled group, where the subject is given CMC-Na2% gel topically, three treatmen group each given noni extract gel in 10%, 25%, and 40% in concentration topically. General anasthesia was done using ketamin 1000mg/10ml about 0.3 ml. Tooth extraction was done with a hemostat and elevator, in line with tooth socket and with an equal force carefully to minimize tooth fracture [11].

Each group was given the gel for 1 minute 2 times a day (morning and afternoon). The animals study were decapitated on the 5th day with inhalation method. Post extraction socket with the surrounding bone was removed, cleaned with NaCL and fixed with buffer formalin 10% for 24 hours. A series of histological assessments was done with Hematoxillin Eosin stain to observe macrofage cell and angiogenesis.

Observations of Macrophage cells and Angiogenesis

Observations was done by evaluating the amount of macrophage cells which was divided into 5 sections, then added together and calculate the mean score. Observation of angiogenesis was done by evaluating the amount of new blood vessels which was divided into 5 sections, then added together and calculate the mean score. The observations of macrophage and blood vessels was done uding binocular microscope (Olympus Type CX31) 400x magnification.

RESULTS AND DISCUSSIONS

Number of Macrophage

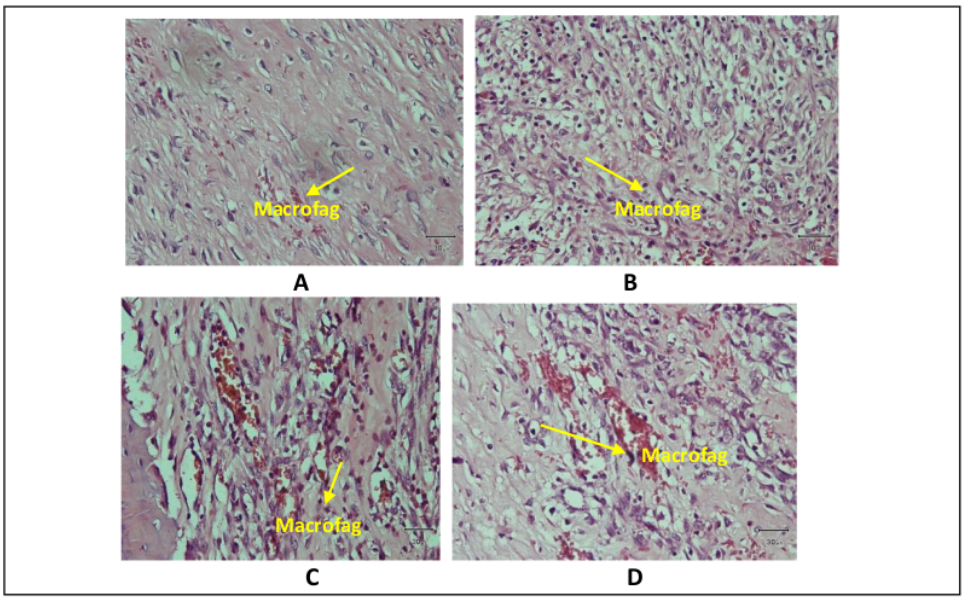

Figure 1: Macrophage a. Control group b. Concentration of 10% c. Concentration of 25% d. Concentration of 40%

The analysis of treatment effect was tested based on the mean value of macrophage between groups after treatment. The analysis result of significance with One Way Anova is shown in Table 1.

Table 1: The difference in the mean value of macrophage between groups (n=5)

Group	Mean value of macrophage	F	*p*
C	33.33 ± 6.74	33.60	0.001
G1	84.30 ± 11.11		
G2	65.90 ± 9.30		
G3	46.50 ± 10.02		

Table 1 shows that the mean value of macrophage in noni extract gel 10% group is higher compared to the other groups. One Way Anova test in Table 1 shows that the mean value of macrophage in 4 groups after treatment is significantly different ($p<0.05$). To determine a different group between groups, a Least Significant Difference (LSD) test was done. Results are shown in the following table 2.

