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Submission date: 07-Mar-2023 07:35PM (UTC+0700)
Submission ID: 2031139833
File name: Taha_Noni_Herbstem_final.pdf (867.83K)
Word count: 2772
Character count: 15419

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ABSTRACT

The objective of the study is to determine the efficacy of noni (Morinda Citrifolia) extract gel to increase the number of macrophage and angiogenesis in mandibles socket after tooth extraction.

In vivo experimental study consist of 32 guinea pigs divided into 4 unpaired groups, first group (control) treated with CMC-Na 2% gel, the rest (treatment groups) treated respectively with noni extract gel of 10%, 25% and 40%. Animals tooth extraction was performed, followed by gel application twice a day (morning and afternoon) for 1 minute, in accordance to the group. Guinea pigs were decapitated on the 5th day, post-extraction's socket and the surrounding bone being removed, fixed and a series of histological examination was conducted. The amount of macrophage cells and angiogenesis were observed and calculated using binocular microscope.

Analytic result shows a significant difference in the amount of macrophage and angiogenesis among the treatment group ($p<0.05$). Noni extract gel with 10% concentration was capable to increase the number of macrophage and angiogenesis compared to the concentration of 25%, 40% and the control group.

It can be concluded that noni extract gel 10% concentration can accelerate the healing process after tooth extraction of guinea pigs, indicated by an increased number of macrophage and angiogenesis. Flavonoid as an active compound found in noni could increase the number of macrophage as an immuno-stimulant by the activation of T-lymphocytes. Saponin accelerates angiogenesis resulting from changes in balance of activator and plasminogen inhibitor, which affects the stimulation of angiogenesis.

Keywords: noni extract, morinda citrifolia; 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 a cellular and biochemical ability to repair the integrity and functional capacity of the tissue system resulting from wound, known as wound healing process [1]. Post extraction wound will heal easily as body physiologic response but sometimes will develop some complications. There is a need for an action or treatment capable to accelerate wound healing after tooth extraction, particularly among for patients requiring immediate denture placement, braces or implant. Incorrect application of orally intake antibiotic to prevent infection will lead to bacterial resistance against

antibiotic. Thus, we are willing to develop topical herb medication as a healing material after tooth extraction.

One of the medicinal trees commonly used by the society and known to be very effective is noni (Morinda Citrifolia). Noni fruit contains alkaloid compound, flavonoid, glycoside, saponin and triterpenoid [2]. Noni fruit contains primary active compound such as anthraquinon, terpenoid compound, antibacterial substance, damnacanthal (anti cancer substance), polysaccharide, scopoletin, ascorbic acid, beta-carotene, I-arginin, xeronin, and proxeronase enzyme from an alkaloid pro-xeronin [3].

One of inflammatory cells that play an important role in wound healing is macrophage. This cell has the ability to phagocyte more bacteria compared to other polymorphonuclear such as neutrophils, before the neutrophil itself become inactive and die. Macrophage has the ability to phagocyte larger particles compared to its size. Macrophage lives for several months even years within the tissue compared to other cell components [4].

The formation of new blood vessels (angiogenesis) is an indicator during wound-healing process [5]. Tissues require nutrition and oxygen supply to allow better proliferation, facilitated by the angiogenesis process. Angiogenesis occurs in proliferation stage during wound healing about 2 days up to 3 weeks after the injury. This is an important natural process, in which during wound healing blood supply is restored to the tissue after injury. The new tissues will receive enough nutrition supply to proliferate [1,6].

Plenty of studies or researches being carried out on noni as a substance in wound healing after tooth extraction. Research [7] shows that noni extract are capable to increase the number of collagen fibers, after tooth extraction in Dawley rats. Another research [8] shows that noni extract are capable to increase the number of fibroblast after tooth extraction in Dawley rats. In this study, we would like to determine the efficacy of noni extract gel to increase the number of macrophage and angiogenesis in mandible socket after tooth extraction.

MATERIALS AND METHODS

Materials

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

Noni extract preparation

Noni fruits are thoroughly washed, sliced thinly and dried in room temperature. Later, dried noni was grinded using blender to achieve powder consistency. Noni powder was macerated using ethanol 95% for 3 days. Result product later been filtered three times using butcner funnel layered by filter paper and placed inside Erlenmeyer tubes. The filtrate achieved was steamed with vacuum rotary evaporator and then heated using water bath at 40^{o}C until rough extract of noni fruit achieved.

Noni extract gel preparation

Gel preparation achieved by mixing CMC-Na2% with sterile aquadest until gel consistency achieved, then added with noni extract until desired concentration achieved.

