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# Effect of *Garcinia mangostana* L. and propolis extracts on the inhibition of inflammation and alveolar bone loss in ligature-induced periodontitis in rats

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The purpose of this study was to evaluate the effect of mangosteen extract complex (MEC; *Garcinia mangostana* L. and propolis extracts) on the inhibition of inflammation and prevention of alveolar bone loss using a ligature-induced periodontitis model. Rat molars were ligatured with silk, and 1 μg/mL of lipopolysaccharide of *Porphyromonas gingivalis* was injected into the buccal and palatal gingivae of the teeth with or without treatment with the MEC. Changes in the expression levels of prostaglandin $E_2$ ($PGE_2$), interleukin-8 (IL-8), inducible nitric oxide synthase (iNOS), matrix metalloproteinase-8 (MMP-8), cyclooxygenase (COX)-1, and COX-2 in gingival tissues were evaluated using enzyme-linked immunosorbent assays. Alveolar bone loss around the ligated molars was examined using micro-computed tomography. The expression levels of $PGE_2$, IL-8, iNOS, MMP-8, COX-1, and COX-2 in gingival tissues were significantly reduced in the group treated with a mixture of 16 μg of mangosteen extract powder and 544 μg of propolis extract powder (ligation [Lig] + lipopolysaccharide extracted from *P. gingivalis* KCOM 2804 [L] + MEC 1:34). Additionally, alveolar bone loss was significantly reduced in the Lig + L + MEC 1:34 group compared with that in other groups. These results indicate that the MEC could be useful in preventing and treating periodontitis.

**Keywords:** Mangosteen extract complex, Anti-inflammatory, Alveolar bone loss

## Introduction

Periodontal disease, characterized by alveolar bone resorption and bacterial pathogen-induced inflammation, is a chronic inflammatory disease. It has a significant impact on oral health [1]. Bacteria in subgingival dental plaque (biofilm) are the major causative agent of periodontal disease. *Porphyromonas gingivalis*, is one of the key pathogens in periodontal destruction and is highly associated with periodontal disease development [2]. *P. gingivalis* induced the secretion of inflammation-inducing molecules, including interleukin (IL)-6, IL-8, prostaglandin $E_2$ ($PGE_2$), inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, and matrix metalloproteinases (MMPs) and causes osteoclast formation in periodontal tissue [3]. Zhang et al. [4] observed that *P. gingivalis* invaded alveolar osteoblasts in a periodontitis mouse model. So, it is necessary to study de-

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velopment of materials inhibiting expression of these inflammatory factors and inhibitory effect of alveolar bone resorption. Development of the therapy has evolved from use of natural materials. Among these, *Garcinia mangostana* L. (mangosteen) and propolis have been shown to have anti-inflammatory and osteogenic effects in the wound healing process [5-7].

Propolis is a natural product produced by the honey bee. It possesses several biological properties, such as antioxidant and anti-inflammatory activities [6,8]. Previous research has shown that ethanol extract of propolis on mouse paw edema inhibited production of nitric oxide (NO) [9]. Additionally, bone mineral density was higher in rats that received oral propolis treatment [8]. Mangosteen extract contains abundant xanthones such as α, β, and γ-mangosteens [10]. Many biological properties such as anti-inflammatory, anti-oxidative damage and antioxidant activities from xanthones have been reported [11,12]. Previous studies have shown that α-mangosteen significantly inhibits NO, $PGE_2$, tumor necrosis factor (TNF)-α, and iNOS production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells [3,13].

Animal models have been used to study pathogenesis of periodontal disease and to develop new treatment and prevention agents. Ligature-induced periodontitis model in rat has been frequently used in periodontitis-related research [14]. Ligature-induced periodontitis in rats is reliable and extensively used to reproduce periodontitis because periodontal anatomy in the molar is similar to that of humans [15]. The purpose of this study was to evaluate the effect of mangosteen extract complex (MEC; *G. mangostana* L. and propolis extract) on inhibition of inflammation and alveolar bone loss in ligature-induced periodontitis model in rat.

