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Original Article

Antcin K suppresses osteoclastogenesis through modulation of the focal adhesion kinase and phosphoinositide 3-kinase pathways and attenuates ligature-induced periodontitis

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KEYWORDS
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Abstract *Background/purpose:* Periodontitis is a chronic inflammatory condition characterized by host-mediated destruction of periodontal tissue and alveolar bone. Elevated proinflammatory cytokines and osteoclast activation are key factors contributing to this breakdown. Antcin K, a triterpenoid derived from *Antrodia cinnamomea*, exhibits immunomodulatory and anti-inflammatory properties. This study aimed to investigate whether Antcin K suppresses osteoclast formation and prevents the progression of periodontitis.
Materials and methods: Receptor activator of nuclear factor κB ligand (RANKL)-induced osteoclastogenesis in murine macrophage cell line RAW 264.7 cells was evaluated following Antcin K

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treatment. RNA sequencing and pathway analysis revealed the involvement of the focal adhesion kinase (FAK)-phosphoinositide 3-kinase (PI3K) signaling axis, which was further validated by Western blotting. *In vivo*, a ligature-induced periodontitis rat model was used to assess osteoclast activity, RANKL/osteoprotegerin (OPG) expression, and alveolar bone preservation following Antcin K treatment.
Results: Antcin K significantly suppressed RANKL-induced osteoclast formation *in vitro*. Transcriptomic and biochemical analyses indicated that inhibition of the FAK-PI3K signaling cascade mediates its suppressive effect. *In vivo*, Antcin K reduced osteoclast numbers, lowered the RANKL/OPG ratio, and alleviated alveolar bone resorption in ligature-induced periodontitis.
Conclusion: Antcin K inhibits osteoclastogenesis by modulating the FAK—PI3K signaling pathway and attenuates alveolar bone loss. These findings suggest that Antcin K may serve as a potential host-modulatory therapeutic agent for the treatment of periodontitis.

Introduction

Periodontal disease is a chronic inflammatory condition affecting the tissues supporting the teeth and remains a major public health concern because of its high prevalence and association with tooth loss.[1,2] Its multifactorial etiology includes poor oral hygiene, smoking, and systemic diseases such as diabetes mellitus.[3,4] Currently, the primary goal of clinical therapies for periodontitis is to eradicate pathogenic bacteria from periodontal tissues using mechanical or antibacterial methods.[5] However, these strategies often fail to suppress the hyperactive immune response that damages periodontal tissues.[6] Among the cellular mediators, osteoclasts—multinucleated cells derived from hematopoietic stem cells—play a central role in alveolar bone resorption and therefore represent a crucial therapeutic target for periodontitis management.

Osteoclasts originate from monocyte/macrophage lineage precursors that migrate from the bone marrow to resorption sites under the regulation of receptor activator of nuclear factor κB (RANK) and its ligand, receptor activator of nuclear factor κB ligand (RANKL).[7,8] The RANKL/RANK axis activates intracellular signaling through tumor necrosis factor receptor—associated factor 6 (TRAF6), which in turn stimulates other signaling molecules.[9] Blocking RANKL signaling effectively inhibits osteoclastogenesis and alleviates bone-destructive diseases, including periodontitis.[10]

Owing to their low toxicity and diverse biological activities, both natural compounds and synthetic molecules derived from natural prototypes have garnered significant attention.[11] *Antrodia cinnamomea* (*A. cinnamomea*), a rare medicinal fungus native to Taiwan, is recognized for its potent anti-inflammatory, hepatoprotective, anticancer, immunomodulatory, and antioxidative properties.[12—14] Research conducted both *in vitro* and *in vivo* has shown that Antcin K, a triterpenoid derived from *A. cinnamomea*, possesses anti-angiogenic and anti-inflammatory properties.[15] In the case of chondrosarcoma, Antcin K prevents metastasis by reducing the production of matrix metalloproteinase-7.[16] In particular, Antcin K showed possible anti-arthritic effects based on its anti-inflammatory properties.[15,17] According to a recent report, Antcin K diminishes skeletal muscle injury and inflammation by boosting interleukin-10 production.[18] However, the effects of osteoclast formation and periodontitis treatments remain largely unknown. Therefore, this study investigated whether Antcin K suppresses RANKL-induced osteoclastogenesis through modulation of focal adhesion kinase (FAK) and phosphoinositide 3-kinase (PI3K) signaling pathways, and whether it could prevent alveolar bone loss in a ligature-induced periodontitis model.

Materials and methods

Antibodies and reagents

The antibodies against phosphorylated FAK (p-FAK), FAK, PI3K p85 subunit (p-p85), and p85 were acquired from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Cell culture supplements were obtained from Invitrogen (Carlsbad, CA, USA). All other reagents were purchased from Sigma—Aldrich (St. Louis, MO, USA).

Cell culture

The murine macrophage cell line RAW 264.7 was purchased from the Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin. The cultures were maintained in a humidified incubator at 37 °C with 5 % CO_2.

Cell viability

Cells were seeded in 96-well culture plates and treated with or without Antcin K at various concentrations for 24 h. After incubation, 0.5 mg/mL of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution

(Sigma–Aldrich) was added to each well and dissolved in dimethyl sulfoxide (DMSO; Sigma–Aldrich). The absorbance was measured at 570 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA).

