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Research Report

Combination of *Aloe vera* and xenograft induction on decreasing of NF-κβ of tooth extraction socket preservation in Cavia cobaya

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ABSTRACT

Background: *Tooth extraction can naturally cause inflammation triggering osteoclast proliferation and alveolar bone resorption. Preservation of the tooth extraction sockets is needed for patients in order to reduce alveolar bone resorption risks. Aloe vera is known to have anthraquinones components, namely Aloin, Aloe emedin, and barbaloin, considered as anti-inflammation. Therefore, to overcome the inflammation, the role of NF-κβ is very significant to decrease nuclear factor kappa β (NF-κβ). As a result, inflammation risks will be decreased.* ***Purpose:*** *The study was aimed to determine the induction effect of combination of Aloe vera and XCB into tooth extraction sockets to reduce inflammation by reducing NF-κβ expression, osteoclasts and osteoblasts.* ***Methods:*** *Forty-eight Cavia cobaya were divided into eight groups, each group consisted of six animals. The mandibular incisors of those Cavia cobaya were extracted and induced with either PEG, XCB, Aloe vera, or the combination of Aloe vera + XCB. Those animals were sacrificed on day 7 and day 30 after the extraction. Then immunohistochemical and histopathology examinations were conducted to observe NF-κβ expression, osteoblasts and osteoclasts.* ***Results:*** *It was known that in group induced with the combination of Aloe vera and xenograft concelous bovine, the growth of osteoblasts was high, while NF-κβ expression and osteoclasts reduced.* ***Conclusion:*** *It can be concluded that the induction of the combination of Aloe vera and XCB into the tooth extraction sockets can reduce NF-κβ expression and osteoclast, as a result, alveolar bone resorption risks decrease, and osteoblast increase.*

Key words: *Aloe vera, NF-κβ, tooth extraction socket, xenograft*

ABSTRAK

Latar belakang: *Trauma mekanis akibat pencabutan gigi asli menyebabkan keradangan. Keradangan memicu proliferasi osteoklas sehingga menyebabkan resorpsi tulang alveolararis. Pada pembuatan gigi tiruan, resorpsi tulang alveolar yang terjadi, sangat tidak diinginkan, sebab resorpsi tulang alveolar mengurangi keberhasilan pembuatan gigitiruan. Diperlukan preservasi soket pencabutan gigi asli pada penderita untuk mencegah terjadinya resorpsi tulang alveolar. Aloe vera mempunyai komponen anthraquinon yaitu aloin, Aloe emodin, barbaloin yang merupakan anti inflamasi yang dapat secara cepat menyembuhkan luka, sehingga berpotensi untuk digunakan pada preservasi soket. Didalam mengatasi keradangan peran NF-κβ sangat berarti, sebab penurunan NF-κβ akan mengurangi terjadinya inflamasi.* ***Tujuan:*** *Penelitian ini bertujuan untuk menguji apakah induksi kombinasi lidah buaya dan XCB ke soket pencabutan gigi dapat mengurangi peradangan dengan mengurangi ekspresi NF - κβ , osteoklas dan osteoblast.* ***Metode:*** *Empat puluh delapan ekor Cavia cabaya yang terdiri dari 8 kelompok, tiap kelompok 6 ekor, kelompok pengisian PEG (kontrol), kelompok pengisian XCB, kelompok pengisian aloe vera dan kelompok pengisian kombinasi aloe vera dan XCB, kelompok ini terdiri dari kelompok 7 dan 30 hari, kemudian diperiksa dengan imunohistokimia ekspresi NF-κβ dan pemeriksaan histologi untuk osteoblas dan osteoklas.* ***Hasil:*** *Kelompok yang diisi kombinasi Alo vera dan xenograft concelous bovine pada soket pencabutan gigi, menunjukan nilai tertinggi dalam pertumbuhan osteoblas dan penurunan pada ekspresi NF-κβ dan osteoklas.* ***Simpulan:*** *Induksi kombinasi Aloe vera*

dan xenograft concelous bovine pada preservasi soket pencabutan gigi dapat menurunkan ekspresi NF-κB dan osteoklas, menurunkan resiko resorpsi tulang alveolar dan meningkatkan osteoblas.

