

Propolis 10%-Gel as a Topical Drug Candidate on Gingivitis

Suryono¹ Nurhidayati Saputri Hasmy² Tia Lovita Pertiwi³ Benni Benjamin⁴
& Ade Ismail A.K⁵

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Propolis is a compound present in honey produced by bees and used for the treatment of wounds as it has anti-bacterial and anti-inflammatory effects. The use of propolis in the field of dentistry is still in the research process. The purpose of this study was to determine the effect of gel contains propolis 10% on healing of Gingivitis. Fifty four male Sprague Dawley rats are used as a model of gingivitis. Gingivitis rat models were randomly divided into three groups. The first group as positive control group treated with aloe vera gel, the second group as negative control group treated with CMC-NA gel, and the third group as treated with propolis 10% gel. Gel application in each group carried out two times a day on days 1,2,3,5,6, and 7. Gingival tissue samples be taken after 24 hours of treatment every day in each group for histological preparats. Hematoxyline Eosin (HE) staining was done to examine neutrophil cells, fibroblasts, and angiogenesis. Data were analyzed using the parametric statistical tests Two-way Anova and LSD (Least Significant Difference). The results showed that gel propolis 10% have the same level of effectiveness with aloe vera gel in reducing significantly the number of inflammatory cells of neutrophils on day 2 and 3 compared with negative controls ($P < 0.05$), increases the number of fibroblasts cells on day 5 and increases the number of new blood vessels significantly on day 5, 6, 7 as compared to the negative control. Propolis 10%-gel have the same effectiveness with aloe vera gel in order to increase the number of fibroblast cells on days 5 and 6. Propolis 10%-gel effective as a topical drug in healing process of gingivitis through the reduction the number of inflammatory cells of neutrophils, increases the number of fibroblasts cells and the formation of new blood vessels (angiogenesis).

Keywords: Propolis, Gingivitis, Neutrophil, Fibroblas, Angiogenesis

¹ Departement of Periodontology, Faculty of Dentistry, Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia & Faculty of Dentistry, Universitas Islam Sultan Agung, Semarang, Jawa Tengah, Indonesia.

² Faculty of Dentistry, Universitas Islam Sultan Agung, Semarang, Jawa Tengah, Indonesia

³ Faculty of Dentistry, Universitas Islam Sultan Agung, Semarang, Jawa Tengah, Indonesia

⁴ Faculty of Dentistry, Universitas Islam Sultan Agung, Semarang, Jawa Tengah, Indonesia

⁵ Faculty of Dentistry, Universitas Islam Sultan Agung, Semarang, Jawa Tengah, Indonesia

1. Introduction

Gingivitis is an inflammatory reaction which occurs in gingiva preceded by the accumulation of plaque dominated by rods and gram-positive cocci such as *Streptococcus mitis*, *S. sanguis*, *A. naeslundii* and species *Eubacteria* (Quirynen et al., 2006) which is able to change the condition of healthy gingiva become stages gingivitis initial – early – established – advanced lesion (Fiorellini et al., 2006). Some of the studies that have done propolis can accelerate the healing of gingivitis according to what is conducted by Amaral (2006) (Amaral et al., 2006) to 4 patients diagnosed with gingivitis and chronic periodontitis given the application of Brazilian propolis 10%.

Propolis is a product of honeybee in the form of a complex mixture of beeswax, a little sugar, and gum of trees collected by honeybees (*Apis mellifera*) from the sap derived from a variety of trees, shrubs, and herbs (Susilo et al., 2009). The main components of propolis are flavonoids and phenolic acids, including CAPE containing up to 50% of the entire composition (Sabir, 2005). The study, whilst emphasizes the antimicrobial and anti-inflammatory properties on the propolis. The anti-inflammatory properties of propolis for their caffeic acid phenethyl ester (CAPE) in propolis. Flavonoids and cinnamic acid derivatives are considered as the main biologically active component in propolis (Gebara et al., 2002., Borrelli et al., 2002).

2. Materials and methods

2.1. Materials

Propolis is a product of honeybee in the form of a complex mixture of beeswax, a little sugar, and gum of trees collected by honeybees from the sap derived from a variety of trees, shrubs, and herbs. Extract Propolis was obtained from east java Indonesia, propolis extract obtained from bee that produced by Al Kautsar trademark which is the company specialized in sector of trigonasp propolis extract's product and distribution. Gel contain aloe vera extract that produced by kalbefarma and gel CMC-Na. Gel contains extracts of propolis has been created by a state university chemistry laboratory Semarang.

2.2. Experimental design

The process of inflammation that occurs in this study is triggered by ligation or silk ligature size 3,0 mm on anterior subgingival area of mandibular incisor (Prasetya et al., 2014) of the experimental animals Sprague-Dawley. The binding was used for 7 days as a retention tool for the plaque bacteria such as bacterie *Streptococcus mitis*, *S. sanguis*, *A. naeslundii* and species *Eubacterium* thus there will be accumulation of bacterial plaque, which in turn can facilitate the onset of gingivitis.