## Materials and Methods

### 1. Bacteria culture and extraction of lipopolysaccharide

*P. gingivalis* KCOM 2804 was used in this study, and was obtained from the Korean Collection for Oral Microbiology (KCOM; Gwangju, Korea). The strain was cultured in tryptic soy broth (TSA; BD Difco Laboratories, Franklin Lakes, NJ, USA) supplemented with 0.5% yeast extract, 0.05% cysteine HCl-$H_2O$, 0.5 mg/mL hemin, and 2 μg/mL vitamin $K_1$ at 37℃ in an anaerobic chamber (Bactron I; Sheldon Manufacturing Inc., Cornelius, OR, USA) with 10% $H_2$, 5% $CO_2$, 85% $N_2$ gas condition. LPS of *P. gingivalis* KCOM 2804 was extracted, according to enclosed protocol using the LPS Extraction Kit (iNtRon, Seongnam, Korea). Extracted LPS was dissolved in sterilized water filtered with 0.22 μm membrane. Extracted LPS was lyophilized and weighed (*P. gingivalis* KCOM 2804 strain LPS extracted, was called pgLPS, and pgLPS is denoted by L).

### 2. Experimental animals

A total of 40 male Wistar rats (weighing 250-400 g) were used in the experiment. In this study, selection and management of the animals, surgical protocol, and preparation procedures, were approved by the Chosun University Institutional Animal Care and Use Committee (CIACUC2017-S0020).

The animals were randomly divided into four groups. The mangosteen complex extracts powder corresponding to each group, were suspended in water and taken into rats. Experimental groups were as follows:

1) Non-ligation (Lig) group (negative control, n = 10): non-ligation + distilled water (DW)

2) Lig + L + DW group (positive control, n = 10): silk-ligation + pgLPS injection + DW

3) Lig + L + MEC 0.5:34 group (n = 10): silk-ligation + pgLPS injection + 8 μg mangosteen extract powder and 544 μg propolis extract powder (0.5:34)

4) Lig + L + MEC 1:34 group (n = 10): silk-ligation + pgLPS injection + 16 μg mangosteen extract powder and 544 μg propolis extract powder (1:34)

### 3. Silk-ligature-induced periodontitis rat model

The experiment was conducted for three weeks and all procedures were performed under anesthesia with intramuscular injection of Zoletil® (20 mg/kg; Virbac, Carros, France) and Rompun® (7.5 mg/kg; Bayer, Ansan, Korea). A 5-0 silk-ligature (0.5-0.6 mm in diameter) was fixed in the cervical part of the maxillary second molar and all ligatures were positioned subgingivally. After ligation, 1 μg/mL pgLPS diluted by phosphate-buffered saline (PBS) was injected into buccal and palatal gingiva of the ligated teeth to induce periodontitis. The pgLPS was reinjected under anesthesia after one and two weeks (Fig. 1).

In experimental groups, mangosteen extract powder and propolis extract powder were suspended in 0.1 mL of sterilized water and administered orally using a feeding needle. In the control groups, the same amount of DW was administered. At three weeks, rats were sacrificed by carbon dioxide inhalation. After sacrifice, gingival tissue around the ligated maxillary sec-

ond molar was obtained. It was stored at −80℃, for analysis of expression of specific inflammatory mediators. Remaining tissues were fixed with 10% formaldehyde for taking micro-computed tomography (micro-CT).

## 4. Expression of inflammatory cytokines in gingival tissues

To measure expression changes of inflammatory mediators ($PGE_2$, IL-8, iNOS, MMP-8, COX-1, and COX-2) in gingival tissue, the ELISA kit of the Mybiosource Company (San Diego, CA, USA) was used, according to manufacturer's instructions. Stored gingival tissue was homogenized with PBS and centrifuged to separate the supernatant. The supernatant was used and analyzed according to manufacturer's instructions. Results are expressed as the mass (ng/mL, pg/mL) or unit (U/L) of each protein present in gingival tissue according to manufacturer's instructions.

## 5. Micro-computed tomography imaging

Collected tissues were scanned with a Quantum GX micro-CT Imaging System (PerkinElmer, Hopkinton, MA, USA) for two minutes at an acceleration potential of 90 kVp, and a beam current of 88 μA. Micro-CT data were reconstructed and formatted using OnDemand3D™ (Cybermed, Seoul, Korea) software. Through this process, three-dimensional structures around the teeth and around the alveolar bone were evaluated in all directions and all widths. Distance between the cemento-enamel junction (CEJ) and coronal plane of alveolar bone crest (ABC) was measured at six sites. It was measured at mesio-buccal, disto-buccal, mid-buccal, mesio-palatal, disto-palatal, and mid-palatal sites of the maxillary left and right second molars.