Kata kunci: *Aloe vera, NF-κβ, soket pencabutan gigi, xenograft*

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INTRODUCTION

Original tooth extraction may cause trauma, triggering inflammation and later stimulating the growth of osteoclasts and the resorption of alveolarar bone. Thus, it is necessary to preserve the extraction sockets in order to reduce the risks of alveolar bone resorption. Alveolar bone resorption can also cause dentures making become unsuccessful. In general, the success of dentures actually depends on retention factor, stabilization factor, and convenience factor of denture use, which can be achieved by supporting anatomical condition related to prominent ridge. Therefore, the prevention of alveolar bone resorption should be conducted during tooth extraction by reserving the extraction socket.

In general surgery, especially oral surgery, and Periodontics, graft has been used to repair bone defects and augmentation.[1-6] The use of graft in long period, according to Pachene *et al*. *cit*. Lanza *et al*.,[7] is not stable even though the design and development of tissue engineering products have benefited the clinical use of various biodegradable polymers for many years. Thus, it takes an effort to tissue engineering for reserving tooth extraction socket by combining *Aloe vera* and graft materials in order to reduce alveolar bone resorption risks and get a prominent ridge.

Aloe vera is considered to have both a function as a biogenic stimulator and hormones stimulating wound healing activity. *Aloe vera* liquid even can prevent scartissue incision, and when the gel is used after surgery, the incision will be healed faster.[8] Similarly, some studies also suggest that there is anthraquinones components in *Aloe vera,* namely *Aloin, Aloe emodin,* and barbaloin, which have important role as anti-inflammatory, anti-bacteria, and anti-virus so that *Aloe vera* can reduce inflammation caused by extraction trauma and also induce extraction wound healing process.[9-14]

To reduce inflammation risk, the physiological role of nuclear factor kappa β (NF-κβ) is very meaningful in immune system. For instance, nuclear factor kappa B can control transcription, cytokines, antimicrobial effector as well as genes that regulate cellular differentiation, life survival and cell proliferation, consequently, it then can regulate various aspects of innate and adaptive immune responses.[15] Lorenzo *et al*.,[16] moreover, said that tumor necrosis factor α (TNF-α) and interlukin 1 (IL-1) can stimulate osteoclast formation. Interlukin-1 can stimulate activities of receptor activator of nuclear factor κβ ligand (RANKL) to induce osteoclastogenesis, and can also be considered as a strong resorption stimuli for prostaglandin synthesis in bones, whereas TNF-α is a potent stimulator of bone resorption. Therefore, the increasing of NF-κβ expression can cause inflammation triggered by the increasing of TNF-α cytokines. As a result, it takes an effort to reduce the inflammation caused by the tooth extraction trauma. Therefore, this research was aimed to determine whether the induction of the combination of *Aloe vera* and XCB into tooth extraction sockets can reduce inflammation by reducing NF-κβ expression, osteoclasts and osteoblasts.

MATERIALS AND METHODS

This research can be considered as an experimental study with a randomized post test control group design. Animals used in this research were male *Cavia cobaya* (guinea pig) with body weight of 300-350 g at the age of 3-3.5 months. In addition, materials used in this research were *Aloe vera* extracts, sterile distilled water, XCB from Dr. Soetomo Hospital tissue bank, absolute alcohol, 70% alcohol, anti NF-κβ p65 monoclonal antibodies (Santa Cruz), reagents for immunostaining kit (Biocare) used in immunohistochemical examination, and reagents for hematoxilen-eosin staining (HE). Thus, indirect immunohistochemical examination could be conducted by using primary antibodies and also secondary antibodies. Furthermore, tools used in this research were a set of tools for immunohistochemical examination techniques, a set of tools for making preparations, micropipette, tip (yellow, white, blue), light microscopy, object glass, and cover glass.

Those experimental animals had been taken care in Biochemistry Laboratory of Faculty of Medicine Universitas Airlangga. Tissue preparations used in this research was conducted in Anatomical Pathology of Dr. Soetomo Hospital. *Aloe vera* extracts was prepared in Physical Chemistry Laboratory of Faculty of Pharmacy, Universitas Airlangga, while the making of freeze dried *Aloe vera* was conducted in Faculty of Biology Laboratory of Science and Technology, Universitas Airlangga. The processes of immunohistochemical staining and HE staining for immunohistochemical examination were conducted in Biochemistry and Biomolecular Engineering Laboratory of Faculty of Medicine, Universitas Brawijaya.

