Topical application of herbal formula for the treatment of ligature-induced periodontitis

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Purpose: The aim of this study was to investigate the therapeutic effects of a herbal formula, PerioH-035, containing *Angelica sinensis*, steamed *Rehmannia glutinosa*, *Angelica dahurica*, *Cimicifuga heracleifolia*, and *Zanthoxylum piperitum* on the periodontal breakdown in a well-established ligature-induced periodontitis model in rats.

Methods: Sprague-Dawley rats were randomly assigned to 1 of 4 groups: NL (non-ligatured), L (ligatured), P1 (ligatured and treated with 1 mg/mL PerioH-035), P100 (ligatured and treated with 100 mg/mL PerioH-035). Periodontitis was induced by placing a ligature around the mandibular first molars. PerioH-035 was topically applied to both sides of the first molar for 2 weeks. The right side of the mandibles was retrieved for micro-computed tomography (CT) and methylene blue staining to analyze alveolar bone loss. The left side of the mandibles was histologically analyzed by TRAP and H&E staining. The MMP-9 mRNA level in gingival tissue was investigated by RT-PCR.

Results: Alveolar bone resorption was significantly reduced in the PerioH-035-treated groups. The number of dense multi-nucleated cells found to be TRAP-positive by staining in the ligatured rats was markedly decreased by PerioH-035 application. In addition, periodontal tissue destruction, especially cementum demineralization, was ameliorated in the P1 and P100 groups. Moreover, gingival tissue from the PerioH-035-treated group showed a decrease in the MMP-9 mRNA level, resulting in recovery of collagen degradation.

Conclusions: These results suggest that PerioH-035 has therapeutic effects on periodontitis, and thus, PerioH-035 shows promise as a treatment for periodontitis.

Keywords: Matrix metalloproteinase 9, Medicinal herbs, Osteoclasts, Periodontitis.

INTRODUCTION

Periodontitis is a common chronic and infectious disease caused by the accumulation of bacteria, leading to the destruction of the surrounding periodontal structure [1]. The fundamental treatment of periodontitis is to reduce the load of subgingival pathogenic bacteria by instrumental debridement by surgical or non-surgical approaches [2,3]. However, complete removal of pathogenic biofilm is impossible since some pathogens are embedded in soft tissue or located in anatomically inaccessible areas. Therefore, antibiotics or antiseptics are often applied as adjuvant treatment for periodontal infection in combination with mechanical instrumentation [4].

Currently, there are two methods of administering antibiotic therapy: locally and systemically. Several studies have demonstrated the beneficial effect of systemic application; however, adverse effects including overdose, bacterial resistance, or gastrointestinal intol-

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erance are frequently reported [5-7]. To overcome these shortcomings, locally delivered drug systems have been sought after, and promising results reported [8-12].

PerioH-035 is a newly combined formula for treating periodontitis, composed of five herbs: radix of *Angelica sinensis*, radix of steamed *Rehmannia glutinosa*, radix of *Angelica dahurica*, rhizome of *Cimicifuga heracleifolia*, and fruit of *Zanthoxylum piperitum*. *Angelica sinensis* has been reported to have diverse biological effects on bone metabolism, inflammation, oxidation, and periodontal regeneration [13,14]. Steamed *Rehmannia glutinosa*, which is a modified form of *Rehmannia* root, has been used to treat inflammatory diseases [15]. *Angelica dahurica* has various types of total coumarins, which were reported to possess anti-inflammatory and anti-oxidant effects [16]. Ferulic acid is regarded as the main active constituent of *Cimicifuga heracleifolia*, which was found to inhibit the production of macrophage inflammatory protein-2 in Raw 264.7 cells [17]. *Zanthoxylum piperitum* is commonly used as a seasoning spice and a traditional medicine for regulating circulation of the blood [18]. Based on previous studies, it was hypothesized that the local application of the combination of these extracts may modulate periodontitis without the initiation of systemic adverse reactions.

The aims of the study were to evaluate the radiographic and histological changes following the local application of PerioH-035 in a ligature induced-periodontitis rat model.

MATERIALS AND METHODS

Preparation of herbal formula

PerioH-035 is composed of the radix of *Angelica sinensis*, radix of steamed *Rehmannia glutinosa*, radix of *Angelica dahurica*, rhizome of *Cimicifuga heracleifolia*, and fruit of *Zanthoxylum piperitum*. Each 100 g of dried herb underwent extraction with 1,000 mL 50% ethanol for 24 hours at room temperature. The extracts of each herb were filtered, evaporated, and lyophilized. A freeze-dryer was used to dry the extracts of each herb. The powders obtained were stored at -20°C until use (*Angelica sinensis* 37.33 g, steamed *Rehmannia glutinosa* 50.12 g, *Angelica dahurica* 34.55 g, *Cimicifuga heracleifolia* 15.03 g, and *Zanthoxylum piperitum* 5.61 g). The final yields of each herb were 37.33%, 50.12%, 34.55%, 15.03%, and 5.61%, respectively. A voucher specimen (PD- PerioH-035) was deposited at our laboratory.

