Original Article

EFFECTIVENESS OF VIRGIN COCONUT OIL IN PERIODONTAL TREATMENT VIA ANALYSIS OF IL-1β AND IL-6

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Abstract

Background: Virgin Coconut Oil (VCO) is a vegetable oil extracted from coconut fleshcontains unsaturated fatty acids in the form of oleic acid and linoleic acid and flavonoids which function as anti-inflammatory. Periodontitis is an inflammatory disease of the periodontal tissues with a high prevalence worldwide. The main etiology of plaque periodontitis is biofilm containing colonies of pathogenic microorganisms. The occurrence of inflammation in the periodontal tissue stimulates the release of inflammatory mediators such as Interleukin-1β (IL-1β) and Interleukin-6 (IL-6). Treatment of periodontitis started from initial therapy, and usually accompanied by additional therapy such as local drug administration. VCO can be used as an alternative anti-inflammatory. **Objectives** is to determine the effect of VCO as a local drug delivery in periodontal treatment through analysis of IL-1β and IL-6. **Methods:** This is an experimental laboratory with a posttest-only control group design. VCO is made from grated fresh coconut and then mixed with NaCMC to get the consistency of the gel. The subjects of this study were 24 male Wistar rats that were induced by periodontitis by injecting Porphyromonas Gingivalis (P. Gingivalis) into the gingival sulcus. Wistar rats were then divided into 3 groups. On the 7th and 14th day, the rats were sacrificed and jaw samples were taken to determine the amount of IL-1β and IL-6 in the regenerative process of the periodontal tissue. **Results:** The amount of IL-1β and IL-6 decreased in all treatment groups, but not as big as the decrease in the VCO group. **Conclusion:** VCO can reduce the amount of IL-1β and IL-6 in the treatment of wistar rats periodontal tissue induced by P. gingivalis.

Keywords: Virgin coconut oil, Periodontitis, IL-1β, IL-6, Periodontal treatment.

Introduction

Periodontal disease is an infection-driven inflammatory disease due to the accumulation of bacteria and debris between the gum line and tooth that affects the supporting tissues and known as a periodontitis.(1,2,3) Periodontitis become one of the main causes of tooth loss which ultimately affects speech, masticatory, and aesthetic function if not treated immediately.(4) Severe periodontal diseases are estimated to occur around 14% of the world's adult population representing more than one billion cases worldwide.(5)

The main cause of periodontal disease is bacterial plaque, especially P. Gingivalis, which is found in deep pockets.(6) The interaction between microbial infection and host response initiates inflammation which results immune response will release cytokines, including TNF-α, IL-1β, IL-6, IL-8 and superoxide anion in human monocyte cell culture.(7) These cytokines act as chemical messengers or intermediaries in communication between cells.(8) Proinflammatory cytokines, such as IL-1β and IL-6 which are proteolytic destroys tissue enzymes, forms pro-inflammatory mediators that play a role in the pathogenesis of various chronic inflammations, immune reactions and periodontal tissue damage, and has strong bone resorption activity and inhibits bone formation.(9)

In patients with periodontal disease results in an increase in the number of peripheral leukocytes, cytokine levels and acute protein phase, but not always significant. Severity and extent disease will exacerbate the increase in leukocytes but the number of leukocytes can reduced with periodontal therapy.(10,11,12)

Periodontal treatment aims to eliminate inflammation of the gingiva, inhibit the course of the disease, prevent further bone damage, and regenerate the supporting tissue affected by periodontitis.(13,14) Initial treatment of periodontitis can be in the form of scaling and root planing plus local drug delivery to improve treatment results, but continuous administration of antibiotics can increase bacterial resistance, allergies and opportunistic infections occur.(15,16)