The procedures of this research, furthermore, consisted of several steps. Forty-eight Cavia cabaya animals were divided into eight groups, each of which consisted of six animals. Before their lower right incisors were extracted by using a special pliers (needle holder), they had intravenously been anaesthetized with ketamine 0.2 cc or 300 g BM.[17] Then, their extraction sockets were induced with either PEG, PEG + XCB, *Aloe vera* + PEG, and a combination of *Aloe vera* + XCB + PEG as much as 0.1 cc of the appropriate volume of the extraction socket, and then stitched.

The sockets of those animals in group 1 and group 2 were induced with polyethylene glycol (PEG), and then examined on day 7 and day 30 after the treatment. Meanwhile, the sockets of those animals in group 3 and group 4 were induced with XCB + PEG, and then examined on day 7th and day 30th after the treatment. The sockets of those animals in group 5 and Group 6 were induced with *Aloe vera* and PEG, and then examined on day 7th and day 30th after the treatment. And, the sockets of those animals in Group 7 and Group 8 were induced with the mixture of *Aloe vera* 500 mg + XCB 500 mg + PEG 24 g, and then examined on day 7th and day 30th after the treatment.[18]

After 7 days and 30 days, those animals were killed, and their jaws were cut off. The preparation consisted of hard materials was decalcified first with 2% nitric acid for approximately 14 days, and then paraffin block preparations were made. Those paraffin blocks were cut with a rotary microtome with a thickness of about 4 microns, and placed on a glass object. Deparaffinization was conducted by dissolving in xylol for 2 x 3 minutes. The rest xylol was washed with absolute alcohol 99%, 95%, 90%, 80%, and 70% for 2 x 1 minutes. Next, the residual alcohol was washed with running water. NF-κβ expression, osteoblasts and osteoclasts were examined by using a light microscope after immunohistochemical staining and HE staining were then conducted.

For the purposes of calculating, moreover, the code of the already coded slides was closed, and a new number was given randomly for each slide. Each slide was then observed with 1000x magnification and 10 fields of view. Afterwards, the results of the observation were written on the worksheet and calculated for their average value per field of view. The results of the calculation were then tabulated and tested with Kolmogorov-Smirnov test and Analysis of variants (ANOVA) test. Finally, to compare the results of the groups, Tuckey HSD Multiple Comparison test was conducted.

RESULTS

The mean (M) and standard deviations (SD) of NF-κβ expression, osteoblasts, and osteoclasts in each treatment groups 7 days and 30 days after the treatment can be seen in Figure 1. Based on Figure 1, it can be said that on day 7th and day 30th , NF-κβ expression was decreased in either group control or all treatment groups. But, the biggest decreasing was in the group induced with the combination of *Aloe vera* and XCB. On day 7th , the number of osteoblasts was increased in all of the groups. The biggest increasing was in the group induced with the combination of *Aloe vera* and XCB. On day 30th , the number of osteoblasts in group control and that in the group induced with XCB were significantly increased, while that in the group induced with *Aloe vera* was slightly decreased. The highest number of osteoblasts was found in the group induced with the combination of *Aloe vera* and XCB. On the other hand, the number of osteoclasts was decreased in all of the groups. But, the smallest decreasing was in the group induced with the combination of *Aloe vera* and XCB. Then NF-κβ expression can be seen in Figure 2.

The results of ANOVA test on NF-κβ expressions on day 7th and day 30th after the examination can be seen in Table 1. The results of ANOVA test on NF-κβ expression on day 7 and day 30 after the examination show that there was significant difference between all of the treatment groups induced with either XCB, *Aloe vera,* or the combination of *Aloe vera* and XCB and group control with $p = 0.001 < 0.05$. After ANOVA test, Tukey HSD Multiple Comparison test was conducted with the following results as seen in Table 2.