Osteoclast differentiation

RAW 264.7 cells (2×10^4 cells/well) were cultured in 24-well plates and stimulated with RANKL at 50 ng/mL (PeproTech, Cranbury, NJ, USA) in the presence or absence of Antcin K. After incubation for 5 days, the cells were stained using a tartrate-resistant acid phosphatase (TRAP) staining kit (Sigma–Aldrich). Cells exhibiting TRAP-positive staining and containing three or more nuclei were identified as mature osteoclasts, following our previous study.[19]

Western blot analysis

Proteins (30 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes in accordance with the procedures described in our previous studies.[20,21] The membranes were blocked with 4 % non-fat milk in phosphate-buffered saline with 0.1 % Tween-20 (PBST) for 1 h at room temperature, followed by incubation with primary antibodies for 1 h and subsequently with horseradish peroxidase (HRP)–conjugated secondary antibodies for 1 h.

Immunoreactive bands were visualized using a Fujifilm LAS-3000 imaging system (Fujifilm, Tokyo, Japan).

RNA sequencing and data analysis

Total RNA was extracted from RAW 264.7 cells treated with or without Antcin K using TRIzol reagent (MDBio, Taipei, Taiwan). RNA sequencing (RNA-seq) was performed by Biotools Co., Ltd. (New Taipei City, Taiwan) using a NovaSeq X platform (Illumina, San Diego, CA, USA). Data analysis was conducted using the Biotools Cloud Platform (https://cloud.toolsbiotech.com/login). Differentially expressed genes identified from the data were analyzed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/pathway.html) to explore potential signaling pathways and associated biological functions.[22]

Ligature-induced periodontitis model

Eight-week-old Wistar rats, purchased from the National Laboratory Animal Center (Taipei, Taiwan), were housed in the animal facility at China Medical University. All experimental procedures were approved by the Animal Research Ethics Committee (approval no. CMUIACUC-2024-029-2). The rats were randomly allocated into three groups as follows: (1) Control group, receiving phosphate-buffered saline (PBS) without ligature placement; (2) Periodontitis group (PD), receiving PBS with ligature placement; and (3) Antcin K-treated group, receiving 30 mg/kg of Antcin K (intraperitoneal injection) with ligature placement.

The ligature placement around the first molar was performed as previously described.[23] Under anesthesia, a sterile 3-0 silk suture was tied around the cervical region of the first molars bilaterally in the maxilla. The suture knot was secured with composite resin on the mesial aspect of the first molar. In the Control group, no ligature was placed on the first molars.

After 7 days of ligation, the rats were euthanized, and both the left and right maxillae were harvested for subsequent analyses.

Micro-computed tomography analysis

Micro-computed tomography (micro-CT) analysis was performed on the left maxilla 7 days after ligation. Fixed specimens were scanned using a micro-CT system (SkyScan 2211, Bruker, Kontich, Belgium) under the following parameters: tube voltage, 65 kV; current, 80 μA; image resolution, 1024 × 1024 pixels and slice thickness of 15 μm.[24,25] The distance from the palatal cemento-enamel junction (CEJ) to the alveolar bone crest (ABC) was assessed according to previously established methods.[24,25] The mean of three measured distances was used as the representative value for each rat. Volumetric measurements of the furcation region were conducted after defining a region of interest (ROI) based on standardized dimensions and anatomical landmarks: (a) a line 0.5 mm apical to the CEJ of the first molar served as the coronal limit, extending vertically 1 mm towards the root apex; (b) a mesial-distal dimension of 3 mm from the mesial CEJ of the first molar; and (c) a buccal-palatal dimension of 2.5 mm.

Histopathological evaluation

The right maxilla was decalcified in a 10 % ethylenediaminetetraacetic acid (EDTA; Cyrusbioscience, Seattle, WA, USA) solution at 4 °C for a duration of four weeks. Following decalcification, the tissues were embedded in paraffin, and bucco-lingual sections measuring 5 μm in thickness were prepared for hematoxylin and eosin (H&E) staining (Sigma–Aldrich). TRAP staining was performed using a commercial kit (Sigma–Aldrich) according to the manufacturer's instructions. Multinucleated cells containing three or more nuclei that exhibited TRAP positivity were identified as osteoclasts. Quantification of osteoclast number and area was performed using ImageJ software (NIH, Bethesda, MD, USA). Osteoclast number was counted using the Multi-point tool, and osteoclast area was measured using the Polygon selection tool. Data from three independent experiments were analyzed. For immunohistochemical (IHC) analysis, tissue sections were incubated with specific primary antibodies following established protocols. Sections were stained with antibodies targeting RANKL (ab9957; Abcam, Cambridge, UK) or OPG (ab734007; Abcam, Cambridge, UK).[26] Briefly, sections were incubated with primary antibodies, followed by secondary antibody binding and 3,3′-diaminobenzidine (DAB) development using a Novolink™ Polymer Detection System (Leica Biosystems, Buffalo Grove, IL, USA). Staining intensity and percentage scores were combined to calculate the final staining index.[27]