VCO is pure coconut oil which is processed through a simple process from fresh old coconut meat without the addition of synthetic chemicals.(17, 18, 19) Coconut fruit (Cocos nucifera) as a raw material for VCO is abundantly available in Indonesia. Indonesia is the main coconut producing country in the world based on 2014-2018 Food and Agriculture Organization (FAO) data, with a contribution of 29.69% of total world coconut production with an average production of 18.04 million tonnes of coconut. (20)

VCO is widely used and utilized in the health sector as an antimicrobial, antiviral, antifungal and antioxidant. (17) VCO contains the main active component, namely lauric acid which is a medium chain triglyceride fatty acid, and also VCO contains unsaturated fatty acids in the form of oleic acid and linoleic acid and flavonoids which function as anti-inflammatory. Other VCO components such as polyphenols and tocopherols function as antioxidants.(17,21,22)

Based on recent studies, VCO has been used to reduce plaque-induced gingivitis. Increasing public awareness in utilizing natural ingredients as medicines has led to an increasingly rapid increase in the production of medicinal plants. This is because drugs derived from natural ingredients has relatively small side effects. (23)

VCO contains saturated fatty acids including Medium Chain Fatty Acid (MCFA) and Medium Chain Triglycerides (MCT). MCFA is lauric acid which has antiprotozoal, antiviral, andantibacterial properties. MCT in VCO can increase immunity against disease and accelerate healing from illness, and can prevent obesity. (23,24,25) There are many studies that use VCO as a treatment for various diseases. In 2017, a study found the effect of VCO on A.Actinomycetemcomitans and P.Gingivalis which are the main bacteria that cause periodontal disease.(24)

The purpose of this study was to determine the effect of VCO as a adjunctive antimicrobial treatment after management of periodontal disease via analysis IL-1β and IL-6 in wistar rats induced P. gingivalis

Methods

This experimental research was conducted in the laboratory animal facility faculty of pharmacy, University Muslim Indonesia. All protocols were approved by the ethical committee faculty of dentistry, Hasanuddin University No. 0078/PL.09/KEPK FKG-RSGM UNHAS/2022. This study used VCO made from coconut which is processed using the cold pressed method, without heating or chemical additions and mixed with NaCMC to produce VCO in the form of a gel to facilitate application during research treatment. Twenty-four male Wistar rats were induced p. gingivalis into the gingival sulcus of the lower anterior teeth and also inserting a silk-ligature in the same places. After 5 days

periodontitis will form on the gingiva of Wistar rats which is characterized by a change in the color of the gingiva and an increase in pocket depth.

Wistar rats were divided into 3 groups, the treatment group receiving initial SRP therapyand VCO gel, the positive control group receiving SRP and metronidazole gel, and the negative control group only receiving SRP. Furthermore, euthanasia was carried out on the 7th and 14th days after treatment, with 4 Wistar rats from each treatment group. Then the Wistar rats were fixedon the workbench for decapitation and separation of the cranium from mandible. Each jaw piece was fixed in 10% buffered formalin, which would then be made into HPA preparations. The expression of IL-1 β and Il-6 was then carried out by immunohistochemical staining using IL-1 β and Il-6 antibodies and viewed under light microscope with 1000x magnification for further calculation of the number of expressions.

Results

The data obtained were tested for normality usingthe Shapiro-Wilk test, then the data was then tested for homogeneity using Levene's test.To analyze the differences between the research groups, one-way ANOVA was used. The results of the analysis were declared significant or there was the difference if the p-value <0.05.

Based on table 1, the immunohistochemical observation the amount of IL-1β in male Wistar rats with periodontitis. In the SRP combination treatment group with VCO gel, the positive control group in the combination of SRP with metronidazole gel, and the negative control group in the form of SRP treatment alone, showed a significance value of p> 0.05 which means that a decrease in the amount of IL-1β on the 7th and 14th days did not significant.

In Figure 1 the bar chart shows the amount of IL-1β in the three groups, on day 7 and 14. On day 14, it can be seen that the amount of IL-1β in all groups has decreased. Although the decrease in all groups did not show significant results, the largest decrease in the amount of IL-1β was found in the treatment group.