In Table 2, it is known that there were significant differences among the treatment groups, especially with group control. However, there was no significant difference

Table 1. NF-κβ expressions on day 7 and day 30 after the examination

Variation	Df	F	P
Among the groups	7	148.570	0.001
Within the groups	40		
Total	47		

Note: df = degrees of freedom; F = Frequency; p = probability

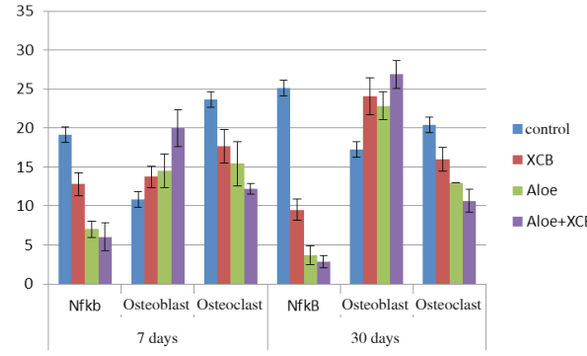

Figure 1. The graphs of NF-κβ expression, osteoblasts, and osteoclasts in group control, group induced with XCB, group Induced with *Aloe vera*, group induced with *Aloe vera* + XCB on day 7 and day 30 after the examination.

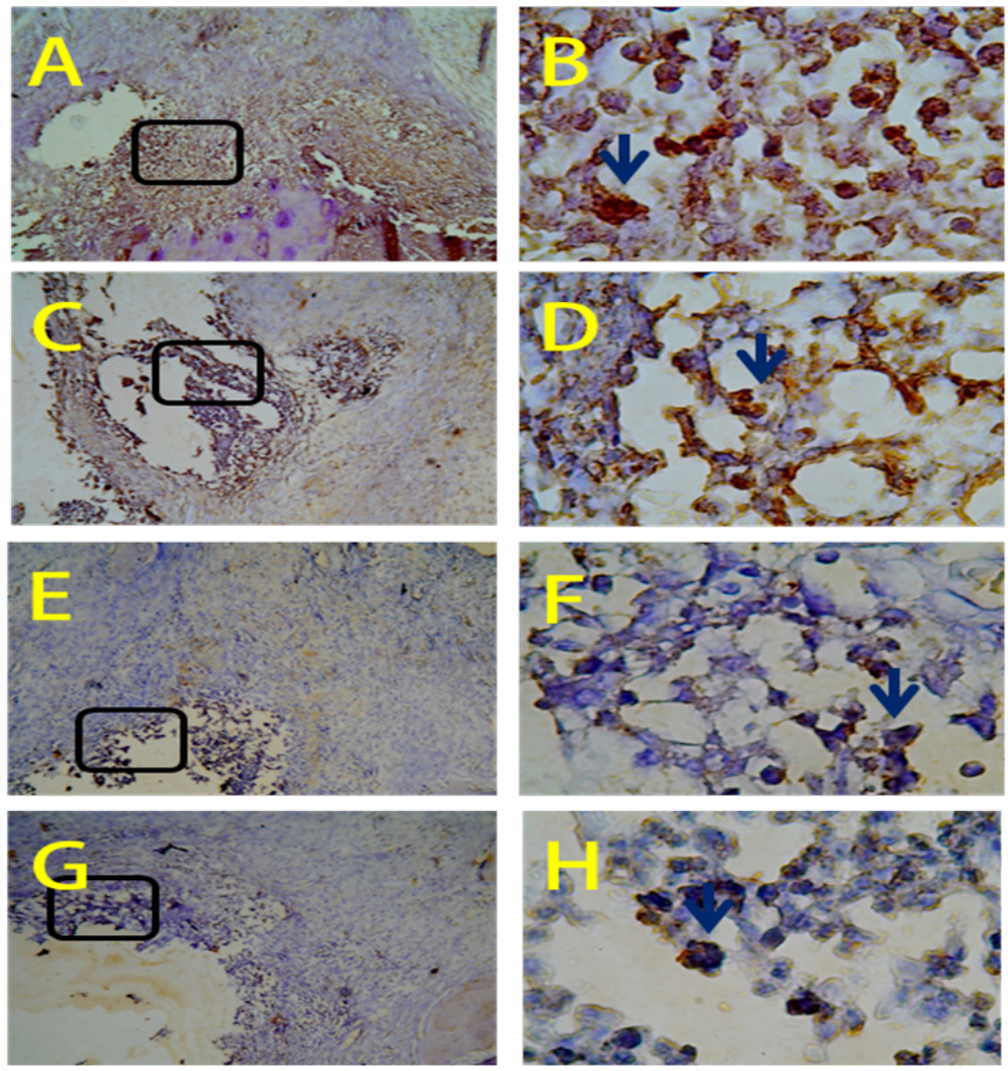

Figure 2. The pictures of NF-κβ expression after immunohistochemical staining.

