

Effectiveness of Mangosteen (*Garcinia Mangostana* L.) Peel Gel on the MMP-8 Levels in Chronic Periodontitis Patients after Scaling and Root Planing

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Abstract

Scaling and root planning (SRP) is considered as initial therapy for periodontitis. Adjuvant therapy is needed to improve the healing response. This study aimed to determine the effect of mangosteen (*Garcinia mangostana* L.) peel extract gel on the MMP-8 levels in the gingival crevicular fluid (GCF) of chronic periodontitis patients after SRP.

This study was a non-randomized controlled trial with a single-blind, pre- and post-controlled design. Thirty-two patients divided into test groups which received (SRP) and application of mangosteen peel extract gel, and the control group was only given SRP. Measurement of the MMP-8 levels of GCF was performed before and after SRP. Data analysis was performed using Mann-Whitney test. p -value <0.05 was considered to be statistically significant.

Mean of the MMP-8 levels in both groups decreased significantly ($p<0.001$). The Mann-Whitney test result showed that there was no difference ($p>0.05$) in the mean of the MMP-8 level before and after SRP between the test group and the control group.

Mangosteen peel gel as the adjuvant therapy after SRP in chronic periodontitis patients had no added value on decreasing the MMP-8 levels, only a trend in reducing the MMP-8 levels.

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Introduction

Periodontitis is chronic inflammation with the primary factor of dental plaque bacteria which stimulates the host reaction in the form of an inflammatory process that causes damage from the attachment of the tooth-supporting tissue. Plaque bacteria will release products such as Lipopolysaccharide (LPS) which will affect the body to release components of antibacterial cells thus activating the immune cells. This activity will increase the production of inflammatory mediators such as cytokines, chemokines, prostaglandins, and proteolytic enzymes such as matrix metalloproteinase (MMP)^{1,2}

MMP is a protease enzyme from zinc and

calcium endopeptidase with the capability to degrade almost all extracellular matrices and their components, including collagen which is the main protein structure in the connective tissue of the periodontium. Naturally, the human body has MMP inhibitors, namely the tissue inhibitor of metalloproteinase (TIMP) which functioned in maintaining the balance in the body's physiological processes. Pathological conditions such as the presence of periodontitis cause an increase in the number of MMPs resulting in increased degradation of extracellular matrix components such as collagen.³ One of the metalloproteinase matrix families, MMP-8 (collagenase-2) is synthesized by differentiating granulocytes in the bone marrow and stored in specific granules from the neutrophil circulation. The collagenase enzyme in MMP-8 is an enzyme that can break down collagen in the tissue remodelling events. The MMP-8 enzyme is produced by bacteria that cause periodontal disease which has the potential to damage the tooth-supporting tissue by breaking down collagen type II. This activity can be detected in the saliva and gingival crevicular sulcus (GSC)

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fluid. The MMP-8 levels have been considered as a key marker in chronic periodontitis to predict the periodontal disease progression. The periodontal disease healing process enzymologically can be described by the decrease in the MMP-8 levels, which play an active role in the pathogenesis of periodontitis.⁴

Scaling and root planing (SRP) is a non-surgical initial therapy for periodontal disease using instruments to restore periodontal tissue health by eliminating plaque, calculus, and endotoxins that cause gingival inflammation on the tooth surface. Study of Konopka et al. (2012)⁵ discovered that the effects of periodontal treatment in the form of scaling and root planing could reduce the MMP-8 levels in the gingival sulcus fluid in patients with chronic periodontitis. SRP treatment sometimes cannot achieve the maximum results, thus required additional chemotherapeutic agents during the therapy phase.^{6,7} The use of natural remedies is currently becoming an adjunctive therapy of choice in dentistry. This practice is done because natural remedies are considered to have relatively fewer side effects than synthetic chemical drugs.