On table 2 it can be seen that the three groups, both the treatment group, positive control and negative control experienced a decrease in the amount of IL-6 between days 7 and 14 even though the significance value was p> 0 ,05.

Based on the bar chart (Figure 4) it can be seen that the average decrease in the amount of IL-6 in the three groups. Although the decrease in all groups did not show significant results where the value of p> 0.05, the largest decrease in the amount of IL-6 was found in the treatment group.

Discussion

Over the years, virgin coconut oil has been used by many people around the world, the beneficial effects of this oil, especially related to its role in nutrition, health and national development. Several properties of this product have been reported in various studies: antibacterial, antiviral, and antimycotic are the main ones. In this study, treatment with VCO gel was carried out on wistar rats induced by periodontitis to see the expressions of IL-1β and IL-6, cytokines that have a role in periodontal disease.(25,26)

Periodontitisis is an inflammatory disease affecting the tooth supporting apparatus caused by interaction of certain microorganisms, host defense mechanism, environment and genetic factors. The biological response in the body is mediated by a complex network of cytokines that play an important role in initiation, development, and modulation hosts of periodontal disease including proinflammatory cytokines such as IL-lβ, TNF-α, MMPs and IL-6 which are proteolytic destroying enzymes. Local formation of proinflammatory mediators can enter the circulation and affect the systemic.(27,28)

IL-1β plays a role in activating the innate immune response by regulating antigen-presenting cell (APC) development (dendritic cells), stimulate IL-6 secretion by macrophages (turn on activates B cells), and has been shown to increase stimulation antigen-mediated from T cells. IL-1β concentration increased at the site affected by gingivitis and periodontitis, and tissue levels of IL-1β correlates with the severity of clinical periodontal disease, IL-

1β upregulates matrix metalloproteinase and decreases regulation of tissue inhibitors of metalloproteinase production and also is effective stimulator of bone resorption, cooperating between PGE2 and IL-1β is an important mediator in periodontal inflammation and bone destruction and is involved in the regulation of tissue response. Animal studies trials show that IL-1β fights pain and alveolar bone resorption. It is clear from the many studies that have been masking of these cytokines that IL-1β plays a fundamental role in pathogenesis of periodontal disease. It is known that IL-1β stimulates bone loss and has an inhibitory effect bone formation. (8, 29,30,31)

IL-6 is cytokines produced by T cells, B cells, monocytes/macrophages, endothelial cells, gingival fibroblasts, osteoblasts and, periodontal ligament cells. It has properties multifunctional and secreted in response to bacterial LPS or IL-1β and TNF-α stimulus. IL-6 in promoting inflammation, may also be involved in the regulation of tissue damage. IL-6 induces the production of MMP inhibitors, suppresses the expression of IL-1β and TNF-α, and induce IL-1 receptor antagonists. (32) Another positive point in support liberation of tissue damage is that IL-6 can stimulate fibroblasts to produce collagen and glycosaminoglycans. IL-6 plays a role in B cell differentiation, T cell proliferation, and acute phase protein expression.(33,34) Besides therefore, IL-6 in synergism with TNF-α is able to induce differentiation osteoclast progenitors directly or stimulate stromal cells to generate RANKL. These cytokines exert important effects in pathogenesis of periodontitis, especially in bone metabolism. During development of chronic periodontitis, several biologic actions may be mediated by IL-6, including hematopoiesis, induction of angiogenesis, activation of immunocytes, and osteoclast differentiation. Presence of IL-6 in serum, crevicular fluid gingiva, and saliva show altered IL-6 production in patients with chronic periodontitis.(35)

Treatment for periodontitis generally falls into two categories, first procedure to halt the progression of the disease is the initial phase therapy consisting of scaling, root planing. improving oral hygiene and second the procedure to regenerate the structures destroyed maintenance or supportive periodontal therapy after active treatment is essential to achieve a successful outcome.(36)

This study showed that there was a decrease in the number of IL-1β and IL-6 cells in inflamed periodontal tissue due to the induction of P.gingivalis bacteria in Wistar rats given VCO gel. Both positive and negative control groups also experienced a decrease number of IL-1β and IL-6 cells, but not as much as a decrease in the number of cells in the VCO gel. This proves that VCO gel is better at helping suppress the inflammatory process, so that it can accelerate tissue regeneration.