Real-time quantitative polymerase chain reaction amplification

Total RNA was extracted from the tissues of the left maxilla using a TRIzol reagent kit (MDBio, Taipei, Taiwan) according to the manufacturer's protocol. RNA quantity and purity were determined by measuring A_{260}/A_{280} ratios with a NanoVue spectrophotometer (GE Healthcare, Chicago, IL, USA). Complementary DNA (cDNA) was synthesized from 1 μg total RNA using an M-MLV reverse transcription kit

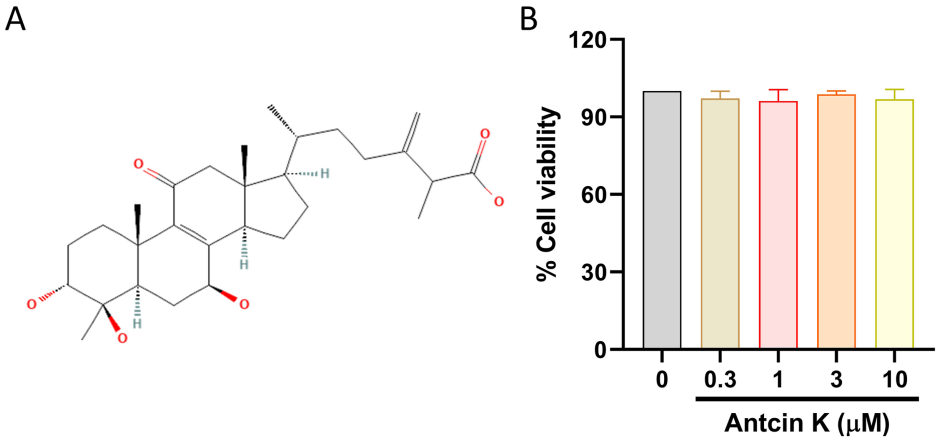

Figure 1 Antcin K did not affect cell viability in RAW264.7 cells. (A) Chemical structure of Antcin K. (B) RAW264.7 cells were treated with Antcin K (0.3—10 μM) for 24 h, and the cell viability was examined by MTT assay. MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

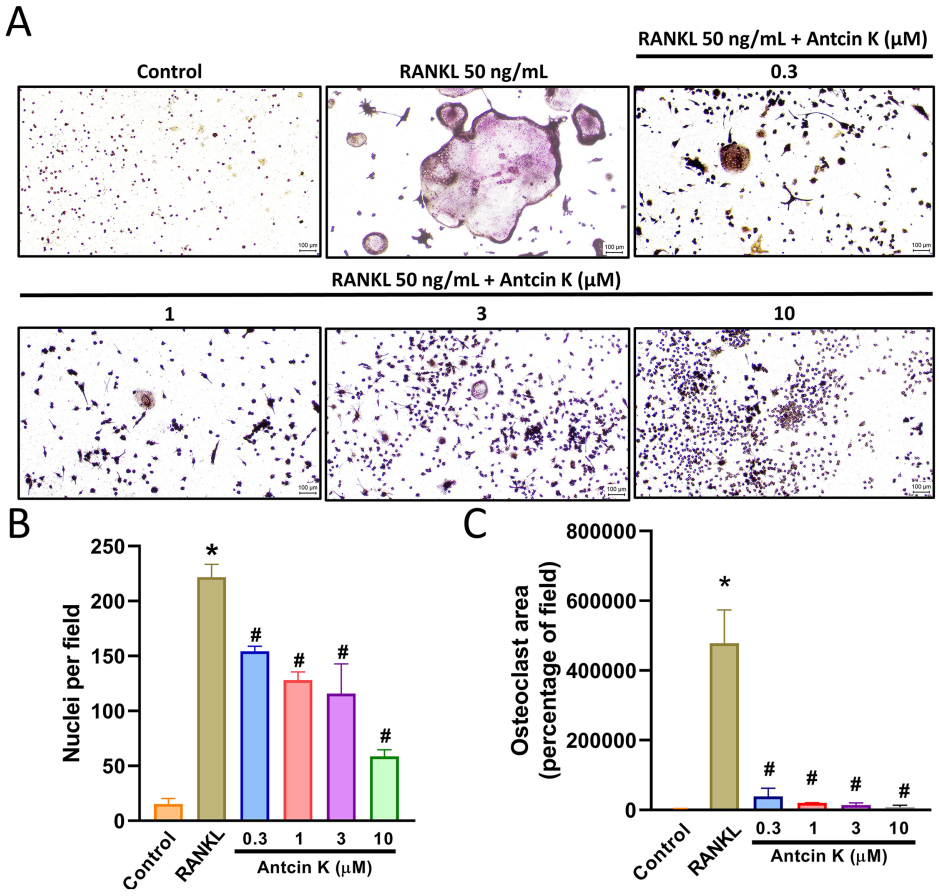

Figure 2 Antcin K inhibits RANKL-induced osteoclast formation. (A) RAW264.7 cells were treated with RANKL and Antcin K for 5 days, and the osteoclast number (B) and area (C) were analyzed by TRAP staining. $^{*}P < 0.05$ compared with the control group. $^{\#}P < 0.05$ compared with the RANKL-treated group. RANKL: receptor activator of nuclear factor-κB ligand; TRAP: tartrate-resistant acid phosphatase.