## 6. Statistical analysis

Data are presented, as average mean ± standard devia-

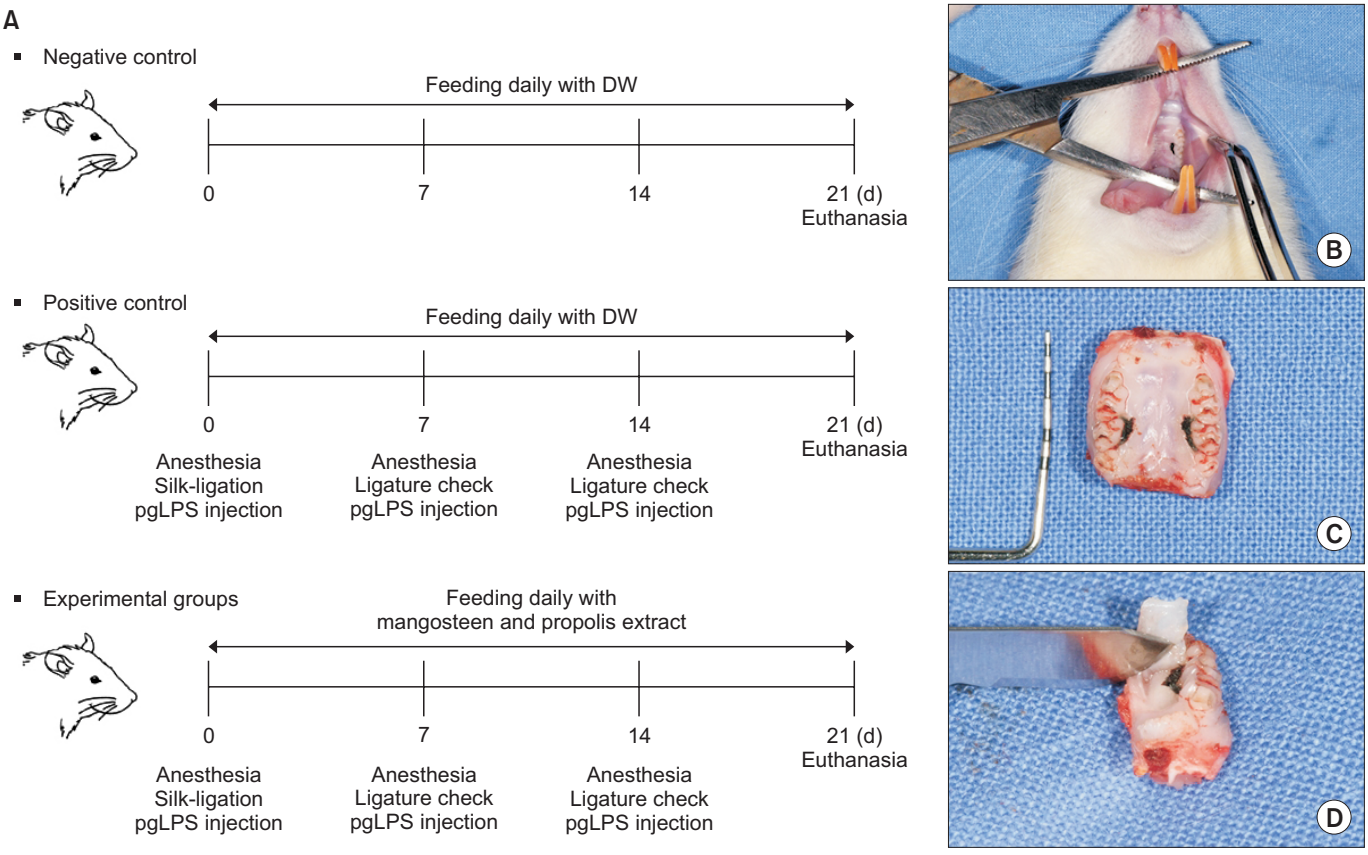


**Fig. 1.** Experimental design of silk-ligature induced periodontitis model in rats. (A) Diagram of study. (B) Silk-ligature placement in cervical part of the second molar. (C) Maxillary tissue of rat. (D) Collection of gingival soft tissue around the second molar area.
DW, distilled water; pgLPS, lipopolysaccharide extracted from *P. gingivalis* KCOM 2804.