Animals

Male Sprague-Dawley rats aged 7 weeks (RaonBio Inc., Yongin, Korea) were housed in standard conditions (12 h light/dark cycle, 20±2°C temperature and 50±5% humidity) with access to food and water ad libitum. All experiments were conducted according to the guidelines of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Care and Use of Laboratory Animals of Kyung Hee University (KHUASP(SE)-14-029).

Ligature-induced periodontitis and sample treatment

The rats were randomly assigned to 1 of 4 groups (n=7, respectively): NL (non-ligatured), L (ligatured), P1 (ligatured and treated with 1 mg/mL PerioH-035), P100 (ligatured and treated with 100 mg/mL PerioH-035). The sample size was determined based on previous studies [19,20]. After adaptation for 1 week, a sterilized 3-0 nylon thread ligature was placed around the cervix of the bilateral mandibular first molars of rats under anesthesia with intraperitoneal injection of a tiletamine/zolazepam mixture (Zoletil 50; Virbac Lab, Carros, France), except in the NL group. For the treatment, 20 mg of 5 herbal powders were mixed and dissolved in 1 mL distilled water (100 mg/mL of PerioH-035). To enhance the viscosity, 1% carboxymethyl cellulose was added to the samples. The normal group, NL, received no treatment and the negative control group, L, was treated with vehicle gel. The PerioH-035-treated groups, P1 and P100, were topically treated with 100 μL of 1 and 100 mg/mL PerioH-035 on the bilateral first molars. The treatments were continued once per day for 2 weeks.

Micro-computed tomography analysis

The left mandibles were analyzed by micro-computed tomography (micro-CT; SkyScan, SCANCO USA, Southeastern, PA, USA), with a minor modification of a previously published protocol after excising the ligatures [21]. Using three-dimensional image reconstructions, the cementoenamel junction (CEJ) and marginal bone crest were drawn manually. Alveolar bone resorption was measured as the distance from the CEJ to the alveolar bone crest.

Methylene blue staining

The right mandibular molar regions were retrieved following the excision of the ligatures and fixed in 10% neutralized formalin for 18 hours. De-fleshed molar samples were stained with 1% aqueous methylene blue (Sigma, MO, USA) for 5 minutes. Digital photography was obtained from a 100 mm macro lens Canon digital camera (Canon, Tokyo, Japan). The root axis of the first molar teeth (three roots) was measured with a computerized densitometry system Image J (NIH, Bethesda, MD, USA). The amount of the alveolar bone loss (ABL) was the average of the values of the measurements from three points along the CEJ to the alveolar bone crest.

Hematoxylin and eosin staining

Following the micro-CT, the left mandibular molar regions were separated and fixed in 10% neutralized formalin for 18 hours. To decalcify, 0.1 M ethylene diamine tetraacetic acid aqueous solution was used for 2 months at room temperature with general shaking. The decalcified molars were embedded in paraffin. 7-μm cut sections were stained with hematoxylin and eosin (H&E). The digital images were acquired using Leica Application Suite (LAS) microscope software (Leica Microsystems, Buffalo Grove, IL, USA). The magnifications used were 40× and 200×.

Tartrate-resistant acid phosphatase (TRAP) staining

The 7-μm cut sections were stained with tartrate-resistant acid phosphatase (TRAP; Sigma, St. Louis, MO, USA) according to the manufacturer's instruction. Naphthol AS-BI phosphate was used as a substrate. The TRAP-stained slides were again stained with hematoxylin to identify the multinuclear cells. The digital images were acquired using Leica Application Suite (LAS) microscope software. The magnification used was 200×.

Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA from the detached gingival tissues was isolated with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. A volume of 1 μg of the total RNA from each sample was isolated and synthesized into cDNA using commercially available cDNA synthesis kits (Invitrogen Corp., Carlsbad, CA, USA) at 45℃ for 60 minutes and then at 95℃ for 5 minutes. The samples were then stored at −20℃ until further use. Amplification of cDNA was conducted with Taq polymerase and primers specific for matrix metalloproteinase (MMP)-9 mRNA and GAPDH. The following primers were used: MMP-9, 5′-GGG ACG CAG ACA TCG TCA TC-3′ (forward) and 5′-TCG TCA TCG TCG AAA TGG GC′ (reverse); and GAPDH, 5′-CCA TCA CCA TCT TCC AGG AG -3′ (forward) and 5′-CCT GCT TCA CCA CCT TCT TG-3′ (reverse). Optimum conditions for RT-PCR were established using routine methods. The conditions used for MMP-9 were 35 cycles of 94℃ for 20 seconds, 60℃ for 30 seconds and 72℃ for 2 minutes, with final extension at 72℃ for 10 minutes. The PCR products were electrophoresed in a 1% agarose gel. Relative mRNA expression was quantified with Quantity One software using the GAPDH mRNA expression level as a reference. Visualized bands were quantified using a computerized densitometry system, Image J.