Table 2: Least Significant difference test on the amount of macrophage between groups (n=5)

Group	G1	G2	G3
C	0.001*	0.001*	0.025*
G1		0.003*	0.002*
G2			0.001*

The amount of new blood vessels (Angiogenesis)

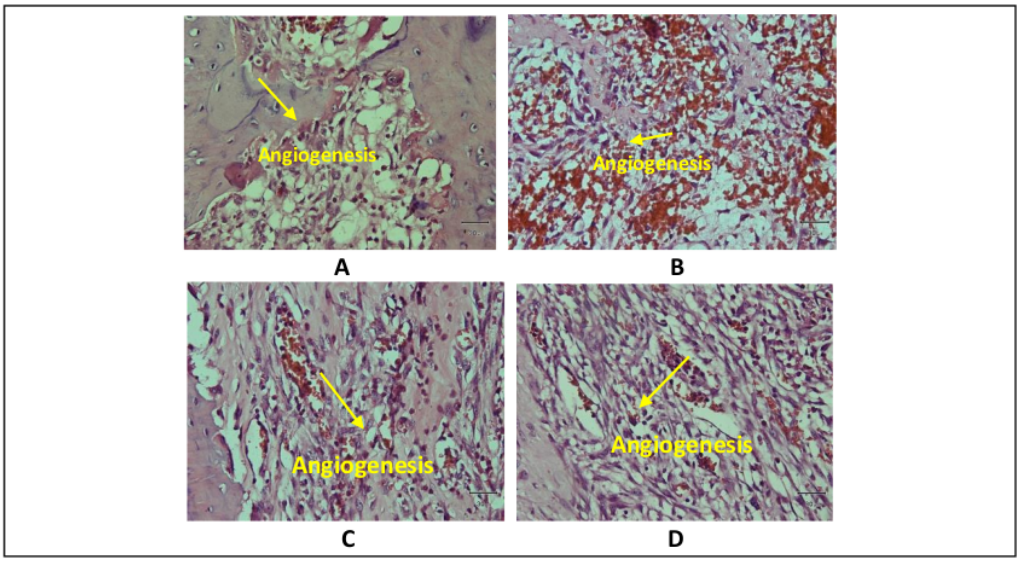

Figure 2: Angiogenesis a. Control group b. Concentration of 10% c. Concentration of 25% d. Concentration of 40%

Analysis of treatment effect was tested based on the mean value of blood vessels between groups after treatment. The analysis result of significance with One Way Anova is shown in Table 3.

Table 3: The difference in the mean value of macrophage between groups (n=5)

Group	Average of capillary blood vessels	F	*p*
C	4.63 ± 0.69	12.58	0.001
G1	7.70 ± 1.23		
G2	5.60 ±0.46		
G3	5.33 ± 1.06		

Table 3 shows that the mean value of new blood vessels (angiogenesis) in noni extract gel 10% group is higher compared to the other groups. One Way Anova test in table 3 shows that the mean value of new blood vessels in 4 groups after treatment is significantly different ($p<0.05$). To determine a different

group between groups, a Least Significant Difference (LSD) test was done. Results are shown in the following Table 4

Table 4: Least Significant difference test on blood vessels between groups (n=5)

Group	G1	G2	G3
C	0.001*	0.081*	0.199*
G1		0.001*	0.001*
G2			0.618*

Based on the results, there is a higher increase in the amount of macrophage and new blood vessels in the noni extract gel 10% group compared to the noni extract gel 25% and 40%. We could say that noni extract gel can increase the number of macrophage and accelerate angiogenesis compared to without the extract gel.

The increase of macrophage possibly caused by the flavonoid content of noni fruit which acts as an immunostimulant through the activation of T-lymphocyte. This cells produce interferon-γ (IFN-γ) which is citokin produced by the activation of T helper-1 (Th-1 in T-lymphocyte and NK cells). Combination of IFN-γ with the stimulation of pro inflammatory causes more macrophage activation [12]. IFN-γ increases $CD4^+$ differentiation to subset Th1 cells and avoiding the proliferation of Th2 cells. Inhibition of Th2 causes delayed production of IL-4 and IL-5, this can result in delayed allergic and inflammatory reaction by eusinophil. IL-4 stimulates B cells to produce IgE which will bond with mast cells, where IL-5 activates eusinophils [13,14].

