The Application of *Andrographis Paniculata* Gel as an Adjunct in The Treatment of Chronic Periodontitis : Clinical and Microbiological Effects

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Abstract

The purpose of this study was to evaluate the clinical and microbiological effects of locally delivered *Andrographis paniculata* (AP) gel as an adjunct to scaling and root planing (SRP) in the treatment of chronic periodontitis. Thirty- two systemically healthy and non-smoking subjects with chronic periodontitis were recruited in this study. The subjects had to comply with the following criteria; having three single-rooted teeth located in different quadrants with probing pocket depth ≥ 5 mm and bleeding on probing, no history of receiving periodontal therapy or antibiotics within 6 months. The experimental sites of three teeth of each subject were randomly assigned to receive either of the three treatments; SRP plus AP gel, SRP plus gel base, or SRP only. The gels were applied at day 0, 1, 2, 3 weeks. Four clinical parameters, i.e., probing pocket depth, clinical attachment level, gingival index, and bleeding on probing were recored at day 0 (baseline), 1 and 3 months after treatments. Subgingival plaque was also taken using paper points at the same time points and subjected to the real-time PCR method (EvaGreen) for quantification of *Porphyromonas gingivalis*. Results showed that clinical changes at the disease sites were significantly improved after received all three treatments ($p<0.05$). When compared the clinical outcomes among the treatments. The clinical outcomes of the SRP plus AP gel-treated sites significantly showed a better improvement ($p<0.05$). For microbiological study, *P. gingivalis* was detected only in eight subjects at baseline. After treatment for 3 months, the bacteria could not be detected in five subjects. In conclusion, the local application of AP gel as an adjunct to SRP showed a better improvement of clinical parameters. These results indicated the benefit of AP gel as an adjunct in the treatment of chronic periodontitis.

Keywords: *Andrographis paniculata gel*, Periodontitis, *Porphyromonas gingivalis*

Introduction

Periodontitis is an infection occurring in the tooth-supporting tissues. The presence of subgingival plaque represents the principal etiologic factor which involves in the initiation and progression of inflammatory periodontitis (Offenbacher, 1996; Zambon, 1996). The treatment of the various forms of periodontitis is based on thorough debridement of the root surfaces to remove calculus, plaque and subgingival microorganisms. The scaling and root planing also for controlling the progression of periodontal diseases. However, this technique need to access to and visibility of the area. In some cases, complete subgingival plaque and calculus removal are hardly achieved and sometimes ineffective (Rateitschak et al., 1992). There are limiting factors such as deep pockets (Rabbani et al., 1981; Caffesse et al., 1986 ; Waerhaug, 1978), furcation areas (Loos et al., 1988; Fleischer et al., 1989; Desanctis & Murphy, 2000) and biofilms in the cementum and dentine (Adriaens et al., 1987). In addition, it may not possible to eradicate bacterial species that can reach epithelial cells and subepithelial connective tissues of the periodontium (Adriaens et al., 1987; Danser et al., 1996). In order to kill the remaining bacteria, systemical and local administration of antimicrobial agent is used as an adjunctive treatment to improve the management of periodontitis (Mombelli & Samaranayake, 2004; Kornman,1993).

The controlled-release of local drug delivery system is designed to slowly release a drug in the treated site for prolonged drug availability and extended drug action. Recently the local drug delivery system containing an antimicrobial traditional herb, *Andrographis paniculata*, has been developed as adjunct to scaling and root planing (Komwatchara, 1996; Narakorn, 1999). The *Andrographis paniculata* gel (AP gel) has been shown to reduce probing depth and improve coronal radiopaque

fill in the AP gel-treated sites at 3 and 6 months (Sirirat & Rojanapanthu, 2003). Moreover, the proportion of the black-pigmented anaerobes was significantly reduced in the AP gel-treated sites, but not in the metronidazole gel-treated site (Atsawasuwan et al., 1998). The AP gel also demonstrated the more consistent increase of cocci and the decrease in percentage of motile rods when compared to 2% minocycline gel during the period of 3-4 months (Boonchaipanichwatana, 2001).