One of the plants often used as a natural remedy is mangosteen (*Garcinia mangostana* L.).⁸ Mangosteen peel extract and its active compounds have pharmacological activities as anti-inflammatory, anti-oxidant, anti-allergic, anti-cancer, and anti-bacterial properties⁹ and has been reported to have inhibitory effects against putative periodontopathogen.¹⁰ It has also been shown to contain various xanthone group compounds. Xanthone compounds can be functioned as an anti-inflammatory agent include alpha-mangostin, gamma-mangostin, garsinon-E and flavonoids. The activity of different xanthone derivative compounds can inhibit nitric oxide (NO), prostaglandin E2 (PGE2) production, and suppress the cyclooxygenase-2 (COX-2) expression.¹¹

Study conducted by Hendiani et al. (2017)¹² regarding the use of mangosteen peel extract gel as adjunctive therapy after SRP in periodontitis patients may reduce probing depth, gingival inflammation, and bleeding on probing. Rassameemasmaung et al. (2008)¹³ and Mahendra et al. (2016)¹⁴ concluded the same results that the administration of mangosteen peel extract gel in chronic periodontitis patients after scaling and root planing was able to improve the periodontal clinical parameters

compared to those given only scaling and root planing. Sung et al. (2019)¹⁵ stated that the effect of mangosteen peel extract and propolis could inhibit inflammation and alveolar bone loss in rats with periodontitis. This present study aimed to determine the effect of mangosteen peel extract gel on the MMP-8 levels in the gingival crevicular fluid of chronic periodontitis patients after scaling and root planing.

Materials and methods

Subject selection

This study is a non-randomized controlled trial with a single-blind and pre- and post-test design. The participants consisted of 32 chronic periodontitis patients who came to the Periodontics Specialist Education Program Clinic of Universitas Padjadjaran Dental Hospital Bandung, West Java, Indonesia. The inclusion criteria were adults aged of 30-60 years, had a periodontal pocket depth of 5-7 mm, had not received periodontal treatment for the past 6 months, had no systemic conditions, did not use any prosthesis and orthodontic devices, and not taking antibiotics and mouthwash for the past 3 months. Each participant signed informed consent as an approval to participate in the study. After recruitment, the patients were divided into the test group and control group. Sixteen patients in the control group were treated with scaling and root planing only, while sixteen patients in the test group were treated with scaling and root planning and mangosteen peel extract gel administered to the depth of periodontal pocket on their teeth sample. This study has been approved by the Health Research Ethics Committee of Universitas Padjadjaran, No. Reg 0319010021.

Mangosteen peel extract

The preparation of the mangosteen peel gel was conducted at the Center for Tropical Biopharmaca Studies Laboratory of Institute of Agriculture Bogor, West Java, Indonesia. The mangosteen was obtained from the Biopharmaca Plantation Conservation Unit of the same institute. The extract was made using the maceration technique with 70% ethanol, then transformed into a gel preparation with a 20% concentration extract. Mangosteen peel extract gel then inserted into vial tubes 5ccs each, and stored at 4°C. Before usage, the gel was transferred into a 1 cc syringe and the application into the pocket

was carried out with a disposable soft rubber tip.

Gingival crevicular fluid collection

The study material was derived from gingival crevicular fluid (GCF) taken on Day 0 and after treatment on Day 15. GCF was taken from periodontal pockets using a paper point. After brushing teeth and rinsing the region of the GSC sample was dried and blocked with a cotton roll, then 3 paper points no.30 (Indodont®, Indonesia) were put one by one into the periodontal pocket for 30 seconds each. Paper points contaminated with blood were discarded. The GCF samples obtained were stored in Eppendorf tubes containing 5 ml phosphate buffer then taken to the Clinical Pathology Laboratory of Dr Hasan Sadikin Hospital, Faculty of Medicine Universitas Padjadjaran with a cool box to be centrifuged and stored in a refrigerator with the temperature of -80°C until the time of the ELISA test.

Treatment procedure

After the initial examination and collection of the GCF on (Day 0), all patients then were treated with SRP for all regions using an ultrasonic scaler and Gracey curette (Osung®, Korea). In the test group, after SRP, the mangosteen peel extract gel was administered to the depth of the pocket (5-7 mm pocket). No prescription of antibiotics or mouthwash was given after treatment. The patient were instructed to maintain oral hygiene. All patients were asked to return on Day 15 after treatment for prophylaxis and GCF collection.