The lauric acid in VCO is known to be very high, this is due to the cold pressed method of making VCO, without heating and adding chemicals so that nutrient levels are maintained. This lauric acid content becomes an antibacterial substance that can kill several microorganisms with cell membranes containing fatty acids, such as Gram-positive and Gram-negative bacteria . Study showed that VCO could inhibit the growth of Streptococcus mutans by degrading it membrane proteins. This research is also supported by other studies which shows that fatty acid scanning kills bacteria through the cell membrane disintegration.(37)

A study by Hasanuddin et al, showed a significant increase of TNF-α and TGF-β in amount after wistar rats induced by periodontitis and treated with VCO gel. Vysakh et al. (2014) demonstrated that oral supplementation polyphenols isolated from VCO inhibit expression proinflammatory cells such as COX2, iNOS, TNF- and IL-6 in mice with arthritis. Another study showed that virgin coconut oil is proven to speed up wound healing time and has the highest percentage in the healing effects of chemical burns in Rattus Novergicus. The study was conducted with excision wound in sprague dawley which proves that VCO can increase fibroblasts proliferation so that the density of collagen fibers increases, and helps quicken tissue regeneration process.(8,37,38)

Result

The histopatological results in all experimental group defined a number of IL-1β and IL-6 cells in the mandibular gingival sulcus wistar rats induced P. Gingivalis.

Conclusion

As a conclusion, this study showed there was decrease of IL-1β and IL-6 due to inflammatory process in inflamed periodontal tissue of Wistar rats given VCO gel.

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TABLES AND FIGURES

Table 1. The mean amount of IL-1β on the 7th and 14th day of each treatment group

Group	Sample	7th day (Mean±SD)	14th day (Mean±SD)	P
Treatment (SRP + VCO Gel)	4	4,00±1,000	1,33±0,577	0,226
Positive control (SRP + Metrodindazole Gel)	4	5,33±1,528	3,00±1,000	0,343
Negative Control (SRP)	4	7,33±1,528	7,00±2,000	1,000

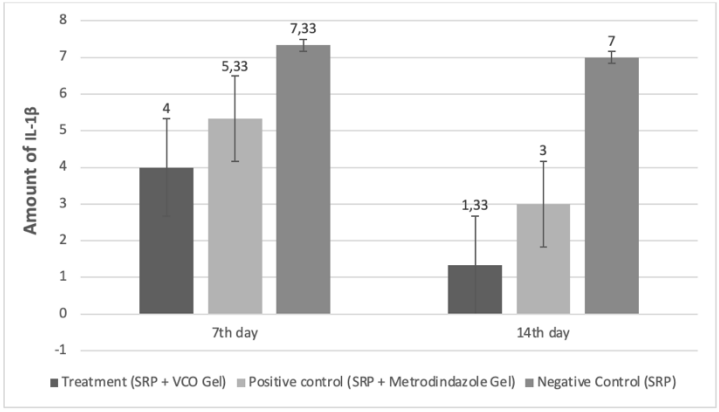

Figure 1. Bar chart of the amount of IL-1β in the three groups on the 7th and 14th days