(Invitrogen), following the manufacturer's instructions. Real-time quantitative polymerase chain reaction (qPCR) was performed using a KAPA SYBR FAST qPCR Kit (Roche, Basel, Switzerland).[28,29]

Statistical analysis

The data are presented as mean ± standard deviation (SD). Statistical significance between experiment groups was

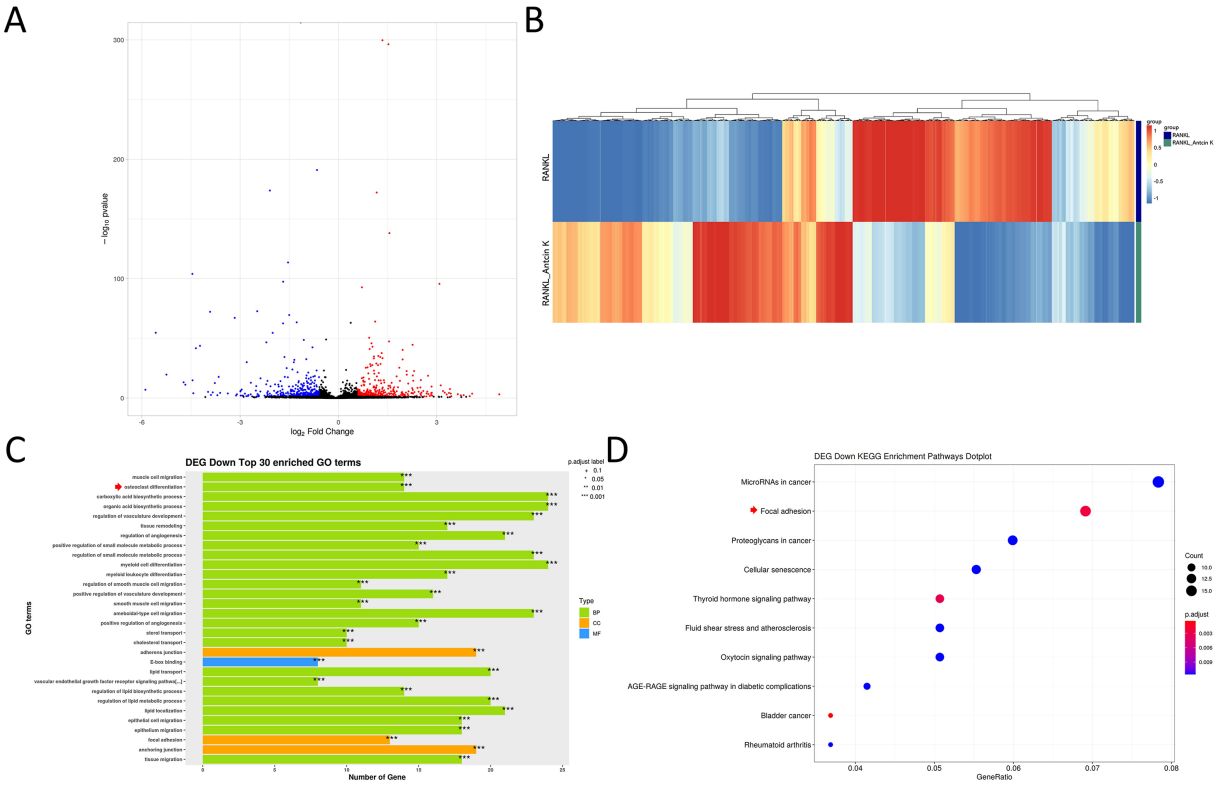

Figure 3 RNA-seq analysis indicated that the focal adhesion pathway is a potential target of Antcin K. (A) The volcano plot shows the fold change in gene expression after Antcin K treatment. (B) The result of a heatmap of RNA sequencing showing differentially expressed genes in RAW264.7 cells with or without Antcin K treatment. (C) The biological processes and cellular function were analyzed by the GO database. (D) Enrichment signaling pathways were analyzed by the KEGG database. RNA-seq: RNA sequencing; GO: gene ontology; KEGG: Kyoto encyclopedia of genes and genomes.