Statistical analysis

Significance was determined by one-way analysis of variance (ANOVA) and Dunnett's multiple comparison tests. In all analyses, $P<0.05$ was taken to indicate statistical significance.

RESULTS

PerioH-035 treatment inhibited alveolar bone loss

Three-dimensional reconstruction from micro-CT images revealed the ABL, and the amount of exposed root surface was about 1.52 times higher in the L group, which was significant (NL = 785.67 ± 208.5 μm; L = 1,197.07 ± 234.22 μm; $P<0.001$), and the treatment of PerioH-035 100 mg/mL significantly inhibited the alveolar bone resorption by up to 15.30% (1,014.04 ± 221.81 μm; $P<0.05$) (Fig. 1). However, there was no statistical difference between L and P1.

Similar results were shown in the methylene blue staining. The rats with ligatures showed significant ABL—about 1.32 times that of NL (NL = 2,720 ± 130 μm; L = 3,610 ± 280 μm; $P<0.001$). It was observed that treatment with PerioH-035 1 and 100 mg/mL (3,003 ± 140 and 2,920 ± 310 μm; 16.81% and 19.11%, respectively) significantly reversed the ABL caused by ligature-induced periodontitis (Fig. 2).

PerioH-035 treatment decreased the number of osteoclast cells

Multinuclear cells, which appeared TRAP-positive cells, were markedly increased by ligature induction as shown in the L group. Increased TRAP-positive cells were reduced by the treatment with

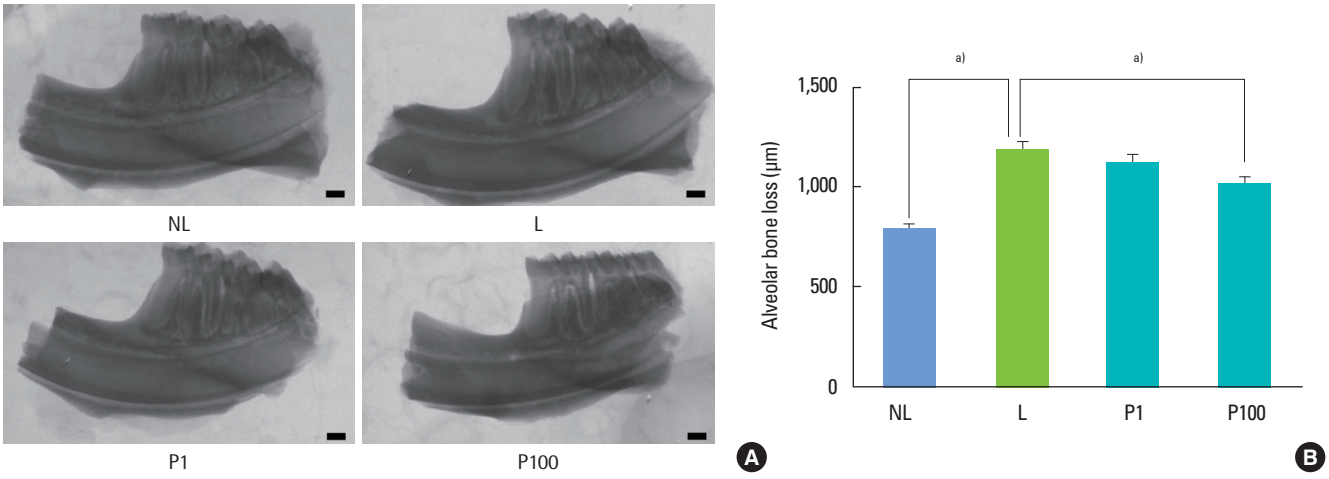

Figure 1. The effect of PerioH-035 on alveolar bone loss indicated by three-dimensional reconstruction from micro-CT images. PerioH-35 is an herbal formula, containing *Angelica sinensis*, steamed *Rehmannia glutinosa*, *Angelica dahurica*, *Cimicifuga heracleifolia*, and *Zanthoxylum piperitum*. Representative micro-computed tomography images are shown (A) and the measurement of the alveolar bone resorption is illustrated (B). The scale bar is 1 mm. Data shown are mean ± S.E.M (NL = 785.67 ± 208.5 μm; L = 1,197.07 ± 234.22 μm; P1 = 1,140.03 ± 261.30 μm; P100 = 1,014.04 ± 221.81 μm). Statistically significant differences between groups ([a])$P<0.001$). NL, non-ligatured; L, ligatured; P1, ligatured and treated with 1 mg/mL PerioH-035; P100, ligatured and treated with 100 mg/mL PerioH-035.

