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RESEARCH ARTICLE

Effect of Mangosteen Peel Extract (*Garcinia Mangosteen*) to Fibroblast Number as Periodontal Disease Therapy

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ABSTRACT

Background: *In Indonesia, periodontitis is among the most prevalent periodontal disease. The dentist may administer extra adjunctive therapy as a gel to treat inflammation and the bacterium that causes periodontitis. The antibacterial and anti-inflammatory xanthones included in mangosteen peel extract gel speed up the inflammatory phase.* ***Objective:*** *This study aimed to determine the effectiveness of mangosteen peel extract gel in increasing the number of fibroblast cells in the healing process of periodontitis in Wistar rats (Rattus norvegicus).* ***Material and Method:*** *This research was an experimental laboratory design with a post-test-only design divided into four groups, 50% mangosteen peel extract gel group, 75% mangosteen peel extract gel group, metronidazole gel group, and metronidazole plus gel group.* ***Result:*** *The research data were analyzed using the One Way ANOVA test and showed a significant value difference with a p=0.000 (p<0.05). However, in the inter-group test with Bonferroni post hoc, it was found that there was no significant difference between the 75% mangosteen peel extract gel group and the metronidazole gel group with a value of p = 0.709 (p>0.05).* ***Conclusion:*** *This study concludes that mangosteen peel extract gel effectively increases fibroblast cells' number in the periodontitis healing process.*

Keywords: *Mangosteen peel extract gel, metronidazole gel, fibroblast.*

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Denta Jurnal Kedokteran Gigi, Februari 2023; Vol.17 No.1; Hal 1-7
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INTRODUCTION

Oral health is a significant concern in dentistry. Basic Health Research in 2018 showed that 57.6% of the Indonesian population had problems with teeth and mouth. The most common dental and oral diseases are dental caries and periodontal disease.[1,2]

The periodontal disease consists of gingivitis and periodontitis. Gingivitis is inflammation of the gingiva. Periodontitis is an inflammation due to bacterial stimulation that causes damage to connective tissue and supporting bone. The main factor causing periodontitis is local factors, the accumulation of bacterial plaque. In addition, systemic factors are predisposing factors capable of influencing the mildest tissue reaction to local irritation. Chronic periodontitis is one of the most common forms of periodontitis.[2,3]

Curettage is mechanical therapy for periodontitis to form new tissue attachments by removing inflamed tissue.[4] Ningsih stated that the wound after curettage involves soft tissue, connective tissue, gingiva epithelium, and hard tissue in the alveolar bone. This condition stimulates the emergence of cytokines as a trigger for the production of phagocytic cells, which will activate fibroblasts in the tissue repair [5]. Fibroblasts are cells that thrive in connective tissue, and their presence is essential for wound healing. After an injury, fibroblast will form within 4 to 7 days and migrate towards the wound after the tissue becomes inflamed.[2,4,5]

Periodontal treatment usually uses a combination of antibiotics as adjunctive therapy. The gold standard for adjunctive treatment in periodontitis is a topical metronidazole gel [6]. Metronidazole gel 25% has antibacterial properties and is effective as adjunctive therapy in periodontal disease.[7] However, the use of antibiotics that are not appropriate in terms of dosage and time can lead to antibiotic resistance, so several experts have conducted various studies on herbal medicines as an alternative to periodontal treatment. One of the herbal ingredients as alternative medicine is mangosteen (*Garcinia mangosteen*), a fruit that is easy to find and often consumed in Indonesia. Unfortunately, the skin is usually thrown to waste.[2,7]

Researches show that mangosteen peel contains xanthones, a substance known to function as antibacterial and anti-inflammatory because it has alpha and beta-mangosteen, which are anti-inflammatory and antibacterial. In addition, xanthones suppress the production of COX enzymes that cause inflammation. This content is the basis of the mangosteen peel, which can be used as a therapeutic drug for periodontal disease.[8]

This study aims to develop additional therapy for periodontitis with herbal ingredients. The study results are expected to improve the quality of health services and the affordability of service costs to reduce the prevalence of periodontal disease.