Fifty-four Sprague-Dawley male rats weighing 175-200 grams were used in this study. Rats were randomly divided into three groups. The first group, as a positive control group namely gel contain aloe vera extract, the second group as a negative control group namely gel CMC-Na and the third group as a treatment group namely gel propolis 10%. Applications of gel in each group carried out 2 times a day on day 1, 2, 3, 5, 6, 7 in a mouse model of gingivitis by means of ligation. Samples in the form of gingival taken after 24 hours of treatment each day and each group. Preparations given colors with HE to see neutrophil cells, fibroblasts and angiogenesis in the healing process of gingivitis.

2.3. Statistical analyses

Analyzed using statistic parametric test of Two-Way Anova with levels of confidence of 95% then continued with analyzing test of Post Hoc namely LSD (Least Significant Difference) to know the presence of PMN neutrofil cells totals, fibroblast cell and angiogenesis in the provision of gel propolis 10% , gel contain aloe vera extract and gel CMC-Na.

3. Results and Discussion

3.1 Average numbers of each group.

Fig 1 Effect of Gel-10% Propolis (blue),CMC-Na (red), and Aloe vera gel (green) on numbers of Neutrophil cells. The values are mean + standar deviation quadruplicate experiments

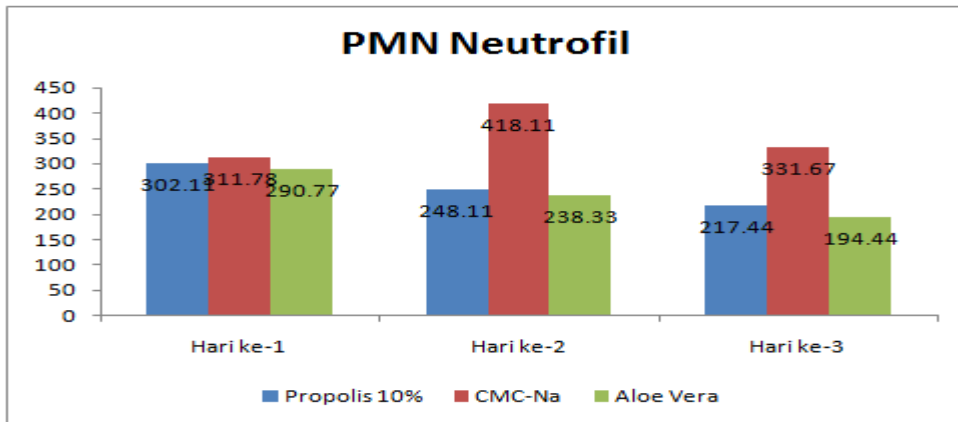


Fig 1I Effect of Gel-10% Propolis (blue),CMC-Na (red), and c (green) on numbers of angiogenesis. The values are mean + standar deviation quadruplicate experiments

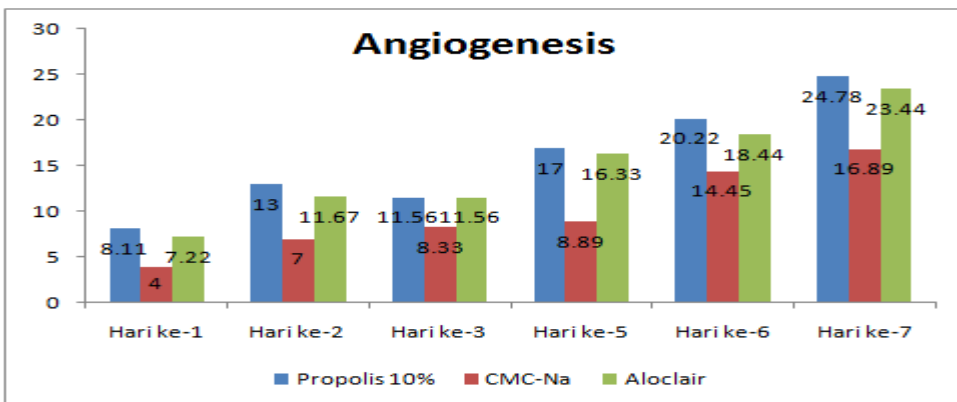
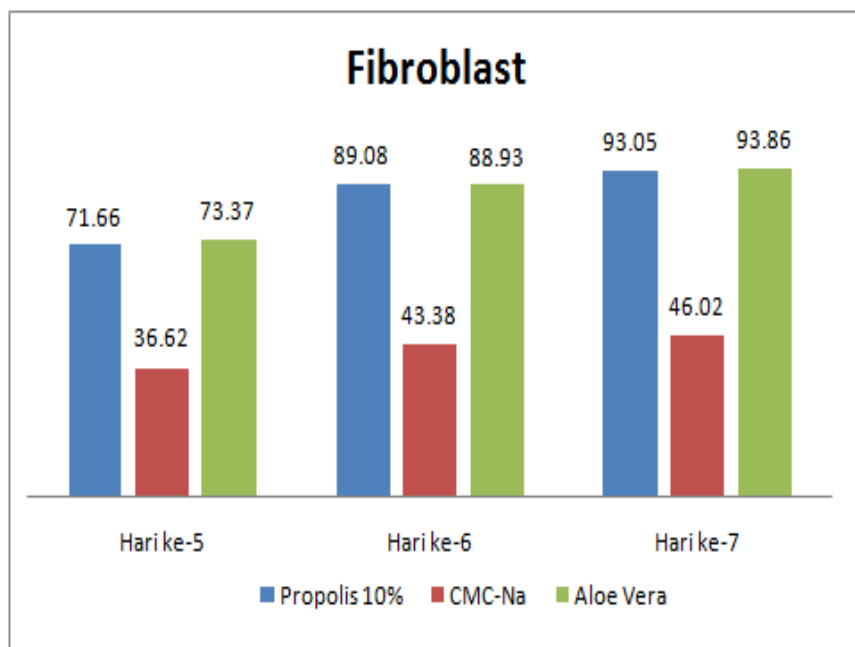