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PerioH-035, especially in the P100 group (Fig. 3).

PerioH-035 treatment maintained the integrity of periodontal structures

In the ligature-induced periodontitis model, severe inflammatory infiltration of the gingival tissues were observed compared with the NL group and exhibited loss of attachment, severe bone resorption, apical migration of junctional epithelium, and cementum demineralization. Several resorption pits were also observed on the cementum surface. However, the treatment with 100 mg/mL PerioH-035 appeared to prevent the destruction of periodontal tissues and the connective attachment was maintained over the cementum lining along the root surface (Fig. 4).

PerioH-035 treatment reduced the MMP-9 mRNA expression in periodontal tissues

The expression of MMP-9 mRNA of gingival tissue in the L group was significantly higher than that in the NL group (NL = 1 ± 0.08; L = 14.1 ± 0.11; $P < 0.05$). However, topical application of PerioH-035 significantly decreased the expression of MMP-9 mRNA in gingival tissues at all concentrations compared with the L group (P1 = 8.7 ± 0.29; P100 = 7.3 ± 0.34; $P < 0.05$) (Fig. 5).

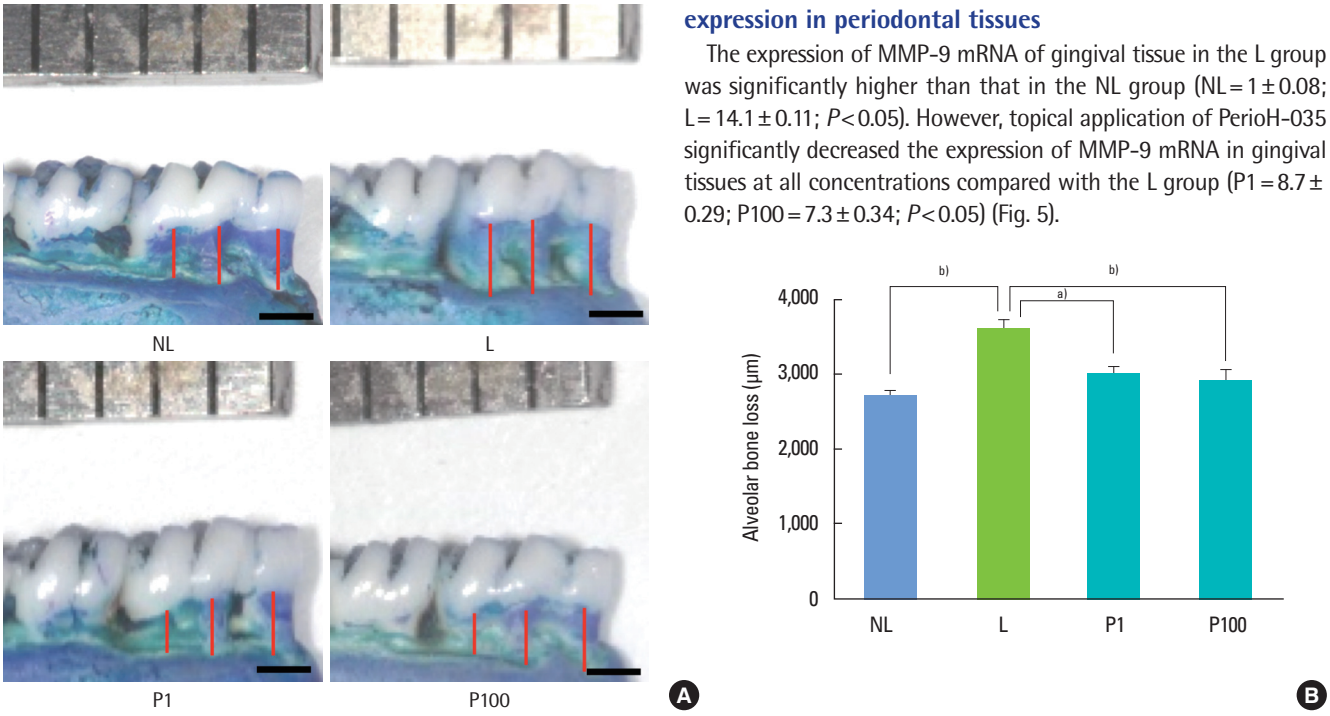

Figure 2. The effect of PerioH-035 on alveolar bone loss indicated by methylene blue staining. PerioH-35 is an herbal formula, containing *Angelica sinensis*, steamed *Rehmannia glutinosa*, *Angelica dahurica*, *Cimicifuga heracleifolia*, and *Zanthoxylum piperitum*. (A) Representative images demonstrate the alveolar bone resorption. The scale bar is 1 mm. (B) The measurement of vertical alveolar bone resorption is illustrated, and the application of P100 has significantly inhibited the alveolar bone resorption. The red lines represent the distance from the cementoenamel junction to the alveolar bone crest. Data shown are mean ± S.E.M (NL = 2,720 ± 130 μm; L = 3,610 ± 280 μm; P1 = 3,003 ± 140 μm; P100 = 2,920 ± 310 μm). Mean values were significantly different among groups. ([a])$P < 0.05$, ([b])$P < 0.01$). NL, non-ligatured; L, ligatured; P1, ligatured and treated with 1 mg/mL PerioH-035; P100, ligatured and treated with 100 mg/mL PerioH-035.