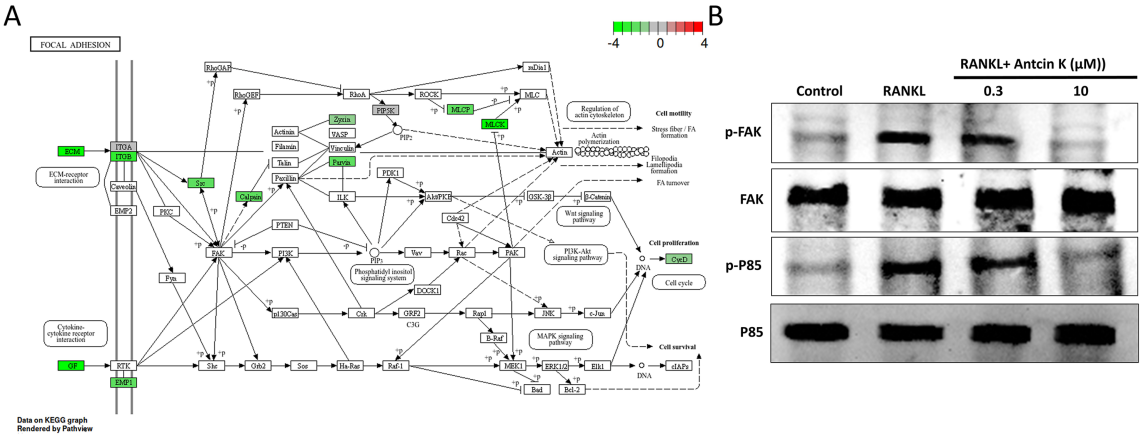

Figure 4 FAK and PI3K pathways are involved in Antcin K's effects. (A) Enrichment diagram showing pathways that involve the FAK and PI3K signaling pathways. (B) RAW264.7 cells were treated with RANKL and Antcin K The FAK and PI3K phosphorylation was examined by Western blotting. $^{*}P < 0.05$ compared with the control group. $^{\#}P < 0.05$ compared with the RANKL-treated group. FAK: focal adhesion kinase; PI3K: phosphoinositide 3-kinase; RANKL: receptor activator of nuclear factor-κB ligand.

evaluated using the two-tailed Student's t-test. Comparisons involving more than two groups were conducted using one-way ANOVA followed by Tukey's post hoc test to determine the significance of differences between groups, with a significance level set at 0.05. All analyses were performed with GraphPad Prism 8.0.1 (GraphPad Software, Boston, MA, USA).

Results

Antcin K inhibits RANKL-induced osteoclast formation

Periodontitis, a condition characterized by excessive alveolar bone resorption, is strongly associated with an increase in both the number and activity of osteoclasts. Consequently, osteoclasts are considered critical therapeutic targets for treating disorders involving alveolar bone loss. We used a macrophage culture system to examine the anti-osteoclast formation effects of Antcin K (Fig. 1A). First, stimulation of RAW264.7 cells with Antcin K did not affect cell viability, nor did it promote cell proliferation, as determined by the MTT assay (Fig. 1B). After being treated with RANKL for five days, RAW264.7 cells developed into large, multinucleated mature osteoclasts. TRAP staining was used to identify osteoclasts, and the number and area of TRAP-positive multinucleated cells were quantified (Fig. 2). Antcin K significantly inhibited RANKL-induced osteoclast differentiation in a concentration-dependent manner.

Antcin K suppresses RANKL-induced FAK and PI3K signaling pathways

To investigate the molecular mechanisms underlying Antcin K's anti-osteoclast effects, we conducted RNA-seq analysis