Subgingival microflora with high proportions of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Actinobacillus actinomycetemcomitans* have been shown to associate with periodontal disease (Slots et al., 1986; Haffajee et al., 1988). Thus, it is suggested that the presence of these bacteria may serve as markers of disease activity (Genco et al., 1986). Microbiological monitoring may be useful as an adjunct to clinical monitoring or as a guide for treatment strategies to achieve good treatment outcomes. *P. gingivalis* has been recognized as a major periodontopathogenic organism (Slots & Listgarten, 1988), and the increase in the subgingival level of *P. gingivalis* has been shown to be associated with the increase in the severity of periodontitis (Socransky et al., 1991). Detection of this anaerobic pathogen by culture method is difficult, expensive and time consuming. Recently, real-time PCR-based detection method is introduced and gained increasing popularity because of its simplicity, speed and high sensitivity (Eick & Pfister, 2002; Lau et al., 2004; Jervoc-Storm et al., 2004; Verner et al., 2006; Boutaga et al., 2006). Therefore, this study aimed to evaluate the effectiveness of AP gel as an adjunct to scaling and root planning. The clinical parameters namely probing pocket depth (PPD), clinical attachment level (CAL), gingival index (GI) and bleeding on probing (BOP) and microbiological parameters, the quantities of *P. gingivalis* detected by real-time PCR method (EvaGreen®), were aassed after treatment with scaling and root planing (SRP) plus AP gel, compared to SRP applied with gel base and SRP only.

Research hypothesis

Using *Andrographis paniculata* gel as an adjunct to scaling and root planing in the treatment of chronic periodontitis enhances the clinical and microbiological outcomes over scaling and root planing with gel base and scaling and root planing only.

Materials and methods

1. Patient selection

Thirty- two patients diagnosed as chronic periodontitis with ages of 30-65 years were participated in this study. Patients included in the study were in generally good health with no systemic diseases and must have at least 3 single-rooted teeth that were the same tooth type and sited on different quadrant. Their initial probing pocket depth was 5 mm or more, present bleeding on probing and show radiographic evidence of bone loss. The depth of pocket among the teeth were not different more than 1 mm. Patients were excluded from the study if they had received periodontal therapy or antibiotics within 6 months prior to the study. Pregnancy, smoking and antibiotic treatment must be avoided during the study. All volunteer patients signed the informed consent form after providing them with detailed information about the clinical trial. The ethical approval was obtained from Mahidol University Institutional Review Board.

2. Clinical procedures

Prior to the study, baseline data were collected from all patients on day 0. These included clinical and microbiological parameters. Using randomized controlled trials, the experimental teeth in each patient were randomly assigned into three treatment groups. The first treatment group received scaling and root planing with AP gel. The second and third treatment group received scaling and root planing with gel base and scaling and root planing only, respectively. Scaling and root planing was performed with hand instruments (Graceycurettes, HuFriedy®, Chicago, IL, USA) and ultrasonic scalers (Sonicflex, KaVo, Biberach, Germany) until all supra and subgingival root surfaces were felt hard and smooth. The pockets were then gently irrigated with 2 ml of 0.9% sterile normal saline solution in a syringe with a 21- gage blunt needle. The AP gel or gel base was gently applied subgingivally into the pocket around the teeth until filled up to the gingival margin. The excess gel was removed by sterile cotton pellets and a suction device. The teeth received scaling and root planing only were served as the controls. Patients were asked to avoid rinsing, drinking and eating for 1 hour after gel application and recalled for the same treatment at 1, 2 and 3 weeks. The parameters were assessed at 1 and 3 months after treatment. The patients received oral hygiene instructions, full mouth scaling, root planing and oral prophylaxis. All treatments were performed by the same periodontist and the clinical parameters were

collected by another periodontist. There were four clinical parameters : The probing pocket depth was measured as the nearest millimeter of the distance from the gingival margin to the base of the pocket with standard periodontal probe (PCPUNC 15, Hu-Friedy®, Chicago, IL, USA).The clinical attachment level was measured as the distance from the cemento- enamel junction to the base of the pocket with the same periodontal probe. Bleeding on probing was performed by gentle probing into the sulcus of the gingival crevice using the same periodontal probe. If bleeding occured within 10 seconds, a positive BOP was recored. All four tooth surfaces were recorded as + or - for bleeding on probing. Gingival index, indicating gingival inflammation, was scored from 0-3 on labial/buccal, lingual, distal and mesial surface of all teeth.