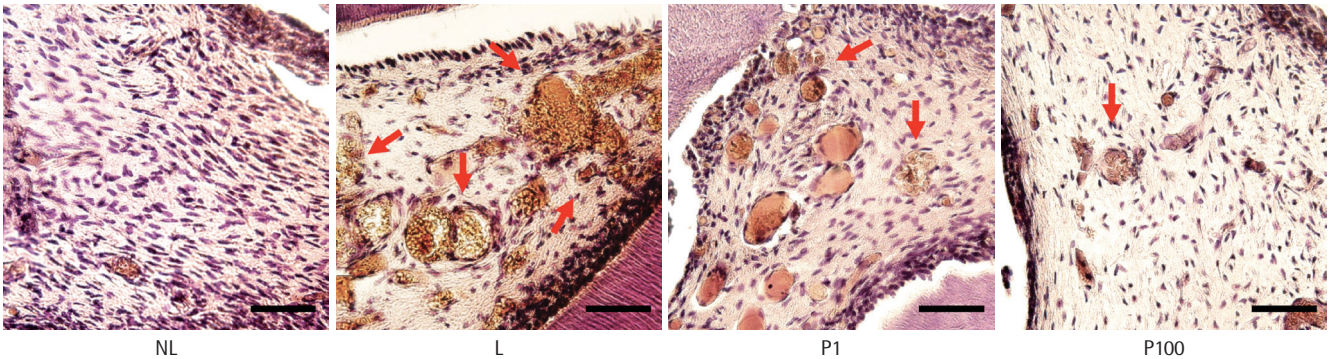

Figure 3. The effect of PerioH-035 on osteoclastic activity indicated by tartrate-resistant acid phosphatase (TRAP) staining. PerioH-35 is a herbal formula, containing *Angelica sinensis*, steamed *Rehmannia glutinosa*, *Angelica dahurica*, *Cimicifuga heracleifolia*, and *Zanthoxylum piperitum*. The arrows indicate TRAP-positive cells. Scale bar = 100 μm. NL, non-ligatured; L, ligatured; P1, ligatured and treated with 1 mg/mL PerioH-035; P100, ligatured and treated with 100 mg/mL PerioH-035.