METHOD

The ethical clearance was obtained from the Health Research Ethics Commission, Faculty of Dentistry, Unissula No. 204/B1-KEPK/SA-FKG/VII/2020. This research was experimental laboratory research with a post-test-only design. The four experimental groups were divided into 50% mangosteen peel extract gel, 75% mangosteen peel extract gel, metronidazole gel, and metronidazole plus gel. The 24 samples were taken from the periodontal ligament of Wistar rats induced periodontitis.

Mangosteen Peel Extract Gel

The process of extracting and making mangosteen peel extract gel begins with drying and grinding to a powder, followed by maceration using 95% ethanol as a solvent for the extraction process. Next, the extraction results were filtered, evaporated, and mixed with a gel base to obtain 50% and 75% concentrations. The gel base was previously made by mixing CMC, aquadest, TEA, propylene, and nipagin.[9]

Denta Jurnal Kedokteran Gigi, Februari 2023; Vol.17 No.1; Hal 1-7
Available at http://journal-denta.hangtuah.ac.id/index.php/jurnal/issue/view/20
DOI: 10.30649/denta.v17i1.1

Preparation and Maintenance of Experimental Animals

Male Wistar rats that fit the inclusion criteria were acclimatized for seven days. Mice were kept in cages measuring 50 x 40 x 40 cm made of plastic tubs and covered with wire at a temperature of 25 - 27 ℃, given a place to eat and drink and a pad of husks. The chaff was replaced every three days. The feed used in raising rats is pellets and drinking ad libitum.[10]

Periodontitis induction

24 male Wistar rats were divided into eight groups, which were adapted for seven days, and then periodontitis was induced in the maxillary and mandibular incisors. Induction of periodontitis using a 3.0 size silk ligature was placed around the maxillary and mandibular incisors. The silk ligature was slightly pressed into the subgingival area and left for about seven days until signs of periodontitis appeared. Signs of periodontitis observed were discoloration, edema, pockets, and gingival recession. The experimental animals were then treated with curettage. They were treated as 50%, 75% mangosteen peel extract gel, metronidazole gel, and metronidazole plus gel in a pocket.[10,11]

Preparation of Tissue Sample and Counting the Number of Fibroblasts

Tissue collection was carried out on the third and seventh days. Experimental animals in each group were sacrificed by euthanasia through the administration of chloroform, and then decapitation was carried out. The tissue was first fixed with 10% buffered formalin for 24 hours, then decalcified using 10% EDTA. After softening, the periodontal ligament was embedded in paraffin and cut with a thickness of 5 m for staining with HE. Furthermore, fibroblast proliferation was counted using a microscope with a magnification of 400 times in three different fields of view, and the average was calculated.[9,11]

Statistical Analysis

The research data were tested for normality using the Shapiro-Wilk test to determine the distribution of the data and the homogeneity test using the Levene test. If the data distribution is normal and homogeneous, then the analysis is carried out using the One Way ANOVA test. On the other hand, if the data distribution is not normal and not homogeneous, then data analysis is carried out using the Kruskal-Wallis non-parametric test.

RESULT

The average value of fibroblast number after treatment with 50% mangosteen peel extract gel, 75% mangosteen peel extract gel, metronidazole gel, and metronidazole plus gel showed in table 1.

Table 1. The difference in the number of fibroblasts between the third day and the seventh day

No	Groups	3rd days	7th days	Sig
1.	50% mangosteen peel extract gel	8,611	10,888	.000
2.	75% mangosteen peel extract gel	10,335	13,668	.000
3.	Metronidazole gel	10,778	14,000	.000
4.	Metronidazole Plus gel	13,500	17,446	.000