Note:
A+B : NF-κβ expression in group control after immunohistochemical examination on day 30;
C+ D : NF-κβ expression in the group induced with XCB after immunohistochemical examination on day 30;
E + F : NF-κβ expression in the group induced with *Aloe vera* after immunohistochemical examination on day 30;
G + H : NF-κβ expression in the group induced with the combination of *Aloe vera* + XCB after immunohistochemical examination on day 30
Blue arrow points to NF-κβ expression

between group XCB on day 30 and group *Aloe vera* on day 7 with p=0.141>0.05. Similarly, there was no significant difference between the Group *Aloe vera* on day 7 and the group *Aloe vera* and XCB on day 7 with p = 0.954>0.05. There was also no significant difference between Group *Aloe vera* on day 30 and Group *Aloe vera* + XCB on day 7 with p=0.203>0.05. Finally, it is also known that there was also no significant difference between group *Aloe vera* on day 30 and group *Aloe vera* + XCB on day 30 with with p=0.983>0.05.

DISCUSSION

This study was aimed to measure NF-κβ expression since it has a main physiological role in immune system as well as in inflammation process.[15] Tooth extraction always causes mechanical trauma triggering inflammation. When exposed to trauma, incompetent macrophage cells and mast cells will increase TNF-α. Most of the immune response is actually regulated by NF-κβ in sitosol and bound into IκB. Consequently, phosphorylation of IκB will occur and then trigger proteasome degradation and NF-κβ releasing to

Tabel 2. The results of Multiple Comparison test on BMP2 expression

Groups	Group control on day 7	Group control on day 30	Group XCB on day 7	Group XCB on day 30	Group *Aloe vera* on day 7	Group *Aloe vera* on day 30	Group *Aloe vera*+XCB on day 7	Group *Aloe vera*+XCB on day 30
Group control on day 7	--	S	S	S	S	S	S	S
Group control on day 30	NS	--	NS	S	S	S	S	S
Group XCB on day 7	S	S	--	NS	NS	S	S	S
Group XCB on day 30	S	S	NS	--	NS	NS	S	S
Group *Aloe vera* on day 7	S	S	NS	NS	--	NS	S	S
Group *Aloe vera* on day 30	S	S	S	NS	NS	--	NS	NS
Group *Aloe vera*+XCB on day 7	S	S	S	S	S	NS	--	NS
Group *Aloe vera*+XCB on day 30	S	S	S	S	S	S	NS	--

Note: S : significant; NS: no significant

translocate to nucleus.[19, 20] Inflammation makes osteoclasts increased, and then proinflammatory cytokines, TNF-α, will also be increased. Consequently, the receptor activator of nuclear factor κβ ligand (RANKL) and the receptor activator of nuclear κβ *(*RANK) will then be increased. RANK and RANKL can be considered as a mediator of osteoclast differentiation signals. Therefore, when osteoclasts increases, bone resorption will occur.[21, 22]

Based on the results of the examination, it is known that NF-κβ expression was decreased in all of the groups. But, the lowest one was in the group induced with the combination of *Aloe vera* and XCB. The results indicate that the decreasing of NF-κβ can reduce inflammation risks. Similarly, a research conducted by Hayden *et al*.[15] also showed that NF-κβ can be considered as an important mediator of local and systemic inflammation, and also can affect on TNF-α as proinflammatory cytokine. Therefore, NF-κβ is essential for the propagation and elaboration of cytokine responses.[15]

In Figure 1, furthermore, it is known that the number of osteoblasts was increased in all of the groups. However, the highest occurred in the group induced with the combination of *Aloe vera* and XCB. It is also known that the number of osteoclasts was decreased. The lowest one was in the group induced with the combination of *Aloe vera* and XCB. Thus, it can indicate that the decreasing of NF-κβ can lead to the decreasing of osteoclast growth. Thereby, bone resorption will be decreased, and the number of osteoblasts will be increased.

The occurrence of bone resorption is actually affected by osteoclast activating factor (OAF).[23-25] NF-κβ (nuclear factor κβ) as a transcription factor involves in various activities, including the regulation of the immune response, the maturation of immune cells, the development of secondary lymphoid organs, and osteoclast genesis.[26] Finally, the study suggested that the induction of the combination of *Aloe vera* and xenograft concelous bovine into tooth extraction sockets could reduce NF-κβ expression. As a result, osteoclasts will decreased, while osteoblasts will increased. Thus, alveolar bone growth then will be improved.

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