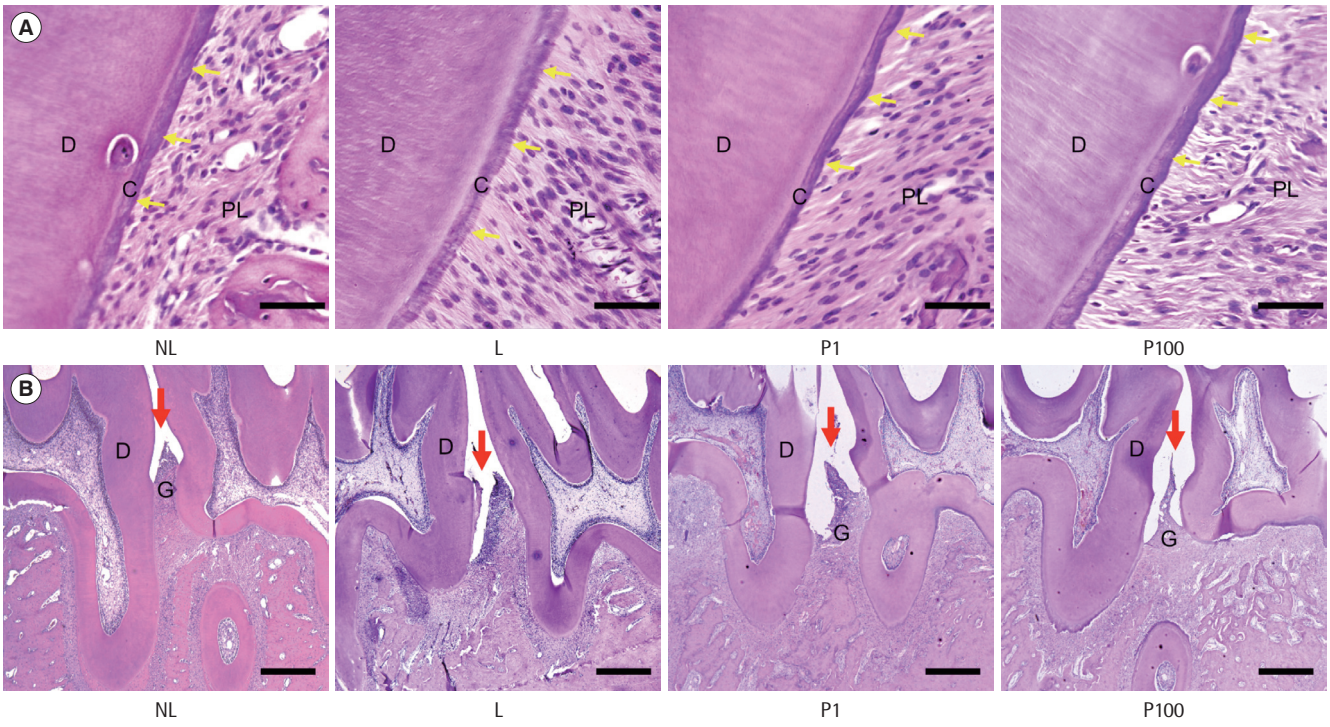

Figure 4. The effect of PerioH-035 on histological changes of the periodontium. PerioH-35 is an herbal formula, containing *Angelica sinensis*, steamed *Rehmannia glutinosa*, *Angelica dahurica*, *Cimicifuga heracleifolia*, and *Zanthoxylum piperitum*. The yellow arrows indicate root cementum (A). The red arrows indicate gingival tissue (B). The thickness and the structure appears intact in the P100 group. Significant destruction of gingival tissue is also visible in the experimental periodontitis groups; however, the P100 group shows the pattern with the least destruction. C, cementum; D, dentin; PL, periodontal ligament; G, gingiva; NL, non-ligatured; L, ligatured; P1, ligatured and treated with 1 mg/mL PerioH-035; P100, ligatured and treated with 100 mg/mL PerioH-035. Scale bar = 100 μm (A) and 400 μm (B). H&E staining.

DISCUSSION

To understand the etiology of periodontal disease and to develop potential periodontal treatment drugs, various animal study models have been developed [22,23]. Among the various experimental periodontitis models, the rat model with ligature-induced periodontitis is one of the most widely used and evidence-based models [24]. The current study model has been utilized frequently in previous studies, and it has been clearly shown that ligature-induced periodontitis resulted in increased alveolar bone resorption and the infiltration of inflammatory cells along with an increase in pathogenic microbes [25]. In the present study, we postulated that the herbal extract compound PerioH-035 would reduce the inflammatory reaction in a ligature-induced model and might prevent periodontal tissue destruction during the induction of experimental periodontitis.

It is well established that bone is remodeled by interactions with bone formation by osteoblasts and bone resorption by osteoclasts [26]. ABL is an especially typical characteristic of periodontitis, evidencing the series of events following alveolar bone resorption [27]. However, topical PerioH-035 application into the mandibular first molar has significantly reduced ABL as shown in three-dimensional reconstructions from micro-CT images and methylene blue staining. In addition, osteoclastic activity, indicated with TRAP-positive brown staining, was decreased by PerioH-035 treatment. These results collectively suggested that PerioH-035 possibly ameliorated alveolar bone resorption via inhibition of osteoclasts, resulting in a decrease in ABL.

Tissue destruction, such as cementum demineralization and gingival collapse, are typical manifestations of the progress of periodontitis [28]. The blurred line regarded as irregular cementum is one of the clinical characteristics of periodontal breakdown. In addition, collagen denaturation in gingival tissue with severe inflammatory infiltrate aggravates alveolar bone destruction [25]. In this study, PerioH-035 application at a 100 mg/mL concentration showed a decrease in bone resorption pits, apical migration of the junctional epithelium, and cementum demineralization, as well as collapsed gingiva, indicating that PerioH-035 was well applied over the cementum lining along the root surface and the connective attachment.

Collagenolytic enzymes such as MMP play a critical role in collagen degradation in the periodontal tissues [29,30]. In particular, MMP-9 enzyme activity has been reported to have a vital relationship with periodontitis by degrading denatured interstitial collagens [31,32]. Ligature application in the lower first molar appeared to induce higher expression of MMP-9 compared to a non-ligatured