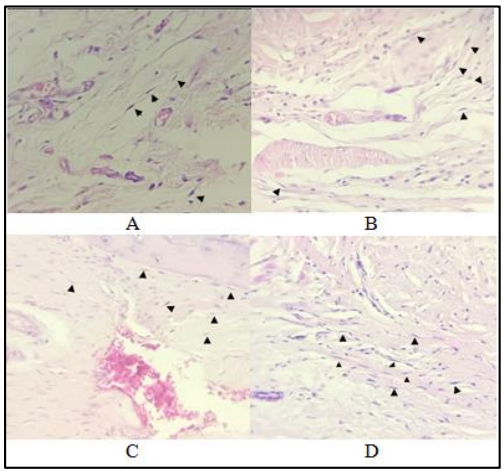

Figure 1. Fibroblast cell on 3rd days A. 50% mangosteen peel extract gel B.75% mangosteen peel extract gel C. metronidazole gel D. metronidazole plus gel.

Denta Jurnal Kedokteran Gigi, Februari 2023; Vol.17 No.1; Hal 1-7
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Based on observations, the highest number of fibroblasts was indicated by the metronidazole plus gel group, followed by the metronidazole gel group, the 75% mangosteen peel extract gel group, and the lowest number was in the 50% mangosteen peel extract gel group. One Way ANOVA test as a comparative test of numerical variables for more than two groups with test results listed in table 2: and Comparison of the number of fibroblasts between the 3rd and seventh days were tested using the unpaired t-test with the results as shown in Table 1.

Table 2. One Way ANOVA on the third day and the seventh day

	Sig.
Between Groups	.000

Table 2 shows a significant result of 0.000 ($p < 0.05$) for observations on the third and seventh days, so it can be concluded that there are significant differences in each group. Furthermore, the Post Hoc Bonferroni test was carried out to find out the significant differences between the treatment groups in table 3.

Table 3. Post Hoc Bonferroni fibroblast cell on the third day

	50% mangosteen peel extract gel on the third day	75% mangosteen peel extract gel on the third days	Metronidazole gel on third days	Metronidazole Plus gel on third days
50% mangosteen peel extract gel on the third day	-	.000	.000	.000
75% mangosteen peel extract gel on the third days	.000	-	.709	.000
Metronidazole gel on third days	.000	.709	-	.000
Metronidazole Plus gel on third days	.000	.000	.000	-

Table 4. Post Hoc Bonferroni fibroblast cell on the seventh day

	50% mangosteen peel extract gel on the seventh days	75% mangosteen peel extract gel on the seventh days	Metronidazole gel on the seventh days	Metronidazole Plus gel on the seventh days
50% mangosteen peel extract gel on the seventh days	-	.000	.000	.000
75% mangosteen peel extract gel on the seventh days	.000	-	1.000	.000
Metronidazole gel on the seventh days	.000	1.000	-	.000
Metronidazole Plus gel on the seventh days	.000	.000	.000	-

Denta Jurnal Kedokteran Gigi, Februari 2023; Vol.17 No.1; Hal 1-7
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Based on table 3, it can be concluded that there were significant differences between the treatment groups on the third and seventh days, except between the 75% mangosteen peel extract gel group and metronidazole gel, which did not show a significant difference.

DISCUSSION

This study aims to determine the effectiveness of the mangosteen peel extract gel on the number of fibroblasts. Fibroblasts have an essential role in wound healing; these cells will migrate toward the wound and proliferate to produce granulation tissue that will close the wound. Observation of the number of fibroblast cells can be done using a microscope from the third day to the seenth day. The number of fibroblast cells has increased after 48 hours of appearance. Fibroblast cells will continue to increase until their peak on day 7.[2,5,12]

The results showed that the mangosteen peel extract could increase the number of fibroblast cells. Due to their dominance in the wound-healing process, fibroblast cells are utilized as markers of wound healing. These cells are formed from the mesenchymal cells that create the fresh matrix required to repair the wounded tissue's structure and function.[13,14]

One Way ANOVA test showed significant data (p = 0.000); mangosteen peel extract gel effectively increased the number of fibroblast cells. The result is consistent with the previous studies that mangosteen peel extract effectively increased the number of fibroblasts in the wound healing process. Caused of mangosteen peel's antibacterial properties can help prevent the growth of plaque and calculus, which are linked to a number of common oral disorders including gingivitis, periodontitis, dental caries, and peri-implantitis.[2,13,14]