The stimulated T lymphocytes during infection will produce limphokines wich will draw macrophages to the needed places and activate them. The function of dead macrophage will be replaced by new ones. The amount of blood monocytes and macrophage will increase drastically when there is an inflammation. Free macrophage aroung the tissue become active during inflammation [15,16]. An increased macrophage activation could also increase the production of cytokines growth hormones, such as VEGF which plays an important role in angiogenesis process. Increased VEGF means faster angiogenesis process because VEGF can induce mitosis in cultured endothelial cells [17].

Faster angiogenesis can also occured because of the saponin content of noni fruits. Saponin has the same effect as b FGF which is important in the formation of new blood vessels, there is also an effect of saponin on the balance of plasminogen inhibitor and activator which directly associated with the stimulation of angiogenesis process. Saponin can also increas the expression of mRNA VEGF on blood vessels endothelial cells [11].

Noni fruits also contain scopoletin, beside flavonoid and saponin which function as an anti-inflammation and anti allergy [18]. Scopoletin can delay the production of myeloperoxidase and prostaglandin E2

(PGE-2) which are mediators for inflammation [19]. Scopoletin also works as an analgesic because of its ability to tie serotonin [20]. Xeronin in noni fruits plays as a subtance to erase the feeling of pain, this is associated with the ability of xeronin to normalize the proteins within the abnormal cells, including brain tissue cells where pain perception comes from [18]. Xeronin can resolve the pain of post tooth extraction.

Anthraquinone compund in noni fruits plays a part in inhibiting the growth of bacteria. The working mechanism of this compound is by disturbing the peptidoglican compossing components on bacterial cell wall, preventing the cell wall to be formed perfectly and this mechanism could lead to cell death. Antibacterial properties of anthraquinone can help the body to avoid infection, fever and all diseases associated with bacteria [21]. This antibacterial properties can prevent infection of post tooth extraction.

Ascorbat and terpenoid compund in noni fruits can help organic synthesis process and accelerate the healing of body cells. Working mechanism of terpenoid compound is the same as phenolic compound, which is to disturb the transportation process of important ion to the bacterial cells. Trepenoid can bond with fat and carbohydrate which causes disturbance in the permeability of bacterial cell wall. Trepenoid compound is an isometric hydrocarbon compound which can be found in fat or essential oil that are important for the body. Ascorbat acid in noni fruits is the source of vitamin C, can delay inflammation, take radical oxygen to block inflammation process. Noni fruits also contain high level of selenium that work as an amazing antioxidant [18]. Vitamin C in antioxidant can increase body immune system from free radicals as well as accelerating wound healing.

CONCLUSION

Based on the results of the research, we can conclude that the application of noni extract gel 10% will increase the quantity of macrophage and new blood vessels (angiogenesis) in mandibular socket of post tooth extraction.

CONFLICTS OF INTERESTS

All authors have none to declare

REFERENCES

1. Miloro M. *Peterson's Principle of Oral and Maxillofacial Surgery* 2nd ed. 2004. London: BC Decker Inc.
2. Gunawan D, Sudarsono, Wahyuono S, Donatus IA, dan Purnomo. *Tumbuhan Obat 2 : Hasil Penelitian, Sifat-sifat dan Penggunaan*. *PPOT* UGM Yogyakarta. 2001:124-132
3. Kamiya K, Tanaka Y, Endang H, Umar M, Satake T. Chemical Constituent of Morinda Fruits Inhibit Cooper-induced Low Density Lipoprotein Oxidation. *Journal Agriculture Food Chemistry*. 2004; 22(19): 5843-5848
4. Guyton AC and Hall CE. *Buku Ajar Fisiologi Kedokteran*. 2008. 11th ed. Irawati et al (penterjemah). Jakarta: EGC
5. Nugroho TS. *Pengaruh Infiltrasi Levobupivakain 0,25% Terhadap Kuantitas Angiogenesis Tikus Wistar Pada Proses Penyembuhan Luka Insisi Hari ke-5*. 2005. Universitas Diponegoro. Semarang.