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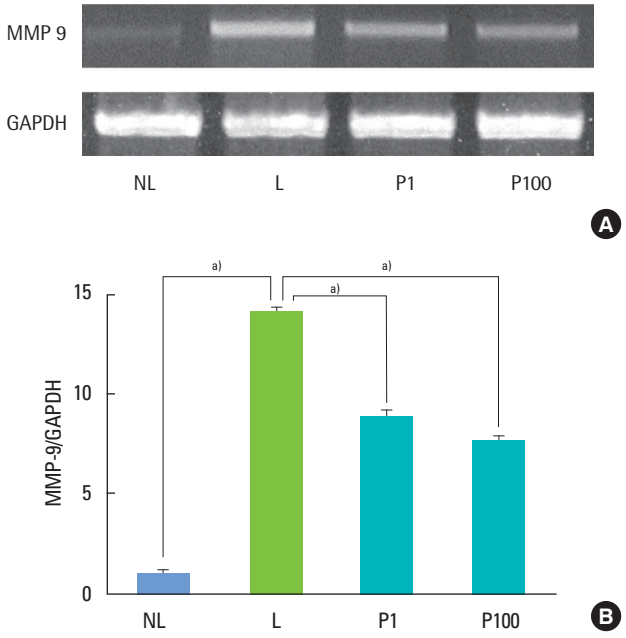

Figure 5. The effect of PerioH-035 on the expression of MMP-9 mRNA. PerioH-35 is an herbal formula, containing *Angelica sinensis*, steamed *Rehmannia glutinosa*, *Angelica dahurica*, *Cimicifuga heracleifolia*, and *Zanthoxylum piperitum*. Polymerase chain reaction results are shown (A), and a dose-dependent decreasing pattern of inverse relationship is observed. The ratio of the expression of MMP-9 in comparison to GAPDH is illustrated (B). The data shown are mean ± S.E.M (NL = 1 ± 0.08; L = 14.1 ± 0.11; P1 = 8.7 ± 0.29; P100 = 7.3 ± 0.34). Application of P1 and P100 has significantly reduced the expression of MMP-9 ($^{a)}P < 0.05$). MMP-9, matrix metalloproteinase-9; NL, non-ligatured; L, ligatured; P1, ligatured and treated with 1 mg/mL PerioH-035; P100, ligatured and treated with 100 mg/mL PerioH-035.

model, and it was demonstrated that MMP-9 was significantly inhibited by PerioH-035. Collectively, collagen degradation in the gingival tissues seems to be reversed along with PerioH-035 treatment via a decrease of MMP-9 expression.

In summary, application of a novel herbal extract, PerioH-035, inhibited alveolar bone resorption by the reduction of osteoclastic activity, maintained the integrity of periodontal structures such as irregular cementum and connective attachment, and lowered collagen degradation in gingival tissues by down-regulating the expression of MMP-9. These results suggest that PerioH-035 has ameliorative effects on the progress of periodontal breakdown and might be utilized for the treatment or the prevention of periodontal diseases.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Socransky SS, Haffajee AD. Evidence of bacterial etiology: a historical perspective. Periodontol 2000 1994;5:7-25.
2. Apatzidou DA, Kinane DF. Nonsurgical mechanical treatment strategies for periodontal disease. Dent Clin North Am 2010;54:1-12.
3. Lindhe J, Westfelt E, Nyman S, Socransky SS, Haffajee AD. Long-term effect of surgical/non-surgical treatment of periodontal disease. J Clin Periodontol 1984;11:448-58.
4. van Winkelhoff AJ, Rams TE, Slots J. Systemic antibiotic therapy in periodontics. Periodontol 2000 1996;10:45-78.
5. Mahmood MM, Dolby AE. The value of systemically administered metronidazole in the modified Widman flap procedure. J Periodontol 1987;58:147-52.
6. Aimetti M, Romano F, Guzzi N, Carnevale G. Full-mouth disinfection and systemic antimicrobial therapy in generalized aggressive periodontitis: a randomized, placebo-controlled trial. J Clin Periodontol 2012;39:284-94.
7. Kapoor A, Malhotra R, Grover V, Grover D. Systemic antibiotic therapy in periodontics. Dent Res J (Isfahan) 2012;9:505-15.
8. Jones AA, Kornman KS, Newbold DA, Manwell MA. Clinical and microbiological effects of controlled-release locally delivered minocycline in periodontitis. J Periodontol 1994;65:1058-66.
9. Paquette D, Oringer R, Lessem J, Offenbacher S, Genco R, Persson GR, et al. Locally delivered minocycline microspheres for the treatment of periodontitis in smokers. J Clin Periodontol 2003;30:787-94.
10. Williams RC, Paquette DW, Offenbacher S, Adams DF, Armitage GC, Bray K, et al. Treatment of periodontitis by local administration of minocycline microspheres: a controlled trial. J Periodontol 2001;72:1535-44.
11. Greenstein G, Polson A. The role of local drug delivery in the management of periodontal diseases: a comprehensive review. J Periodontol 1998;69:507-20.
12. Goodson JM, Offenbacher S, Farr DH, Hogan PE. Periodontal disease treatment by local drug delivery. J Periodontol 1985;56:265-72.
13. Zhao H, Alexeev A, Sharma V, Guzman LD, Bojanowski K. Effect of