tion. Statistical analysis was performed, using SPSS (version 12; SPSS Inc., Chicago, IL, USA). To compare the control and experimental groups, the Mann–Whitney U test was used. Statistical significance was determined by $p < 0.05$ (95% confidence level).

## Results

### 1. Expression of inflammatory cytokines in gingival tissues

Expression level of inflammatory cytokines, such as $PGE_2$, IL-8, iNOS, MMP-8, COX-1, and COX-2 in gingiva tissues had a tendency to increase in the group Lig + L + DW than that of group Non-Lig. In the group Lig + L + MEC 1:34, expression levels of $PGE_2$, IL-8, iNOS, MMP-8, COX-1, and COX-2 were significantly decreased compared to the groups Non-Lig, Lig + L + DW, and Lig + L + MEC 0.5:34 (Fig. 2). Compared with the group Lig + L + DW, expression levels of $PGE_2$, IL-8, iNOS, MMP-8, COX-1, and COX-2 in the group Lig + L + MEC 1:34 were significantly reduced and in particular, expression of iNOS was downregulated by 91.1% ($p < 0.05$) (Fig. 2).

Considering these results, the group Lig + L + MEC 1:34 was most effective in inhibiting expression levels of $PGE_2$, IL-8, iNOS, MMP-8, COX-1, and COX-2 in gingival tissues of ligature-induced periodontitis rats model. The inhibition of inflammatory cytokines in gingival tissues of rats model suggested that Lig + L + MEC 1:34 may be associated with decreased