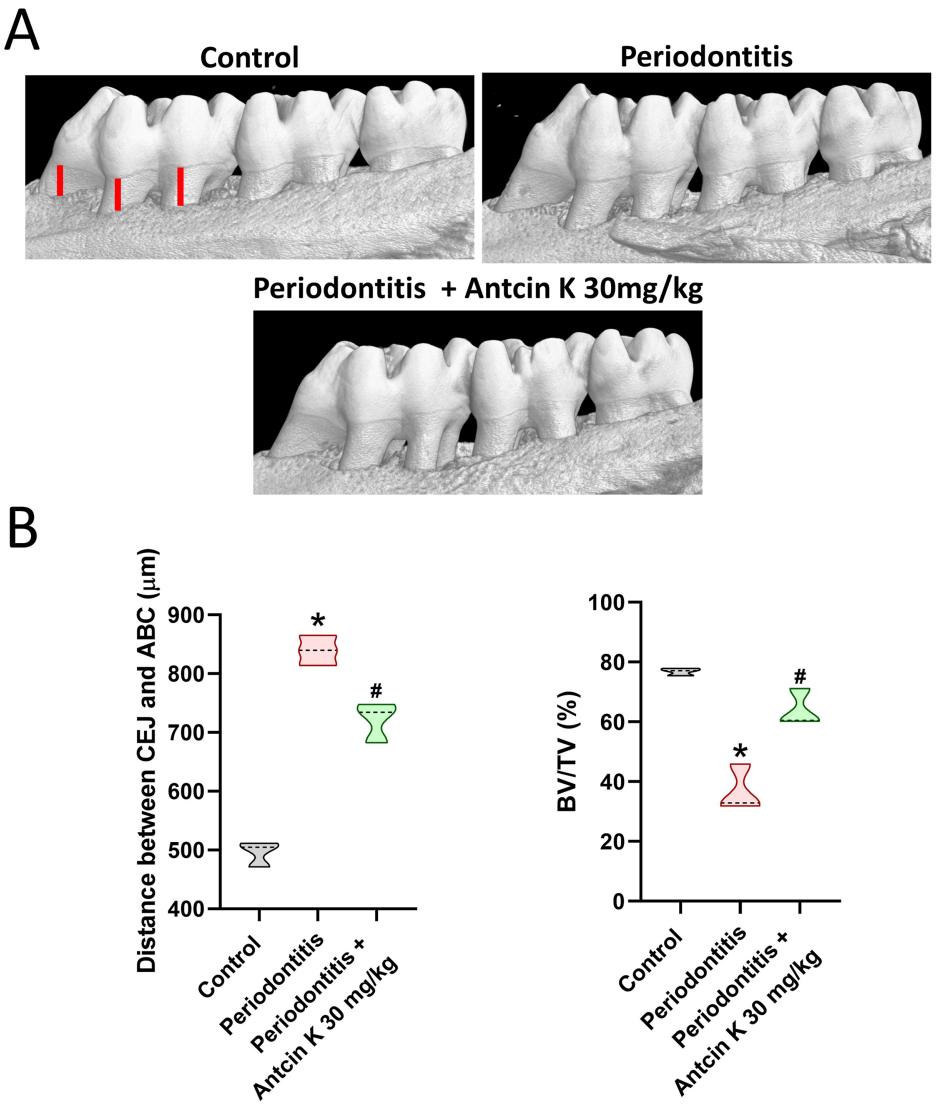

Figure 5 Antcin K inhibits ligature-induced periodontitis *in vivo*. (A) Micro-CT images from the left side of the maxilla of control, PD, and Antcin K-treated groups. (B) Quantitative analyses of the CEJ-ABC distances and BV/TV data. $^{*}P < 0.05$ compared with the control group. $^{\#}P < 0.05$ compared with the PD group. Micro-CT: micro-computed tomography; PD: periodontitis group; CEJ—ABC: cementoenamel junction—alveolar bone crest; BV/TV: bone volume/tissue volume.

on RAW 264.7 cells treated with and without Antcin K. Changes in gene expression following Antcin K treatment were visualized in volcano and heatmap plots (Fig. 3A and B). Biological process analysis using the Gene Ontology (GO) indicated involvement of osteoclast differentiation (Fig. 3C). Antcin K was found to mediate the focal adhesion signaling pathway (Fig. 3D), which involves the FAK and PI3K pathways, as revealed by KEGG enrichment pathway analysis (Fig. 4A). Antcin K treatment of RAW 264.7 cells inhibited the phosphorylation of FAK and PI3K induced by RANKL (Fig. 4B). These findings indicate that Antcin K reduces osteoclast generation through the FAK and PI3K signaling pathways.

Antcin K inhibits ligature-induced periodontitis *in vivo*

To assess the therapeutic effects of Antcin K *in vivo*, a ligature-induced periodontitis model was used. Micro-CT analysis showed that the CEJ to ABC distances were elevated in the ligature group, while Antcin K treatment significantly reduced these ligature-enlarged CEJ-ABC distances (Fig. 5A and B). Furthermore, the ligature group exhibited a significant reduction in bone volume to total volume (BV/TV) (Fig. 5B). H&E and TRAP staining revealed that Antcin K prevented the ligature-induced thinning and discontinuity of the junctional epithelium, as well as osteoclast formation (Fig. 6A and B). Additionally, IHC and qPCR analysis showed a significant increase in RANKL and a reduction in OPG production in the periodontal tissue of the ligature group (Fig. 6C and D). This upregulated RANKL/OPG ratio was markedly reduced in the Antcin K-treated group (Fig. 6D).

Discussion

Periodontal disease is a condition characterized by tissue destruction resulting from the interplay between periodontopathogenic bacteria and the host immune response. Certain oral bacterial populations in dental plaque are precursors to periodontal disease development; however, once the disease is initiated, additional factors influence its progression and complicate treatment.[30] Major contributors to periodontal degradation include increased levels of proinflammatory cytokines and osteoclast activity. Precursor osteoclast cells differentiate into mature osteoclasts in