Mangosteen peel contains xanthones that function as antibacterials, such as metronidazole. In addition, Mangosteen peel extract has anti-inflammatory properties such as metronidazole plus. The antibacterial and anti-inflammatory properties of mangosteen peel extract and metronidazole gel will accelerate the inflammatory process by inhibiting the synthesis of inflammatory mediators and increasing the number of fibroblast cells.[1,2,7]

Mangosteen peel extract gel with 75% concentration was more effective than 50% (Table 3) following research conducted by Putri Khairani *et al*. that the higher the concentration of the mangosteen peel extract, the higher the compounds contained in the mangosteen peel to strengthen its antibacterial and anti-inflammatory effects. This effect arises due to the presence of xanthones as an anti-inflammatory with the ability to inhibit cyclooxygenase enzymes and lipoxygenase enzymes from suppressing the inflammatory process, characterized by decreased pain and fever.[12]

The effectiveness of the 75% mangosteen peel extract gel had no significant difference with metronidazole gel, so it can be concluded that 75% mangosteen peel extract gel had the same effect as metronidazole gel as the gold standard in the treatment of periodontitis to increase the number of fibroblast cells in the healing process. It can help fibroblast cells to be produced more quickly through increased expression of TGF-β, which is in charge of stimulating chemotaxis and fibroblast cell proliferation which will start the formation of granulation tissue and make the wound appear reddish.[12,14,15]

In addition, topical anti-septic 10% w/v povidone-iodine did not effectively cause fibroblast cells to migrate as well as mangosteen peel extract gel did. For the wound-healing, fibroblast cell migration is an important phenomenon.[14] Metronidazole plus gel had the highest effectiveness in increasing the number of fibroblast cells among the other groups. This is because the metronidazole plus gel has additional content in the form of mefenamic acid as an anti-inflammatory which can maximize the work of the metronidazole plus gel. Mefenamic acid is an analgesic, Non-Steroidal Anti-Inflammatory Drug (NSAID), which is often used to treat inflammation by inhibiting the cyclooxygenase enzyme and suppressing

Denta Jurnal Kedokteran Gigi, Februari 2023; Vol.17 No.1; Hal 1-7
Available at http://journal-denta.hangtuah.ac.id/index.php/jurnal/issue/view/20
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inflammation.[1,7] Anti-inflammatory activity will stimulate macrophages to produce cytokines and growth factors that can induce fibroblast cells to proliferate.[6,7,16] Antibacterial in mangosteen peel has the exact mechanism of action as 25% metronidazole, namely interacting with bacterial DNA molecules causing loss of helix structure and breaking DNA strands resulting in damage to DNA synthesis in the bacterial cell nucleus, which causes bacteria to be killed or die. The death of the bacteria that causes periodontitis is a core phase of the healing process. This phase helps prevent excess bacterial colonization from preventing a more severe infection. Therefore, the inflammatory phase can occur quickly.[1,7,14] Puteri *et al.* stated that an increase in the number of fibroblast cells indicated that the concentration of mangosteen in the tissue was safe or not toxic. This statement is by the results of the study, which showed an increase in the number of fibroblasts from third day to seventh in each group.[8] Mangosteen peel extract gel could be recommended as an alternative herbal used as an additional therapy in the treatment of periodontitis because of its antibacterial and anti-inflammatory properties, which are generally used in the treatment of periodontitis. This research confirms that mangosteen peel waste can be used as herbal-based therapy in dentistry.

CONCLUSION

Mangosteen peel extract gel effectively increased the number of fibroblasts in the rat model of the periodontitis healing process.

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Denta Jurnal Kedokteran Gigi, Februari 2023; Vol.17 No.1; Hal 1-7
Available at http://journal-denta.hangtuah.ac.id/index.php/jurnal/issue/view/20
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Denta Jurnal Kedokteran Gigi, Februari 2023; Vol.17 No.1; Hal 1-7
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