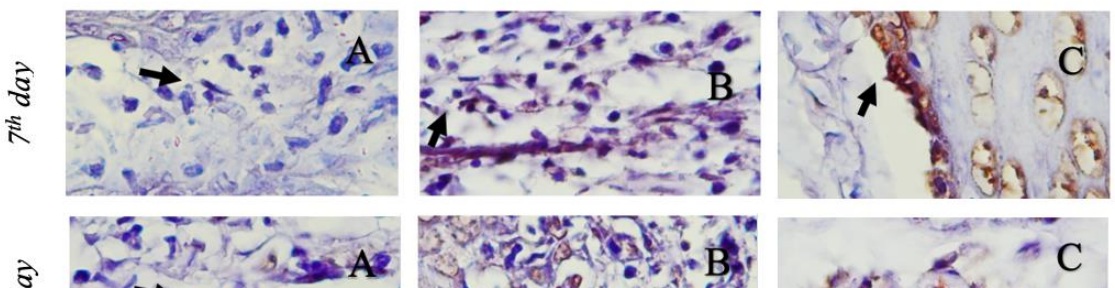

Figure 2. Amount of IL-1β on day 7 and 14 (A) SRP + VCO Gel combination treatment group, (B) Positive control group SRP + Metronidazole gel combination, (C) SRP negative control group

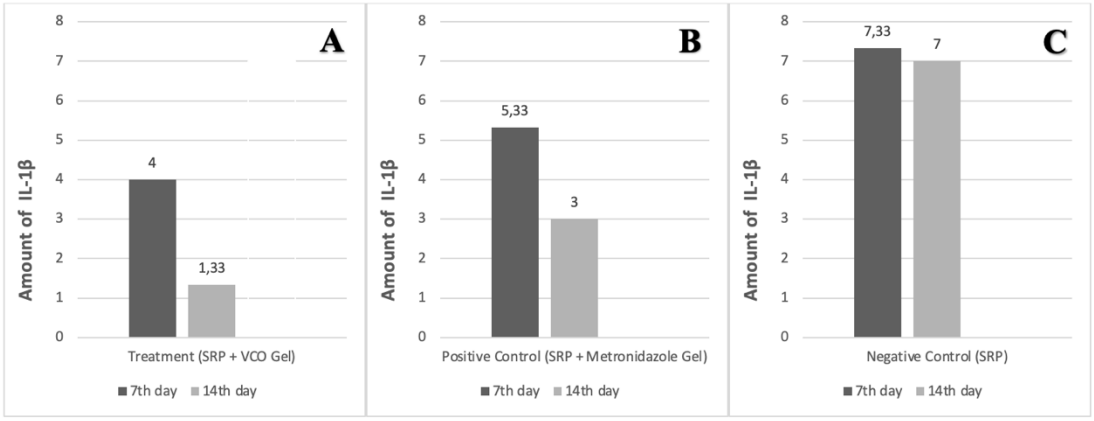

Figure 3. Bar chart of decreased IL-1β on the 7th and 14th day in the three groups, where the treatment group (A) experienced a greater decrease compared to the positive control group (B) and the negative control group (C)

. **Table 2.** The mean amount of IL-6 on the 7th and 14th day of each treatment group

Group	Sample	7th day (Mean±SD)	14th day (Mean±SD)	P
Treatment (SRP + VCO Gel)	4	3,00±1,000	1,67±1,155	0,664
Positive control (SRP + Metrodindazole Gel)	4	4,00±1,000	3,00±1,000	0,858
Negative Control (SRP)	4	7,33±1,528	5,67±0,577	0,452

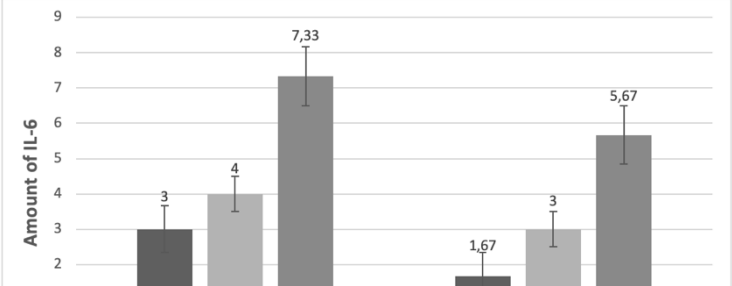

Figure 4. Bar chart of the amount of IL-6 in the three groups on the 7th and 14th days