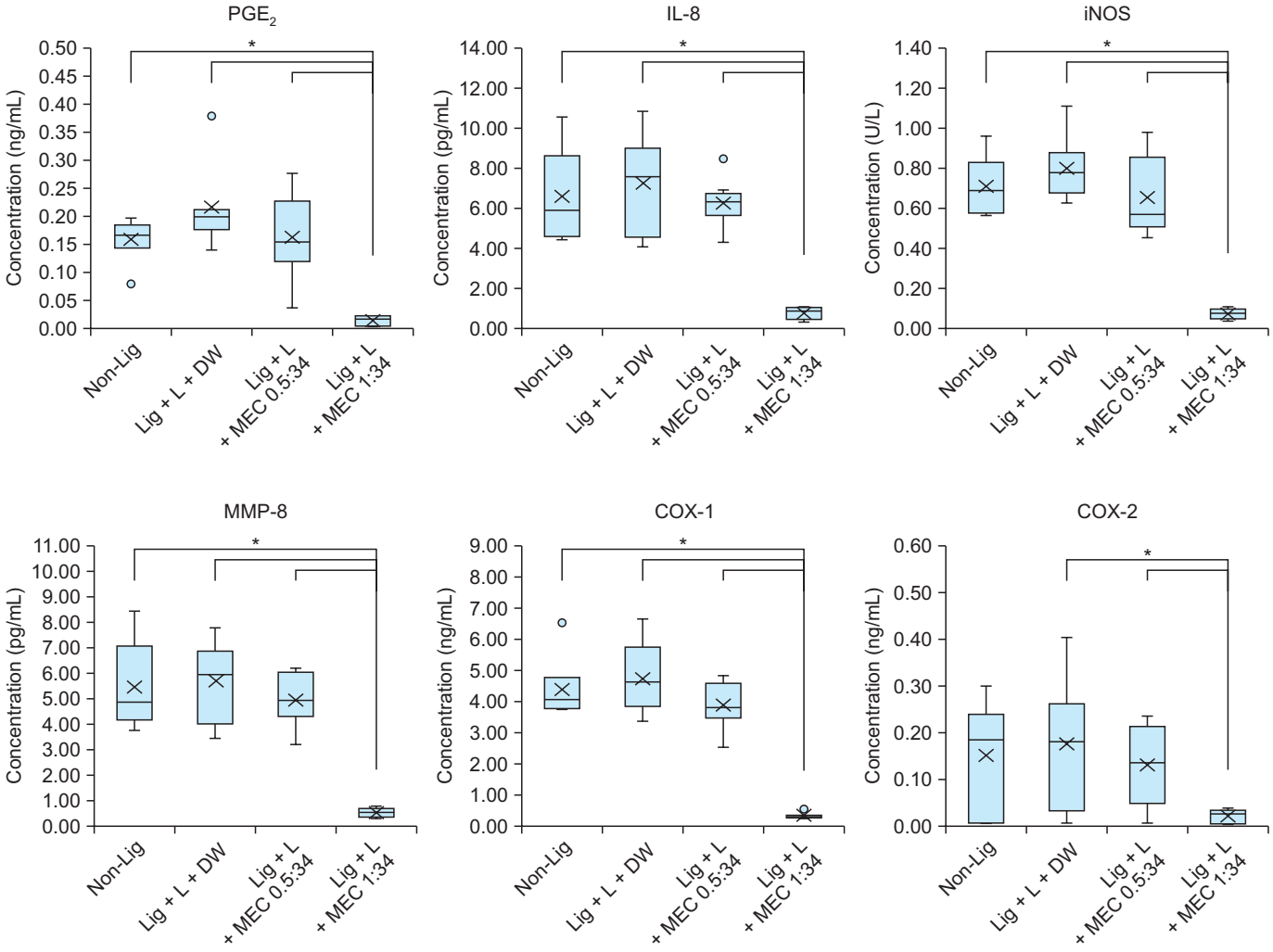


**Fig. 2.** Expression of inflammatory cytokines ($PGE_2$, IL-8, iNOS, MMP-8, COX-1, and COX-2) in gingival tissues.
Lig, ligation; L, lipopolysaccharide extracted from *P. gingivalis* KCOM 2804; DW, distilled water; MEC, mangosteen extract complex; $PGE_2$, prostaglandin $E_2$; IL-8, interleukin-8; iNOS, inducible nitric oxide synthase; MMP-8, matrix metalloproteinase-8; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2.
*$p < 0.05$; Mann–Whitney U test.