3. Microbiological procedures

Subgingival plaque samples were collected from three sites per a patient on day 0 before the clinical examination, and at 1, 3 months after treatment for microbiologic evaluation. Before samples collection, each tooth was isolated with cotton rolls and all supragingival plaque was removed with sterile scalers, then gingival and tooth surface was dried. The gingival crevicular fluid sample was obtained by insertion a standardized sterile paper point (# 35, DiaDent®) without forcing into the deepest part of periodontal pocket, it was left in situ for 20 seconds. After removal from the pocket, the paper point was immediately transferred to a microtube containing 200 ul of sterile deionized water, and then stored at -20°C until use.

Porphyromonas gingivalis strainW50 was provided from the Department of Oral Microbiology, Faculty of Dentistry, Mahidol University. The bacteria were cultured on anaerobe basal agar (Oxoid®) supplemented with 5% v/v blood, incubated at 37°C for 3-5 days in an anaerobic jar (Oxoid®). The bacterias were then inoculated in Schaedler broth (BBL) supplemented with vitamin K, incubated at 37°C in an anaerobic jar for two days.

A mineral oil drop was added to each sample to avoid evaporation during the boiling step. The plaque samples and the bacterial suspensions were boiled at 100 °C for 10 min by Dry Block Heating Thermostate. Then, the solutions were transferred to clean microcentrifuge tubes without mineral oil and centrifuged at 15,000xg for 5 min. The supernatant containing the bacterial DNA was kept at -20°C until use as a PCR template.

FimA gene of *P. gingivalis* was used to test the specificity of the assay. The nucleotide sequence of primers and the length of PCR products were shown in Table 1.

Table 1 The primers for PCR in this study

Gene	Specificity	Primer sequence (5′ to 3′)	Name	Position
fimA gene	*P. gingivalis*	ATC TGA ACG AAC TGC GAC	PgF	2-19
		GTT CTG CCT CGT TGT CTT	PgR	172-155

Specificity of the primers was checked with web 'Basic Local Alignment Search Tool' program (BLAST®, NCBI home page http://ncbi.nlm.nih.gov/blast).

The numbers of bacterial cells were quantitative by real-time PCR performed in CFX96 Real-Time PCR System (Bio-Rad,USA) and SsoFast™ EvaGreen® Supermix (Bio-Rad,USA). Each PCR amplication was carried out in a total volume of 10µl, consisting of 1µl of DNA template and 9 µl of reaction mixture, according to the manufacturer's instruction using optimization of real-time PCR condition.

For each run, 1 µl of sterile distilled water instead of DNA solution was used as the negative control. DNA extracted from ten-fold serial dilutions of *P. gingivalis* strain W50 ranging from $8x10^4$ to $8x10^0$ cells were used to prepare the standard curve and test sensitivity of the primer.

Each amplification reaction was performed in the CFX96 Real-Time PCR System with the cycling conditions as follows:Enzyme activation at 98°C for 2 minutes, denaturation at 98°C for 5 seconds, annealing/ extension at 54°C for 10 seconds followed by 54 PCR cycles and melting curve at 65°C to 95°C. The melting peak was used to determine the specificity of the PCR product. Accumulation of the PCR products was detected in each cycle by monitoring the increase in fluorescent density of the reporter dye double strand DNA-binding Eva Green. The fluorescence level was generated at a specific PCR cycle.The cycle threshold value (C_T) indicates the cycle where the fluorescence detected from

the amplicon of the quantity of the target gene exceeded the preset threshold. The C_T was converted into the number of bacterial cells using the standard curve established the bacterial-specific DNA. Fluorescent data were analyzed with Bio-Rad CFX Manager software version 1.6 (Bio-Rad, USA).

Sensitivity of the primers was performed to determine the minimum of bacterial-specific DNA concentrations that CFX96 Real-Time PCR System (Bio-Rad,USA) could detect. The $8x10^3$-$8x10^0$ solutions of *P. gingivalis* strain W50 were used to test sensitivity of primer by using 1 µl of each solution. Then, it was added to 10 µl by mixing with 9 µl of reaction mixture following manufacturer's instructions that was mentioned above. The standard curve was constructed from the solution of $8x10^3$-$8x10^0$ *P. gingivalis* strain W50 cells. Absolute quantification of the target bacteria in clinical samples was performed using *P. gingivalis* (W50) as the controls. Standard curves were made with this control and used to convert cycle threshold scores into the number of bacterial cells.