Assessment of MMP-8 level

The MMP-8 levels of the GCF samples were analyzed using the ELISA test (Elabs Science Biotechnology Inc., USA) with sandwich method principle. The GCF samples obtained were placed in Eppendorf tubes containing 5 ml phosphate buffer (pH 7.2), then centrifuged for 1 hour at room temperature. All ELISA procedures were carried out according to the manufacturer's instructions. The MMP-8 content values were calculated from standard curves in nanograms with a sensitivity of 0.01 ng/mL. Examination of the MMP-8 levels was conducted at the Clinical Pathology Laboratory of Dr Hasan Sadikin Hospital, Faculty of Medicine Universitas Padjadjaran, Bandung, West java, Indonesia.

Data analysis

All data obtained from the measurement of the number of MMP-8 formed was tabulated.

Data were tested for normality with the Shapiro-Wilk test. Analysis of the MMP-8 levels of GCF in chronic periodontitis patients was conducted using the Mann-Whitney test if the data were not normally distributed. The significance of the test results was determined based on the p-value<0.05.

Results

Demographic characteristics of respondents can be seen in Table 1.

Patients' Characteristics	Groups			
	Test (n= 16)	Percentage (%)	Control (n=16)	Percentage (%)
Age (yo)				
Mean ± Std. deviation	42.0		44.6 ±	
Range	±7.3		6.8	
Sex	31 – 56		35 – 57	
Male		50%		37.5%
Female	8	50%	6	62.5%
Occupation	8		10	
Employed		68.8%		50%
Unemployed/Housewives	11	31.2%	8	50%
	5		8	

Table 1. Patients' characteristics (n = 32).

The MMP-8 levels between the test group (treated with SRP, followed by the application of mangosteen peel extract gel) and the control group (treated with SRP alone) were measured by the ELISA test; then statistical calculations were performed. The average MMP-8 levels obtained in both groups are presented in Table 2. The average MMP-8 levels before (D0) and after scaling and root planing (D15) in the test group and the control group based on the Wilcoxon test both experienced a significant decrease (p<0.05).

Group	MMP-8 level		P-value*
	D0 data	D15 data	
Test			
Mean (SD)	0.61 (0.53)	0.24 (0.19)	p<0.001 (S)
Median	0.39	0.16	
Range	0.08 – 1.93	0.06-0.71	
Control			
Mean (SD)	0.54 (0.34)	0.26 (0.21)	p<0.001 (S)
Median	0.57	0.21	
Range	0.05 – 1.40	0.01- 0.71	

Table 2. The average MMP-8 levels before (D0) and after scaling and root planing (D15) in the test and control groups.

Notes: *based on the Wilcoxon test results (p<0.05); S = significant.

Comparison of the mean difference of the MMP-8 levels before and after scaling and root planing between the test group and control group

can be seen in Table 3. Based on the normality test (Shapiro-Wilk test), the data were not normally distributed, so the Mann-Whitney test was conducted. Statistical results of the Mann-Whitney test obtained the p-value=0.895 (p>0.05) thus can be concluded that there was no significant difference between the mean difference of the MMP-8 levels before and after scaling and root planing between the test group and control group.

Group	Mean difference	Standard deviation	Z _{count}	P-value*
Test	0.37	0.49	-0.132	0.895 (NS)
Control	0.28	0.26		

Table 3. Comparison of mean difference in the MMP-8 levels before and after scaling and root planing between the test and control groups.

Notes: *based on the Mann-Whitney test results (significant in the p-value<0.05);

NS: Not significant.

Discussion

This study aimed to evaluate the effect of topical application of the mangosteen peel extract gel applied in the periodontal pocket of chronic periodontitis patients on decreasing the MMP-8 levels of the gingival sulcus fluid after SRP (test group) compared with the MMP-8 levels of the chronic periodontitis patients treated with SRP alone (control group).

Laboratory results of the present study indicated that the average of MMP-8 level from D0 of the treatment up to D15 after SRP in both groups were decreasing (Table 2). This result was consistent with the study of Marcaccini et al. (2009)¹⁶ and Konopka et al. (2012)⁵ which suggested that SRP can reduce the MMP-8 levels. The mean difference of the MMP-8 levels before and after treatment in the test group was 0.37, and the mean difference of the MMP-8 levels in the control group was 0.28 (Table 3). These values showed that the mean difference of the MMP-8 levels before and after SRP in the test group was higher than the control group, but statistically insignificant when compared to the control group. Provision of adjunctive treatment to the test group after SRP in the form of application of mangosteen peel extract gel which has anti-inflammatory properties was proven to be able to reduce the MMP-8 levels in the test

group.