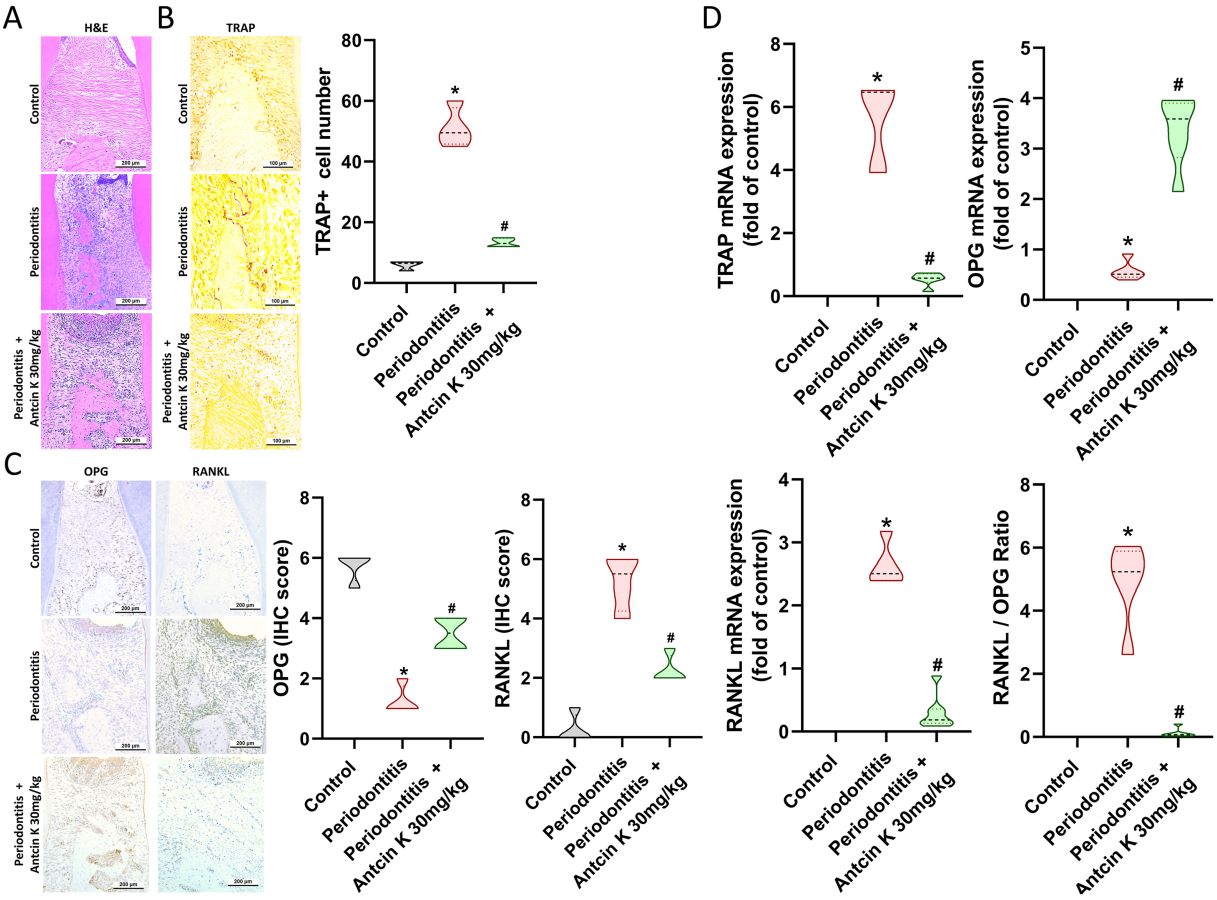

Figure 6 Antcin K inhibits osteoclast formation and RANKL/OPG ratio in ligature-induced periodontitis model. (A) H&E staining, (B) TRAP staining, (C) IHC and (D) qPCR analysis and scoring of RANKL and OPG in the maxilla. *$P < 0.05$ compared with the control group. #$P < 0.05$ compared with the PD group. H&E: hematoxylin and eosin; TRAP: tartrate-resistant acid phosphatase; IHC: immunohistochemistry; qPCR: real-time quantitative polymerase chain reaction; RANKL: receptor activator of nuclear factor-κB ligand; OPG: osteoprotegerin; PD: periodontitis group.

response to proinflammatory cytokines, leading to pathological changes and ultimately the destruction of affected tissues.[31] Thus, inhibiting osteoclast differentiation and bone resorption is a critical strategy for managing periodontal disease. In this study, we demonstrated that Antcin K inhibits osteoclast differentiation from macrophages and reduces osteoclast formation in a ligature-induced periodontitis model *in vivo*. Therefore, Antcin K is a promising candidate for reducing osteoclast activity and treating periodontal disease.

The dynamic balance between osteoblasts and osteoclasts, which regulates the continuous processes of bone formation and resorption, is essential for maintaining skeletal homeostasis.[32,33] For individuals with osteoporosis, arthritis, or periodontitis, bone loss is a significant concern. Reduced bone density, compromised bone structure, and altered microarchitecture are hallmarks of bone loss that increase the risk of fractures.[34,35] Osteoclasts, activated by RANKL, play a critical role in both physiological and pathological bone resorption.[33,36] In this study, we utilized the widely used RANKL-induced osteoclastogenesis model in RAW264.7 cells. RANKL stimulation for five days induced osteoclast differentiation in RAW264.7 cells, as confirmed by TRAP staining to quantify osteoclast number and area. Antcin K treatment significantly suppressed RANKL-mediated osteoclast formation *in vitro* and reduced osteoclast activity *in vivo*. Additionally, Antcin K altered the RANKL/OPG ratio, as demonstrated by IHC staining and qPCR analysis. These findings provide evidence that Antcin K prevents the progression of periodontal disease by inhibiting osteoclast formation.

Investigating potential molecular mechanisms is a critical process in drug discovery. In this study, RNA-seq of Antcin K-treated RAW264.7 cells revealed that the focal adhesion signaling pathway—particularly involving FAK and PI3K—was significantly affected. The FAK–PI3K signaling cascade plays an essential role in multiple cellular processes, including inflammation and differentiation.[15,37] Antcin K treatment reduced RANKL-induced phosphorylation of FAK and PI3K, confirming that this pathway mediates its anti-osteoclastogenic effects. These results indicate that modulation of the FAK–PI3K signaling axis underlies Antcin K's inhibitory effects on osteoclastogenesis.

For many years, the ligature-induced experimental periodontitis model has been a reliable and widely used model.[38] Ligation of the cervical area leads to significant plaque accumulation and sulcular epithelial ulceration.[39] This process triggers a host immune response, resulting in increased inflammatory cell infiltration and alveolar bone resorption. In the current study, Antcin K attenuated ligature-induced increases in CEJ-ABC distances and bone loss. Notably, Antcin K also reduced the ligature-induced increase in osteoclast number and RANKL levels in the treated group. These findings collectively demonstrate that Antcin K prevents the progression of periodontal disease in a ligature-induced periodontitis model. The proposed mechanism is illustrated in the schematic diagram (Fig. 7).