In vivo study

Based on the research plan, animals (32 guinea pigs, 3 months age, body mass of 250-350 gr and healthy) used as the samples. Guinea pigs divided into 4 groups, firstly control group, applied with CMC-Na2% gel topically, the rest are treatment group, topically applied with noni extract gel 10%, 25%, and 40% each. General anaesthesia was carried out using ketamin 1000mg/10ml for 0.3 ml. Tooth extraction done gently using haemostat and elevator in line with tooth socket with equal force to minimize tooth fracture [11].

Gel was applied to each group for 1 minute twice a day (morning and afternoon). Guinea pigs were decapitated on the 5th day under inhalation method. Post extraction socket and the surrounding bone were removed, cleaned using NaCL solution and fixed using buffer formalin 10% for 24 hours. A series of histological assessments done under Haematoxylin Eosin staining, to observe macrophage cell and new blood vessels (angiogenesis).

Macrophage cells and angiogenesis observation

Observations done by counting the number of macrophage cells, which was divided into 5 fragments, then added together, and the mean score was calculated. Angiogenesis observation done by counting the number of new blood vessels, which was divided into 5 fragments, then added together, then the mean score was calculated. Macrophage and blood vessels observation was done under binocular microscope (Olympus Type CX31) with 400x magnification.

RESULTS AND DISCUSSIONS

Number of Macrophage

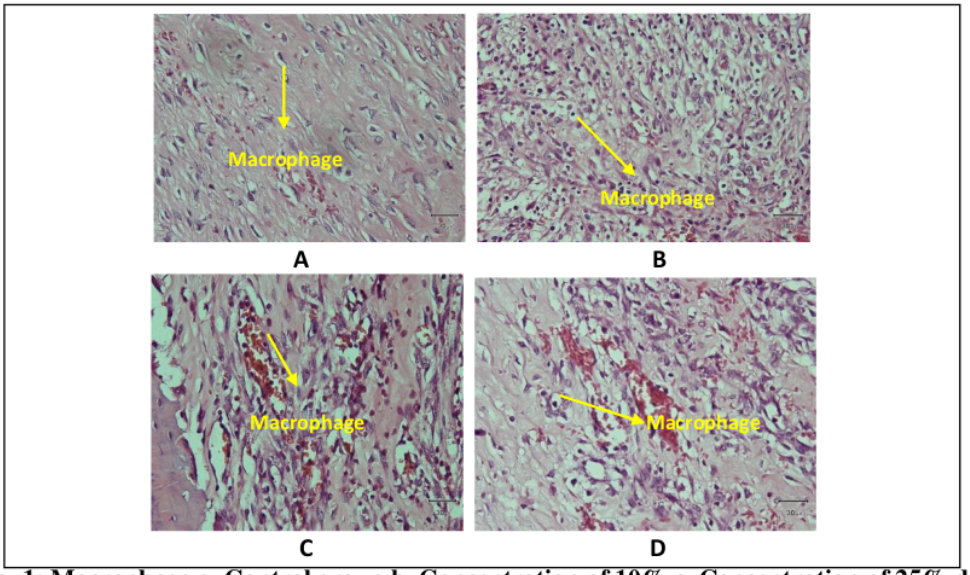

Fig. 1: Macrophage a. Control group b. Concentration of 10% c. Concentration of 25% d. Concentration of 40%

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

Table 1: 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 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 is significantly different ($p<0.05$) after treatment. To determine a different group between groups, Least Significant Difference (LSD) test was done. Results shown in the following Table 2.

Table 2: Least Significant Difference (LSD) test on number 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)

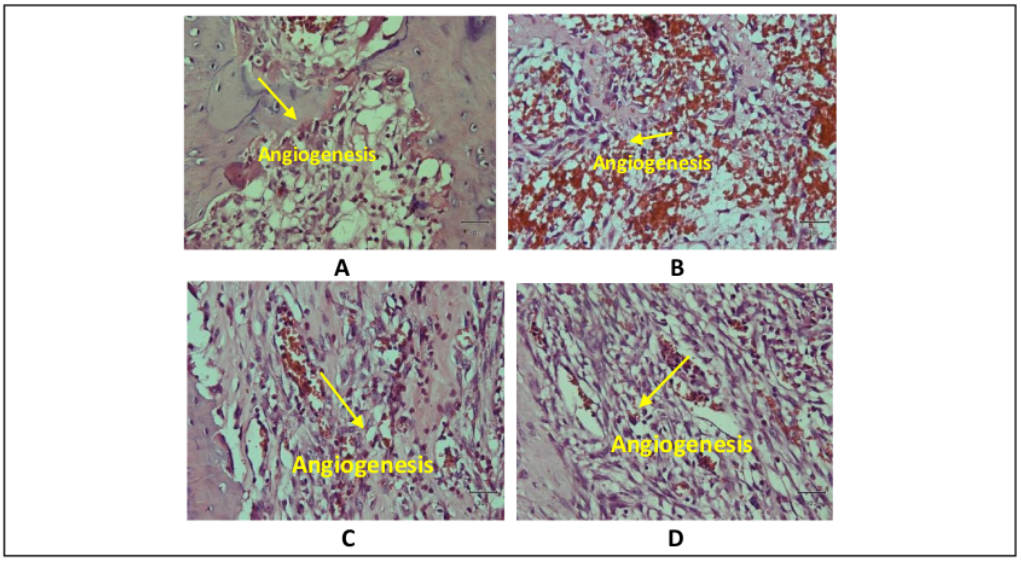

Fig. 2: Angiogenesis a. Control group b. Concentration of 10% c. Concentration of 25% d. Concentration of 40%