Fig 1II Effect of Gel-10% Propolis (blue),CMC-Na (red), and Aloe vera gel (green) on numbers of fibroblast cells. The values are mean + standar deviation quadruplicate experiments



3.2 Effect of propolis 10% in gingivitis healing

The results showed that the propolis gel 10% has the same effectiveness with aloe vera gel in reducing the number of inflammatory cells namely PMN neutrophils on day 2 and 3 with $P < 0.05$ compared with negative controls. This is because the main components of propolis are caffeic acid (3-acid, 4-dihydroxycinnamic) phenethyl ester (CAPE) and quercetin which is an active biological substance that the propolis has anti-inflammatory and antioxidant (Gebara et al., 2002., Rajo et al., 2013) and contains such as galangin, pinobanksin, pinocembrin and polyisoprenylated benzophenone acting as antibacterial (Suryono et al., 2003). CAPE is able to inhibit Nuclear Transcription Factor Kappa B (NF- κ B) and releases IL-1 β penetrated by NF- κ B and stimulant IL-2 which stimulates the proliferation of T cells itself, while quercetin could affect cyclooxygenase path. Both of these compounds are equally acting to deter lipooxygenase and cyclooxygenase (Sabire et al., 2005).

Lipoxygenase is the main enzyme neutrophil compound which later on will produce leukotrienes, while cyclooxygenase produces prostaglandins that will be the mediator in the inflammatory reaction. The existence of such barriers will affect the production of leukotrienes, decreased production of leukotrienes will affect the activity of neutrophils phagocytosis and calprotectin production (Riyanti et al., 2009) which will suppress the inflammatory process. Lipoxygenase pathway inhibition by quercetin and flavonoids and cyclooxygenase by CAPE that can suppress prostaglandin endoperoxidase synthase at high concentrations depending on the hydrophilicity and structure, will reduce the occurrence of vasodilation of blood vessels and blood flow will be reduced subsequently the migration of leukocytes (PMN) to the area of inflammation also decreased (Sabire et al., 2005., Gebara et al., 2002., Rajo et al., 2013) Thus it is also followed by an increased number of fibroblasts cells on days 5 and 6.

Flavonoids are capable of regulating cell function by stimulating the production of TGF- β which may increase chemotaxis and proliferation of fibroblasts in the wound area. The more fibroblasts at the injured area, the synthesis of collagen immediately started so as to accelerate the wound healing process (Taqwimetc, 2010).

Flavonoids and CAPE are the most potent natural components on propolis, and has been compared with COX inhibitor indomethacin (IM) and nordihydroguaiaretic LOX inhibitor acid (NDGA) and found to have the same effect as IM and NDGA (Rajooetc, 2013). Results also showed that propolis gel increases the number of new blood vessels significantly on days 5,6,7 compared to the negative control and had a mean greater than the positive control group on day 7. Propolis contains flavonoids that work to stimulate the production of growth factors and its works by increasing the production of growth factors in particular transforming growth factor β (TGF- β) and vascular endothelial growth factor (VEGF) which stimulate fibroblast proliferation and stimulates the formation of blood vessels (Damayanti etc, 2012., Taqwimetc, 2010). Saponins can stimulate angiogenesis through the increased activity of endothelial cell migration presentation and increases the secretion of vascular endothelial growth factor (VEGF), which became an important mediator in the formation of blood vessels. This content can improve the amount of angiogenesis higher than the positive control group.

4. Conclusion

This study concluded that the using propolis 10%-gel stimulate the healing process of gingivitis model on Sparague Dawley through a reduction in the number of PMN and increased the number of fibroblast and new blood vessels.

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