4. Statistical analysis

The variables were the scores for probing pocket depth, clinical attachment level, bleeding on probing and gingival index. The data were analyzed using the statistical package SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). The different clinical evaluation of the three treatments were compared by Kruskal-Wallis test and further analysis using Multiple Comparison for nonparametric statistic, except bleeding on probing which was analyzed by Fisher's Exact test. For within comparison of each treatment, the difference between baseline and each follow up visit was analyzed by Friedman test and further analyzed by Multiple Comparison for pairwise comparison. However, Cochran'Q test was used to assess the significant difference of bleeding on probing over time within treatment and McNemar test was used for pairwise comparison. The level of statistical significance was considered at $p<0.05$ in all tests.

Results

Thirty-two systemical healthy and non-smoking subjects with chronic periodontitis, 7 males and 25 females aged 34 to 66 years (mean age 50.31±8.09 years), were recruited in the study. These subjects were employees from Prasat Neurological Institute. No subjects were excluded during the study. No side effects occurred in any of the subjects and no one complained about the bitter taste after AP gel application.

Clinical evaluation

Four clinical parameters were collected to evaluate and compare the clinical changes of each treatment group. These were probing pocket depth, clinical attachment level, gingival index and bleeding on probing.

1. Probing pocket depth (PPD)

Mean PPD values at baseline, 1 month and 3 months from the treated sites of three treatments were shown in Table 2. Means PPD at baseline of all treatments, i.e., SRP plus AP gel, SRP plus gel base, and SRP only were not significantly different with the mean range of 5.41 to 5.50 mm. However, the significant differences of PPD between treatments at 1 month and 3 months were found ($p<0.05$). Multiple comparison was used to show the significant difference of PPD between SRP plus AP gel and SRP plus gel base, SRP plus AP gel and SRP only at 1 and 3 months ($p<0.05$) as shown in Table 2.

After treatment, all groups showed the significant reductions of PPD at 1 month and 3 months compared to baseline ($p<0.05$) (Figure 1).

Table 2 Means and standard deviations of probing pocket depth (mm) of all treatments at different time intervals.

Treatment	Time		
	Baseline	1 month	3 months
SRP+AP gel	5.47±0.72	* 3.09±0.89	* 2.78±0.71
SRP+gel base	5.50±0.76	3.63±1.09 *	3.47±1.11 *
SRP only	5.41+0.71	3.53+1.02	3.53+0.88

SRP was scaling and root planing

AP gel was *Andrographis paniculata* gel

* Statistical significance of difference between the treatments ($p<0.05$)

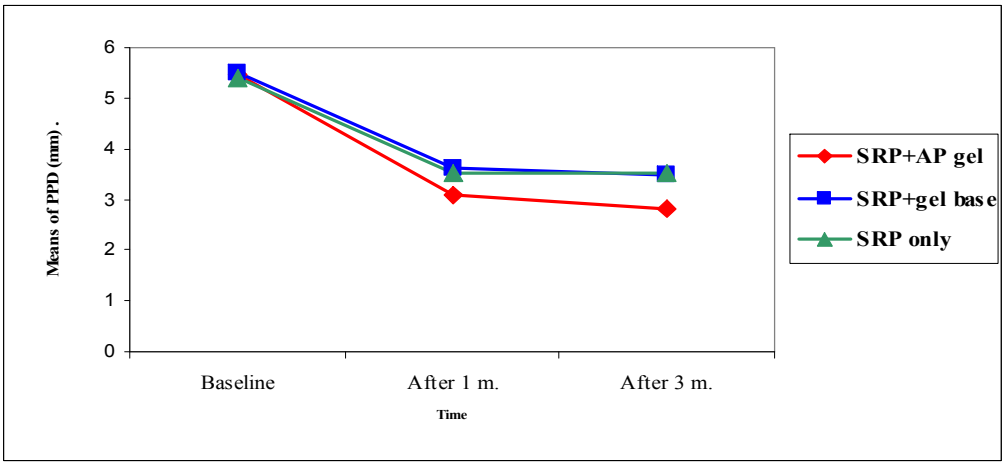

Figure 1 Means of probing pocket depth of 3 treatments at baseline, 1 month and 3 months after treatment.

2. Clinical attachment level (CAL)

Means of clinical attachment level at baseline, 1 month and 3 months from the treated sites in three treatments were shown in Table 3. CAL at baseline of all treatments were not significantly different with the means range of 5.41 to 5.88 mm. In addition, when compared between treatments using Multiple comparison for pairwise comparison, the significant difference of CAL was found between SRP plus AP gel and SRP plus gel base, SRP plus AP gel and SRP only at 3 months after treatment ($p<0.05$) as shown in Table 3.