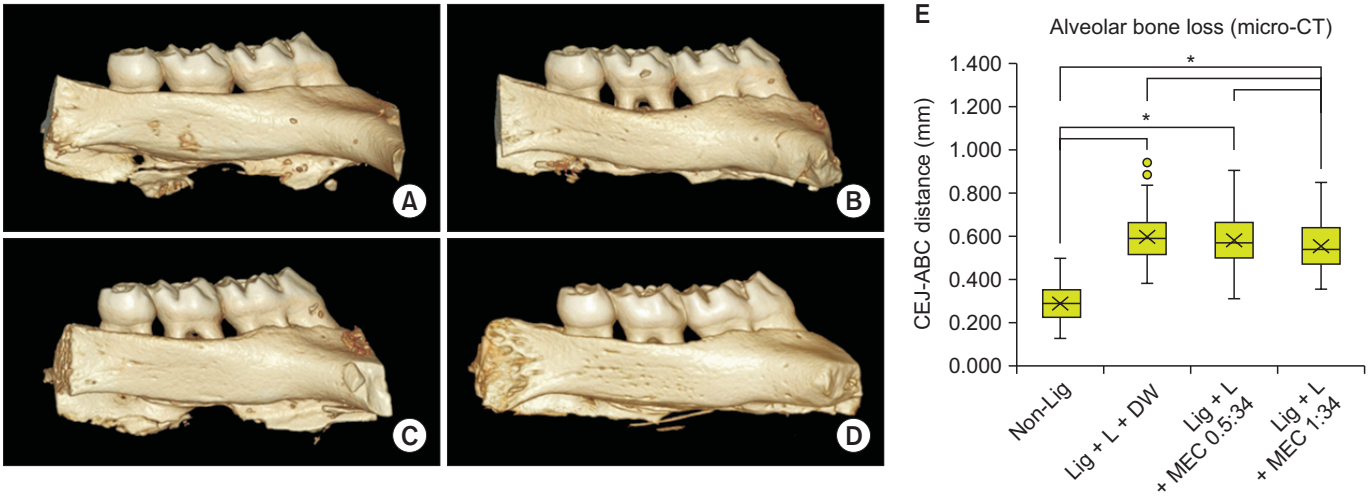


**Fig. 3.** 3-Dimensional micro-computed tomography (micro-CT) image of alveolar bone loss around the maxillary second molar. Non-Lig group (A), Lig + L + DW group (B), Lig + L + MEC 0.5:34 group (C), Lig + L + MEC 1:34 group (D), and distance between cemento-enamel junction (CEJ) and alveolar bone crest (ABC) (E).
Lig, ligation; L, lipopolysaccharide extracted from *P. gingivalis* KCOM 2804; DW, distilled water; MEC, mangosteen extract complex.
*$p < 0.05$; Mann–Whitney U test .

progression of periodontitis and inhibition of alveolar bone resorption.

### 2. Alveolar bone loss in ligature-induced periodontitis rats model

Alveolar bone loss was significantly increased in the group Lig + L + DW compared to the group Non-Lig. In contrast, it was reduced in the groups Lig + L + MEC 0.5:34 and Lig + L + MEC 1:34 compare to the group Lig + L + DW (Fig. 3). To evaluate degree of periodontal destruction, the distance between the CEJ and ABC was expressed as the amount of alveolar bone destruction. Distance from the CEJ to ABC in the groups Lig + L + MEC 0.5:34 and Lig + L + MEC 1:34 was significantly reduced by 4.2% and 13%, respectively, compared to the group Lig + L + DW ($p < 0.05$) (Fig. 3). These data showed that MEC exhibited an inhibitory effect on alveolar bone loss by marked decrease of CEJ-ABC distances in rats by ligature-induced periodontitis.

## Discussion

Periodontitis, one of the most common diseases in adults, is an inflammatory disease caused by periodontal pathogens in plaque on the surface of teeth. These pathogens promote production of inflammatory cytokines such as $PGE_2$, and cytokines IL-1, IL-6, IL-8, TNF-$\alpha$, and MMPs resulting in bone destruction [16,17].

Results of this study showed that MEC was significantly inhibited expression levels of inflammatory mediators ($PGE_2$, iNOS, MMP-8, IL-8, COX-1, and COX-2) in gingival tissues induced periodontitis by silk-ligature and injection of pgLPS. Especially, the expression $PGE_2$ in the group Lig + L + MEC 1:34 markedly decreased than that in the group Non-Lig. In previous study, MEC 1:34 showed anti-inflammatory and *in vitro* bone formation effects in human gingival fibroblasts and human osteosarcoma cell lines (in submission). This result suggests that MEC 1:34 may alleviate the inflammatory state of rat by reducing the expression of $PGE_2$ at the basic level. $PGE_2$ is known to be an important inflammatory mediator causing alveolar bone destruction and osteoclast formation [18].

Degree of alveolar bone loss is one of the indicators of severity of periodontal disease [19]. Previous studies have shown that 500 μg/mL mangosteen extract did significantly inhibition, in LPS-induced bone destruction by osteoclast [20]. It has been reported that the group treated with 100 mg/kg propolis in rats with expanded premaxillary suture reduced alveolar bone loss and a number of osteoclast compared to the control group [21]. Micro-CT study showed significant reduction of alveolar bone loss (13%) in the group Lig + L + MEC 1:34. This study validates for the first time that MEC (mixture of mangosteen and propolis) reduced alveolar bone loss through inhibition of inflammatory responses in rats with ligature- and pgLPS-induced periodontitis. These results suggest that MEC

could be useful in preventing and treating periodontitis.

## Acknowledgements

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## Conflicts of Interest

Dae Sung Lee is a co-register on patent (No.: 10187939-80000, Korea) entitled "Pharmaceutical composition for enhancing osteogenesis comprising *Garcinia mangostana* extract and propolis extract" and on patent (No.: US 10,022,412 B2 and JP 6387467 B2) entiled "Composition for preventing or alleviating periodontal diseases, containing, as active ingredients, mangosteen extract or α- or γ-mangosteen."

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