SBD.4A--a defined multicomponent preparation of Angelica sinensis--in periodontal regeneration models. Phytother Res 2008; 22:923-8.
14. Lim DW, Kim YT. Anti-osteoporotic effects of Angelica sinensis (Oliv.) Diels extract on ovariectomized rats and its oral toxicity in rats. Nutrients 2014;6:4362-72.
15. Lee B, Shim I, Lee H, Hahm DH. Rehmannia glutinosa ameliorates scopolamine-induced learning and memory impairment in rats. J Microbiol Biotechnol 2011;21:874-83.
16. Deng GG, Wei W, Yang XW, Zhang YB, Xu W, Gong NB, et al. New coumarins from the roots of Angelica dahurica var. formosana cv. Chuanbaizhi and their inhibition on NO production in LPS-activated RAW264.7 cells. Fitoterapia 2015;101:194-200.
17. Sakai S, Kawamata H, Kogure T, Mantani N, Terasawa K, Umatake M, et al. Inhibitory effect of ferulic acid and isoferulic acid on the production of macrophage inflammatory protein-2 in response to respiratory syncytial virus infection in RAW264.7 cells. Mediators Inflamm 1999;8:173-5.
18. Li X, Kim HY, Cui HZ, Cho KW, Kang DG, Lee HS. Water extract of Zanthoxylum piperitum induces vascular relaxation via endothelium-dependent NO-cGMP signaling. J Ethnopharmacol 2010; 129:197-202.
19. Lee J, Choi YY, Kim MH, Han JM, Lee JE, Kim EH, et al. Topical application of Angelica sinensis improves pruritus and skin inflammation in mice with atopic dermatitis-like symptoms. J Med Food. Forthcoming 2015.
20. Choi YY, Kim MH, Lee JY, Hong J, Kim SH, Yang WM. Topical application of Kochia scoparia inhibits the development of contact dermatitis in mice. J Ethnopharmacol 2014;154:380-5.
21. Park JC, Su C, Jung IH, Choi SH, Cho KS, Kim CK, et al. Mechanism of alveolar bone loss in a collagen-induced arthritis model in mice. J Clin Periodontol 2011;38:122-30.
22. Garcia de Aquino S, Manzolli Leite FR, Stach-Machado DR, Francisco da Silva JA, Spolidorio LC, Rossa C Jr. Signaling pathways associated with the expression of inflammatory mediators activated during the course of two models of experimental periodontitis. Life Sci 2009;84:745-54.
23. Baker PJ, Dixon M, Evans RT, Dufour L, Johnson E, Roopenian DC. CD4(+) T cells and the proinflammatory cytokines gamma interferon and interleukin-6 contribute to alveolar bone loss in mice. Infect Immun 1999;67:2804-9.
24. Bak EJ, Kim JH, Lee DE, Park BH, Ryu JH, Cha JH, et al. Inhibitory effect of SPA0355, a thiourea analogue, on inflammation and alveolar bone loss in rats with ligature-induced periodontitis. Int J Oral Biol 2012;37:63-8.
25. Napimoga MH, Benatti BB, Lima FO, Alves PM, Campos AC, Pena-Dos-Santos DR, et al. Cannabidiol decreases bone resorption by inhibiting RANK/RANKL expression and pro-inflammatory cytokines during experimental periodontitis in rats. Int Immunopharmacol 2009;9:216-22.
26. Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 1999;20:345-57.
27. Zhou T, Chen D, Li Q, Sun X, Song Y, Wang C. Curcumin inhibits inflammatory response and bone loss during experimental periodontitis in rats. Acta Odontol Scand 2013;71:349-56.
28. Page RC. Milestones in periodontal research and the remaining critical issues. J Periodontal Res 1999;34:331-9.
29. Smith PC, Muñoz VC, Collados L, Oyarzún AD. In situ detection of matrix metalloproteinase-9 (MMP-9) in gingival epithelium in human periodontal disease. J Periodontal Res 2004;39:87-92.
30. Santos J, La VD, Bergeron C, Grenier D. Inhibition of host- and bacteria-derived proteinases by natural anthocyanins. J Periodontal Res 2011;46:550-7.
31. Kudalkar MD, Nayak A, Bhat KS, Nayak RN. Effect of Azadirachta indica (Neem) and Aloe vera as compared to subantimicrobial dose doxycycline on matrix metalloproteinases (MMP)-2 and MMP-9: An in-vitro study. Ayu 2014;35:85-9.
32. Kusano K, Miyaura C, Inada M, Tamura T, Ito A, Nagase H, et al. Regulation of matrix metalloproteinases (MMP-2, -3, -9, and -13) by interleukin-1 and interleukin-6 in mouse calvaria: association of MMP induction with bone resorption. Endocrinology 1998;139:1338-45.