After treatment 1 and 3 months, the CAL of all groups showed significant reduction, compared to baseline level ($p<0.05$) (Figure 2).

3. Gingival index (GI)

Means of gingival index at baseline, 1 month and 3 months from the treated sites of three treatments were shown in Table 4. GI of all groups at baseline were not significantly different with the means range of 1.84 to 1.91. Comparison between treatments using Multiple Comparisons showed significant difference of gingival index between of SRP with AP gel and SRP with gel base, SRP with AP gel and SRP only at 3 months ($p<0.05$) as shown in Table 4.

Table 3 Means and standard deviations of clinical attachment level (mm) of all treatments at different time intervals.

Treatment	Time		
	Baseline	1 month	3 months
SRP+AP gel	5.53+0.72	3.63+1.24	* 3.22+0.97
SRP+gel base	5.88+1.04	4.25+1.67	4.19+1.55 *
SRP only	5.41+0.84	4.16+1.72	3.88+1.41

* Statistical significance of difference between the treatments ($p<0.05$)

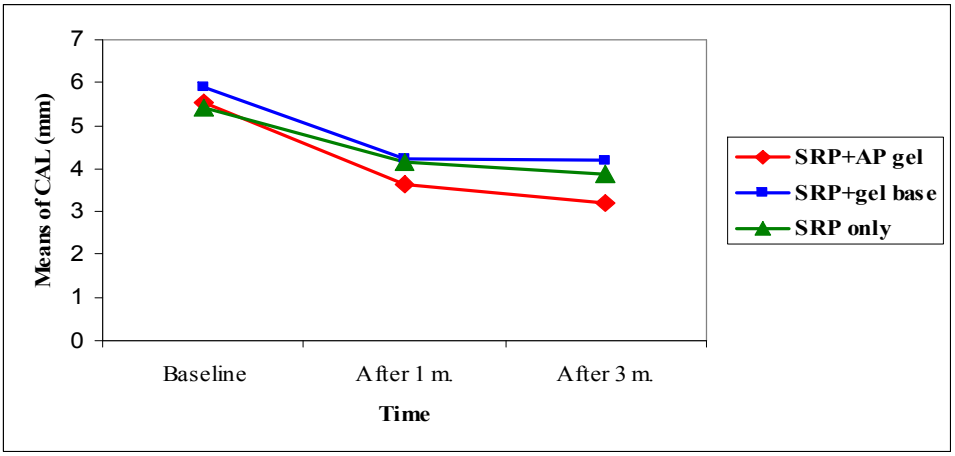

Figure 2 Means of clinical attachment level of 3 treatments at baseline, 1 month and 3 months after treatment.

Table 4 Means and standard deviations of gingival index of all treatments comparing at different time intervals.

Treatment	Time		
	Baseline	1 month	3 months
SRP+AP gel	1.88+0.34	1.22+0.49	* 1.00+0.25 *
SRP+gel base	1.84+0.45	1.53+0.51	1.22+0.42 *
SRP only	1.91+0.39	1.34+0.48	1.28+0.46

* Statistical significance of difference between the treatments (p<0.05)

The significant reduction of gingival index at 1 month and 3 months compared to baseline data were observed in all treatment groups (p<0.05), as shown in Figure 3.

4. Bleeding on probing (BOP)

Percentages of bleeding on probing at baseline, 1 month and 3 months from the treated sites in all groups were shown in Table 5. Bleeding on probing of all groups at baseline were not significantly different with the range from 93.75 to 100%.

When compared among treatments, the sites treated with SRP plus AP gel showed significant decreased of percent BOP compared to the other two treatments at 1 month and 3 months after treatment (p<0.05), as shown in Table 5.

Using McNemar Test, percentages of BOP of the sites treated with SRP plus AP gel and SRP only post treatment 1 month and 3 months were significantly decreased when compared to the baseline (p<0.05), However, percentages of BOP of the sites treated with SRP plus gel base was significantly decreased only at 1 month after treatment as shown in Figure 4.