Analysis of treatment effect done based on the mean value of blood vessels between groups after treatment. Significance analysis result done with One Way Anova, showed in Table 3.

Table 3. Difference in mean value of macrophage between groups (n=5)

Group	Mean value 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 the mean value of new blood vessels (angiogenesis) in noni extract gel 10% group (G1) 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 number 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 process compared to other group without the application of extract gel.

The enhancement on the number of macrophage, most likely caused by flavonoid content in noni fruit which as an immuno-stimulant through the activation of T-lymphocyte. This cells produce interferon-γ (IFN-γ), a cytokine produced by the activation of T helper-1 (Th-1 in T-lymphocyte and NK cells). Combination between IFN-γ and the stimulation of pro inflammatory enhance the activation of macrophage. IFN-γ increases $CD4^+$ differentiation to subset Th1 cells and block Th2 cells proliferation. Inhibition of Th2 result to delayed production of IL-4 and IL-5; lead to delayed allergic and inflammatory reaction by eusinophil. IL-4 stimulates B cells to produce IgE that will bond with mast cells, where IL-5 activates eusinophils.

Stimulated T lymphocytes during infection will produce a number of limphokines, which will draw macrophages to injury area and activate them. The new ones will replace the role of dead macrophage. The number of blood monocytes and macrophage will increase significantly during inflammation. Free macrophage within the tissue will become active during inflammation [15, 16]. Enhancement of macrophage activation could increase the production of cytokines and growth hormones, such as VEGF

that plays an important role during angiogenesis process. Increased VEGF indicates faster angiogenesis process because VEGF induce mitosis in cultured endothelial cells [17].

Faster angiogenesis might occur because of the saponin content in noni fruits. Saponin has the same effect as b FGF, which is important during tube formation of new blood vessels; besides, effect of saponin on the balance of plasminogen inhibitor and activator which directly associated with the stimulation of angiogenesis process, has been found. Saponin could enhance the expression of mRNA VEGF in blood vessels endothelial cells [11].

Noni fruits contain scopoletin, beside flavonoid and saponin, as an anti-inflammation and anti allergy [18]. Scopoletin can block the production of myeloperoxidase and prostaglandin E2 (PGE-2), which are mediators for inflammation [19]. Scopoletin also works as an analgesic due of its ability to bind with serotonin [20]. Xeronin in noni fruits act as a painkiller, this is associated by the ability of xeronin to normalize the proteins within the abnormal cells, including brain tissue cells, origins of pain perception [18]. Xeronin can resolve the pain after tooth extraction.

Anthraquinone compound in noni fruits plays a role during inhibition bacterial growth. The working mechanism of this compound is by disturbing the peptidoglican composing components in 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 prevent infection, fever and bacteria-associated diseases [21]. These antibacterial properties can prevent infection after tooth extraction.

Terpenoid compound and ascorbic acid in noni fruits could help during organic synthesis process and accelerate body cells healing. Working mechanism of terpenoid compound is similar as phenol compound, which is to disturb the transportation process of important ion to the bacterial cells. Trepenoid could bond with fat and carbohydrate, which will disturb the permeability of bacterial cell wall. Trepenoid compound is an isometric hydrocarbon compound, which can be found in fat or essential oil that very important for the human body. Ascorbic acid in noni fruits is the source of vitamin C. Vitamin C can delay inflammation; transport radical oxygen to block inflammation process. Noni fruits contain high level of selenium that work as an amazing antioxidant [18]. Vitamin C and antioxidant can increase the body immune system and protect from free radicals as well as accelerating wound healing.

CONCLUSION

Based on the study, it can be concluded that noni extract gel 10% concentrations can accelerate the healing process after tooth extraction of guinea pigs, indicated by an increased number of macrophage and angiogenesis. Flavonoid as an active compound found in noni could increase the number of macrophage as an immuno-stimulant by the activation of T-lymphocytes. Saponin accelerates angiogenesis resulting from changes in balance of activator and plasminogen inhibitor, which affects the stimulation of angiogenesis

CONFLICTS OF INTERESTS

All authors have none to declare

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