6. William, Tsakayannis D, Vinchent W. *Angiogenesis: A Control Point for Normal and Delayed Wound Healing*. 2003. Supplement Contemporary Surgery
7. Sridjaja C. Uji Gel Buah Mengkudu (Morinda citrifolia Linn) terhadap pertumbuhan sabut kolagen pasca pencabutan gigi pada *dawley rats*" (Skripsi). 2009. Surabaya. Universitas Airlangga.
8. Khoswanto C. The Effect of mengkudu gel (*Morinda citrifolia* Linn) in accelerating the escalation of fibroblast post extraction. *Dental Journal*. 2010; 43(1).
9. Hartini IGAA. Pemberian ekstrak etanol daun belimbing wuluh (*Averrhoa Bilimbi Linn)* secara topikal konsentrasi 10% lebih meningkatkan jumlah fibroblast daripada 20% dan 40% untuk penyembuhan luka gingiva tikus putih jantan (*Rattus norvegicus*). Tesis. 2011. Denpasar. Universitas Udayana.
10. Prasetya BY, Wientarsih I, Priosoeryanto BP. Aktivitas Sediaan Gel Ekstrak Batang Pohon Pisang Ambon dalam Proses Penyembuhan Luka pada mencit. *Jurnal Veteriner*. 2010;11(2): 70-73
11. Permatasari N, Pasaribu R, Razaq KA. Efektifitas Ekstrak Ginseng Asia (Panax ginseng) Dalam Meningkatkan Jumlah Pembuluh Darah Pada Soket Mandibula Pasca Pencabutan Gigi Rattus norvegicus. *Majalah Kesehatan FKUB*. 2012.
12. Diegelmann RF, Evans MC. Wound Healing : An Overview of Acute, Fibrotic and Delayed Healing. *Frointers in Bioscience*. 2004; 9: 283-9
13. Baratawidjaja KG dan Rengganis I. *Imunologi Dasar*. 2012. 10th ed. Balai Jakarta: Penerbit FKUI.
14. Jeon DW, Ahn WS, You SJ, Chae GT, Shim YB, Chun HJ. Induction of Classical Activation of Macrophage in Vitro by Water Soluble Chitin. *Aplied Surface Science Journal*. 2012; 262: 134-9
15. Adityatama AP, Arijani E, Irmawati A. Peningkatan Jumlah Makrofag pada Proses Penyembuhan Luka Pasca Pencabutan Gigi Marmut (*Cavia cobaya*) Akibat Pemberian Gel Ekstrak Meniran (*Phyllanthus niruri Lynn)*. *Oral Biology Dental Journal*. 2010. 2(2): 1-4
16. Permatasari N, Prasetyaningrum N, Genna Y. Efek Pemberian Jus Buah Belimbing Manis (Averrhoa carambola L) Terhadap Peningkatan Jumlah Sel Makrofag Pada Soket Gigi Tikus (Rattus novergicus) Strain Wistar Pasca Pencabutan. *Majalah Kesehatan FKUB*. 2013.
17. Frisca, Sardjono CT, Sandra F. Angiogenesis: Patofisiologi Dan Aplikasi Klinis. *JKM*. 2009;8(2): 174-188.
18. Waha MG. *Sehat Dengan Mengkudu*. 2008. Jakarta: PT.Kesaint Blanc.: 1-10
19. Kim SW, Jo BK, Jeong JH, Choi SU, Hwang YI. Induction of Extracellular Matrix Synthesis in Normal Human Fibroblast by Anthaquinone Isolated from Morinda citrifolia (Noni). *J Med Food*. 2005; 8(4):552-5
20. Bangun AP dan Sarwono B. *Khasiat dan Manfaat Mengkudu*. 2002. Agromedia Pustaka. Jakarta
21. Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK, Anderson G. Morinda citrifolia (Noni): A Literature Review and Recent Advances in Noni Research. *Acta Pharmacol Sin*. 2002;23(12): 1127-1141

NONI EXTRACT GEL (MORINDA CITRIFOLIA) INCREASED THE NUMBER OF MACROPHAGE AND ANGIOGENESIS IN MANDIBULAR SOCKET AFTER TOOTH EXTRACTION (IN VIVO TEST)

ORIGINALITY REPORT

10%	10%	1%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	scialert.net Internet Source	8%
2	jurnal.unpad.ac.id Internet Source	1%
3	mail.medwelljournals.com Internet Source	1%

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		