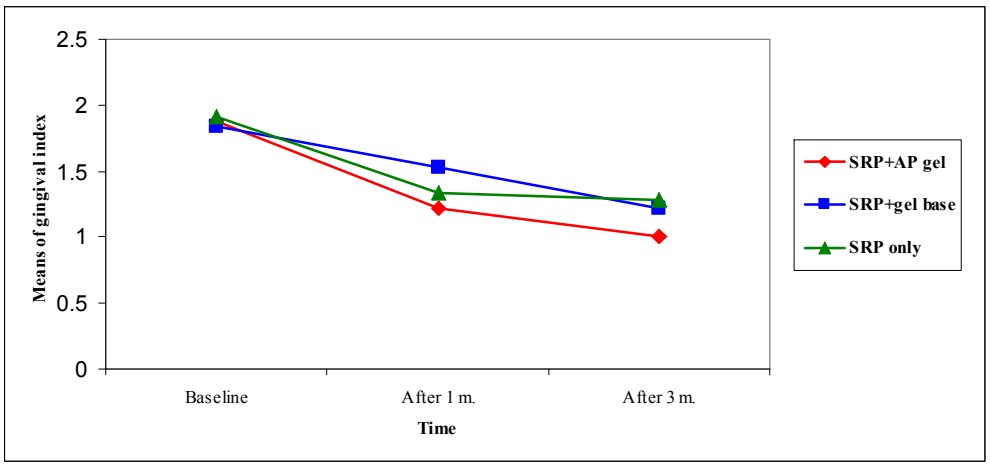

Figure 3 Means of gingival index of 3 treatments at baseline, 1 month and 3 months after treatment.

Table 5 Percentages of bleeding on probing of all treatment groups comparing at different time intervals.

Treatment	Time		
	Baseline	1 month	3 months
SRP+AP gel	100.00	* 53.13 *	* 46.88 *
SRP+gel base	93.75	68.75	81.25
SRP only	96.88	75.00	78.13

* Statistical significance of difference between the treatments (p<0.05)

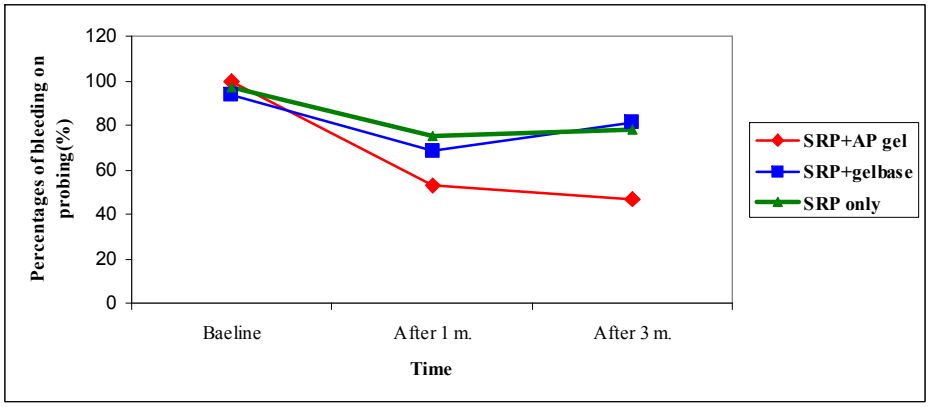

Figure 4 Percentages of bleeding on probing of 3 treatments at baseline, 1 month and 3 months.

Microbiological evaluation

For microbiological assessment, this study used real time PCR method (EvaGreen®), CFX96 Real-Time PCR System (Biorad, USA) was used to detect quantities of *P. gingivalis* in each treatment groups at baseline, 1 month and 3 months after treatment.

Porphyromonas gingivalis was detected only eight subjects from total of thirty-two subjects (25%). The numbers of the bacteria (cells/µl) detected in all treatments were shown in Table 6.

Numbers of *P. gingivalis* at baseline of five cases, i.e., No.15, 18, 20, 23 and 28 ranged from 10.19 to 52.35 cells/µl. Case No.1 ranged from 152.2 to 579.1 cells/µl, case No.9 from 80.89 to 180 cells/µl, and case No. 29 from 279.3 to 1391 cells/µl. The numbers of bacteria were calculated from the standard curve.

Table 6 Numbers of *Porphyromonas gingivalis* (cells/µl) of all treatments at various time.