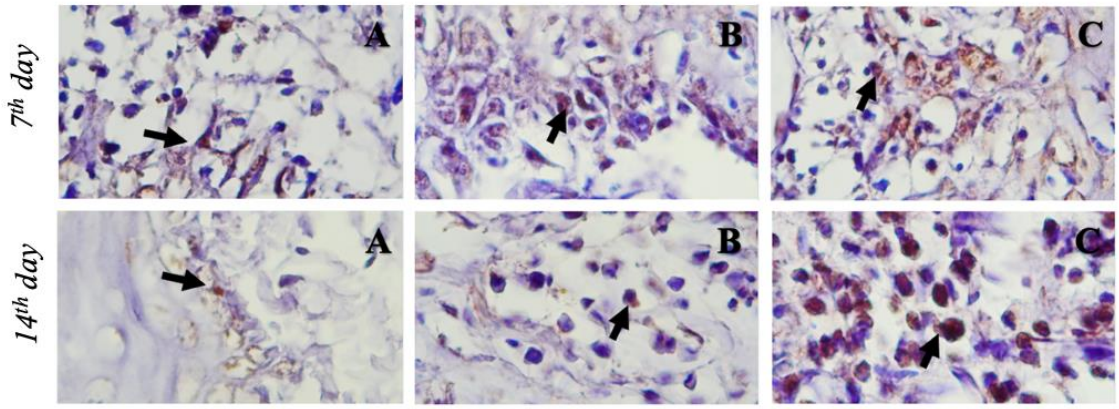

Figure 5. Amount of IL-6 on day 7 and 14 (A) SRP + VCO Gel combination treatment group, (B) Positive control group SRP + Metronidazole gel combination, (C) Negative control group (SRP)

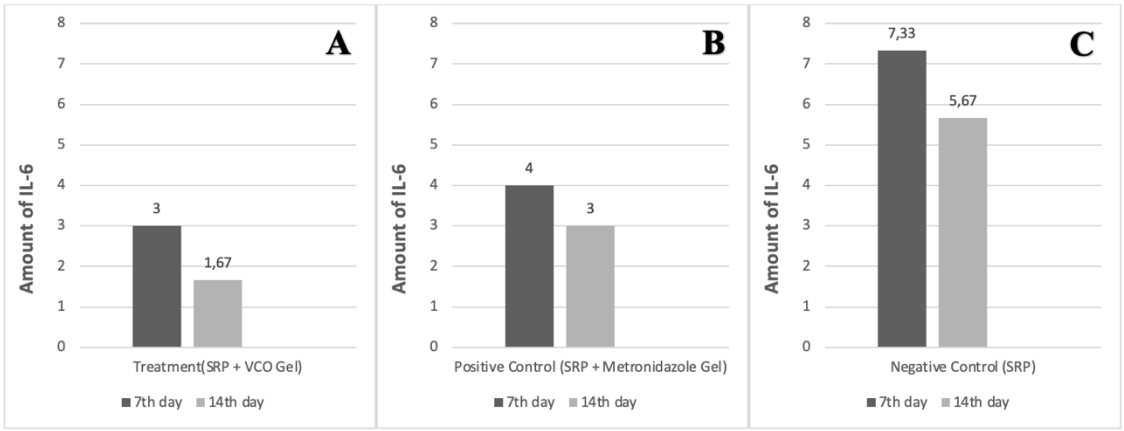

Figure 6. Bar chart of decreased IL-6 on the 7th and 14th day in the three groups, where the treatment group (A) experienced a greater decrease compared to the positive control group (B) and the negative control group (C)