The MMP-8 level is one of the biomarker parameters in assessing the presence of inflammation in the periodontal tissue by the research conducted by Al-Majid et al. (2018)⁴ which stated that MMP-8 is an inflammatory biomarker in periodontitis and periimplantitis. Active MMP-8 levels can also be used to predict and determine the progression of periodontitis. According to Mauramo et al. (2017)¹⁷ and Kasuma et al. (2018)¹⁸, MMP-8 levels are higher in periodontitis patients compared to gingivitis patients and healthy patients. MMP-8 is found in serum, saliva, and gingival crevicular fluid. According to Kinney et al. (2014)¹⁹, MMP-8 levels in GCF are more specific than saliva particularly for assessment of local treatment outcomes. Examination of the MMP-8 levels in the present study was performed using the ELISA test, carried out on the sample from D0 and D15. Following the statement of Marcaccini et al. (2009)¹⁶ which stated that 15 days is the beginning of an effective MMP-8 decrease and reaches a very significant reduction on the 90th day after SRP. Research conducted by Konopka et al. (2012)⁵ showed a decrease in the MMP-8 levels 30 days after SRP.

SRP was able to reduce the MMP-8 levels in periodontitis patients, as suggested from the research of Marcaccini et al. (2009)¹⁶ and Konopka et al. (2012)⁵. SRP also able to change the bacteria composition in the pocket, thus inhibits the activity of periodontal tissue collagenase enzymes and reduce the MMP-8 levels. The effectiveness of SRP can be improved by locally applied additional therapy on the periodontal pockets. An adjunctive treatment which is effectively reducing the MMP-8 levels is the administration of doxycycline drugs, both orally and topically. Routine use of antibiotics for a long time is contraindicated and can cause a person to experience antibiotic resistance so that many people are now turning to use herbal remedies as an alternative treatment because of the fewer side effects, economic, and easy to obtain.

The mangosteen plant, which is nicknamed as “the queen of fruits”, has a reasonably high nutritional content in each of its parts. The peel contains xanthone, which is a bioflavonoids agent with properties such as antioxidants, antibacterial, hypo-allergenic, antitumor, antihistamine, and anti-inflammatory.⁵

Sung et al. (2019)¹⁵ discovered that the effect of mangosteen peel extract supplemented with propolis could inhibit inflammation and alveolar bone loss in rats with periodontitis. Also, α -mangostin, as a derivative of xanthone, contained on the mangosteen peel, can prevent the activity of the cyclooxygenase-2 (COX-2) enzyme, which is a marker of inflammation. Lee et al. (2010)²⁰ suggested that α -mangostin can suppress the MMP-2 and MMP-9 expression in breast cancer cells, also, a study conducted by Shih et al. (2010)²¹ indicated that α -mangostin could also suppress the MMP-2/MMP-9 expression in the lung cancer cells.

The difference of decreasing MMP-8 levels that was not significant in the present study could be caused by taking the period of the study of 15 days, which might affected the amount of MMP-8 levels decrease because the MMP-8 levels experienced a significant decrease on the 90th day (3 months) after SRP.¹⁶ However, from the result of the laboratory observation on the mean MMP-8 level after treatment, there was a more significant decrease in the test group given the application of mangosteen peel extract gel.

The use of herbal remedies in the form of extracts containing many active compounds work synergistically and interact antagonistically. Most of the compounds present in extracts of herbal remedies are multitarget agents that modulate the activity of proteins, nucleic acids, and biomembranes in a less specific way thus can be interpreted that the extract achieving the healing target in a slow period.²² One of the limitations of the present study was the measurement of GCF volume. The authors did not employ a Periotron[®] device. Periotron[®] is a device used to measure the gingival sulcus fluid volume in a more accurate way, and its value has been calibrated using the human serum as a standard. This limiting factor can be one of the causes of the result differences between the present and previous studies.

Conclusions

Under limitations of this present study, it can be concluded that application of mangosteen peel gel after SRP in chronic periodontitis patients had no added value in reducing the MMP-8 levels of GCF. However, there was a trend of decreasing amount of the MMP-8 levels

in the group administered with mangosteen peel gel compared to the group without the application of mangosteen peel gel.

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Declaration of Interest

The authors declare no conflict of interests.

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