	SRP+AP gel			SRP+gel base			SRP only		
Case No.	Baseline	1month	3 months	Baseline	1month	3 months	Baseline	1month	3 months
1	152.2	ND	253.3	203.2	ND	329.8	579.1	ND	417.3
9	180.0	261.3	ND	80.89	387.8	ND	110.7	208.2	525.2
15	52.35	ND	ND	13.56	ND	ND	15.28	ND	ND
18	33.58	ND	ND	10.79	8.34	ND	10.84	ND	ND
20	10.19	ND	ND	12.53	9.94	9.64	64.87	10.55	ND
23	27.45	ND	ND	42.76	ND	ND	20.82	ND	ND
28	42.00	49.55	25.89	69.24	78.96	107.6	47.38	179.8	94.84
29	1175	ND	ND	1391	796	ND	279.3	ND	ND

ND = Not detected

Discussion

Generally accepted that scaling and root planing are treatment of choice in periodontitis. In some cases that treatment failure occurred due to the incomplete removal of subgingival plaque and calculus, antimicrobial agents are given as an adjunct. Recently AP gel containing *Andrographis paniculata*, a medicinal plant with antimicrobial activity has been formulated (Narakorn, 1999) and used locally in periodontal pocket. However, at least one clinical study had failed to show the significant benefit of AP gel (Rassameemasmaung et al., 1998). This might be due to the low viscosity of the gel rendering the rapid diffusion of the active ingredient below the therapeutic levels. The new formula of AP gel with increased viscosity has been developed and used in this study. The efficiency of AP gel was evaluated by assessing clinical and microbiological parameters at disease sites.

Randomized single blinded controlled trial was used in this study. Three non-adjacent sites of each periodontitis patient were randomly assigned to one of the following treatments; 1) SRP with AP gel, 2) SRP with gel base, 3) SRP only. The parameters were monitored at day 0 (baseline), 1, and 3 months after treatment. Results showed that the clinical parameters

including PPD, CAL, GI and BOP were improved in all treatments. The results showed that all treatments were effective method of therapy for periodontal disease that resulted from the removal of microbial products, calcified deposits and contaminated from the root surface as well as reducing gingival inflammation, pocket depth and improving or maintaining attachment level (Hughes & Caffesse, 1978; Lisgarten et al., 1978; Mousques et al., 1980; Axelsson & Lindhe, 1978). Results from the comparison among treatments showed that the clinical outcomes of the group treated with gel base as an adjunct to SRP was similar to that treated with SRP only. Thus, it might concluded that this gel base formulation did not have effect on decreasing inflammation or healing promotion. However, the clinical parameters of the group treated SRP with AP gel was found to be significantly improved when compared with the other groups. It showed that the treatment with using the AP gel as an adjunct to scaling and root planing improved periodontal condition.

There are other studies that showed the advantage of using AP gel as an adjunct in periodontal treatment. Amornchat et al (1991) demonstrated that extracted *Andrographis paniculata* with 95% ethanol exhibited inhibitory activity against *P.gingivalis.* Atsawasuwan et al. (1998) also found that proportions of the black-pigmented anaerobes were significantly reduced in the pockets treated AP gel but not in those treated with metronidazole gel. The study by Boonchaipanichwatana (2001) found that the AP gel had a more consistent increase in percentage of cocci and a decrease in percentage of motile rods over the period of 3-4 months compared to 2% minocycline gel. The effect of AP gel on repairing periodontium was demonstrated by Noppamassiri (2009). They found AP gel and AP extract can enhance alkaline phosphatase activity and induction of mineralized nodule formation in gingival tissues at AP gel treated sites. These results suggested that AP gel and AP extract can promote the differentiation of human PDL cells into bone-forming cells. In addition, Sirirat & Rojanapanthu (2003) revealed that AP gel can improve the radiopaque fill in the periodontal defects at 3 and 6 months after loading AP gel. Results of previous and present study clearly showed that AP gel as an adjunct to scaling and root planing improved the clinical parameters of the periodontitis when compared to the SRP only. These suggest AP gel as benefit as an adjunct to scaling and root planing for treatment periodontal disease.

Regarding the microbiological parameter, the number of *P. gingivalis* in periodontal pocket was used as the marker since the bacteria has been considered as one of the major periodontal pathogens. The quantification of *P. gingivalis* was performed by real-time PCR method using EvaGreen®. PCR technique theoretically can be detected even the presence of a single copy of the target DNA. However, in this study, *P. gingivalis* could be detected only in eight patients (25%) from the total of thirty two periodontitis patients. The low detection rate might be explained by several factors, for example, the characteristics of patients most were localized chronic periodontitis, well-educationed as well as had ever treated periodontitis. The previous study (Fox et al., 1994) demonstrated that socio-economic factors that related to high periodontal disease experience are low education, low income and rural domicile, contrary the characteristics of almost patients in this study. Thus, *P. gingivalis* of subjects was not detected or very rare, particularly when the probing pocket depths were reduced to 2-3 mm after treatment. It was in agreement with the study of Wara-aswapati et al. (2009) demonstrated that the detection of P.gingivalis in non-periodontitis sites of the chronic periodontitis patients were markedly low and appeared to be similar to those of the non-periodontitis control subjects. The sampling technique is another factor that might has major impact on the detection and enumeration of the bacteria (Jervoe-Storm et al., 2007). Several studies showed the significant number of subgingival bacteria using paper point sampling (Jervoe-Storm et al., 2007; Renvert et al., 1992), notwithstanding the subgingival bacteria at apical portion of the narrow pockets may be difficult to reach due to the softness of paper points (Baker et al., 1991). Since the restricted number of patients in this study is the reason that the screening amount of *P. gingivalis* in each experimental teeth before treatment could not be done. However, based on the results obtained from eight subjects positive for *P. gingivalis*, five of them showed the decrease number of *P. gingivalis* in all groups after treatment 1 and 3 months. Among other 3 cases, case No.1 showed the recolonization of *P. gingivalis* at 3 months after treatment in all groups while case No. 9 showed the recolonization of *P. gingivalis* at 1 month after treatment in all groups. Besides case No. 28 showed the recolonization of *P. gingivalis* at 1 and 3 months after treatment in all groups. Clinical study (Kuphasuk et al., 2004) investigating the concentration of AP gel in gingival crevicular fluid showed that the concentration

of andrographolide at 24 hours after loading could be detected in the periodontal pocket at the concentration of 201.964 μg/ml in only one tooth from 15 teeth. Kuphasuk et al. (2008) examined the concentration of andrographolide in gingival crevicular fluid, saliva and blood plasma after application of AP gel into the periodontal pocket following treatment. They found that andrographolide could be detected in gingival crevicular fluid on the first hour and at 24 hours only two cases still had andrographolide at the concentration of 0.969 ± 2.9638 μg/ml. The andrographolide in saliva were found up to 1½ hours at the concentration of 0.2740 ±0.5354 μg/ml. It could not be detected in the blood plasma. The study presented that AP gel did not sustain in periodontal pocket for a long time. It is not surprised that *P. gingivalis* could be recolonizd in the pocket treated with AP gel after 1 or 3 months.

Since conventional periodontal therapy consists of mechanical debridement to disrupt the subgingival biofilms and appropriate home care oral hygiene. However, to achieve consistent success, it demands the compliance of the patients to take care their oral hygiene. The classical experimental gingivitis studies (Loe & Theilade, 1965) showed that clinical symptoms of gingivitis developed in students with clinically healthy gingival within two to three weeks if dental plaque was allowed to accumulate. When adequate oral hygiene measures were resumed, the gingival inflammation subsided within a week. The microbiological study (Magnusson et al., 1984) has shown that if supragingival plaque control is deficient, recolonization of subgingival biofilm may occur within in 4 to 8 weeks after scaling and root planing in sites with deep pockets.

Conclusion

This study presented clinical and microbiological results after treating the disease sites with *Andrographis paniculata* (AP) gel as an adjunct to scaling and root planing, comparing to those treated with SRP plus gel base and SRP only. The AP gel and gel base were applied at day 0, 1, 2 and 3 weeks. Four clinical parameters, i.e., probing pocket depth, clinical attachment level, gingival index and bleeding on probing were measured at day 0 (baseline), 1 and 3 months after treatments. Microbiological parameter was exhibited by quantification of *Porphyromonas gingivalis* in subgingival plaque by the real-time PCR method (EvaGreen®). The results showed that clinical changes at the disease sites were significantly improved after received all three treatments. When compared the clinical outcomes between the sites treated with AP gel and the sites treated with gel base and SRP only, the clinical outcomes of the AP gel-treated sites significantly showed a better improvement. In addition, the numbers of *P. gingivalis* in some sites were decreased and could not be detected at 1 and 3 months after treatment. In conclusion, the results indicate the benefit of local application of AP gel as an adjunct to SRP in the treatment of chronic periodontitis.

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