In conclusion, we demonstrated that Antcin K suppresses RANKL-induced osteoclastogenesis by modulating the FAK–PI3K signaling pathway and mitigates alveolar bone loss *in vivo*. These results suggest that Antcin K may serve

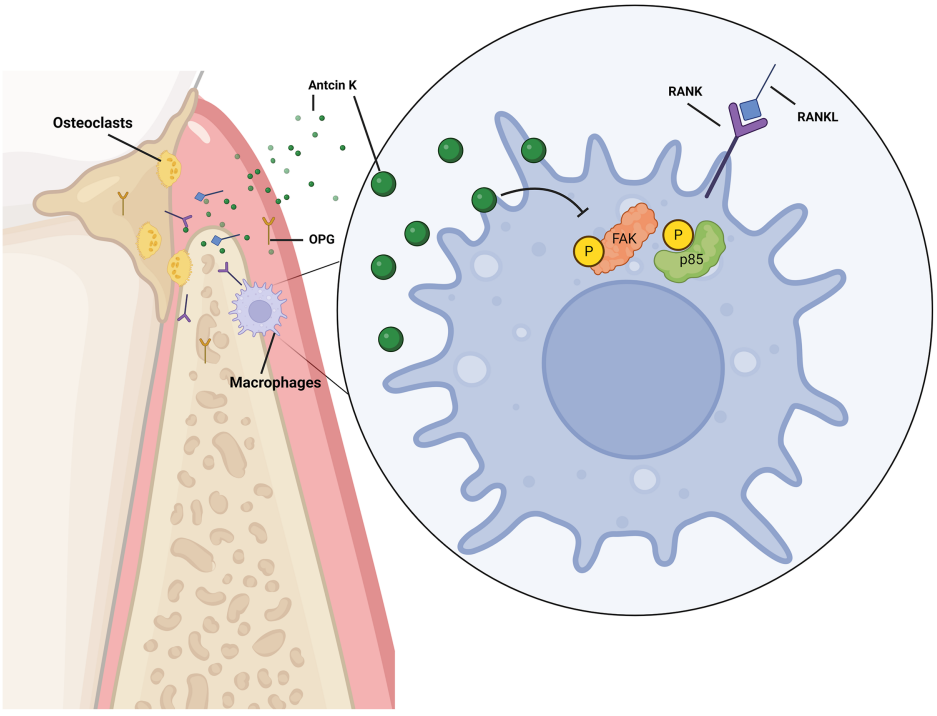

Figure 7 Illustration depicting the effects of Antcin K on osteoclast formation during periodontal disease progression. Antcin K suppresses RANKL-induced osteoclastogenesis from macrophages through the FAK and PI3K signaling pathways. Antcin K also antagonizes periodontal disease development in a ligature-induced periodontitis model *in vivo*. RANKL: receptor activator of nuclear factor-κB ligand; FAK: focal adhesion kinase; PI3K: phosphoinositide 3-kinase.

as a novel natural compound for the treatment of periodontitis.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgments

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References

1. Loos BG, Van Dyke TE. The role of inflammation and genetics in periodontal disease. *Periodontol 2000* 2020;83:26–39.
2. Nazir M, Al-Ansari A, Al-Khalifa K, et al. Global prevalence of periodontal disease and lack of its surveillance. *Sci World J* 2020;2020:2146160.
3. Arigbede AO, Babatope BO, Bamidele MK. Periodontitis and systemic diseases: a literature review. *J Indian Soc Periodontol* 2012;16:487–91.
4. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. *Nat Rev Dis Primers* 2017;3:17038.
5. Graziani F, Karapetsa D, Alonso B, Herrera D. Nonsurgical and surgical treatment of periodontitis: how many options for one disease? *Periodontol 2000* 2017;75:152–88.
6. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet* 2005;366:1809–20.
7. Soysa NS, Alles N. Osteoclast function and bone-resorbing activity: an overview. *Biochem Biophys Res Commun* 2016;476:115–20.
8. Eriksen EF. Cellular mechanisms of bone remodeling. *Rev Endocr Metab Disord* 2010;11:219–27.
9. Kobayashi N, Kadono Y, Naito A, et al. Segregation of TRAF6-mediated signaling pathways clarifies its role in osteoclastogenesis. *EMBO J* 2001;20:1271–80.
10. Kızıldağ A, Arabacı T, Albayrak M, et al. Therapeutic effects of caffeic acid phenethyl ester on alveolar bone loss in rats with endotoxin-induced periodontitis. *J Dent Sci* 2019;14:339–45.
11. Rajendran P. Unveiling the power of flavonoids: a dynamic exploration of their impact on cancer through matrix metalloproteinases regulation. *Biomedicine (Taipei)* 2024;14:12–28.
12. Ganesan N, Baskaran R, Velmurugan BK, Thanh NC. Antrodia cinnamomea-An updated minireview of its bioactive components and biological activity. *J Food Biochem* 2019;43:e12936.
13. Chen YY, Chou PY, Chien YC, et al. Ethanol extracts of fruiting bodies of Antrodia cinnamomea exhibit anti-migration action in human adenocarcinoma CL1-0 cells through the MAPK and PI3K/AKT signaling pathways. *Phytomedicine* 2012;19:768–78.
14. Huang TT, Wu SP, Chong KY, et al. The medicinal fungus Antrodia cinnamomea suppresses inflammation by inhibiting the NLRP3 inflammasome. *J Ethnopharmacol* 2014;155:154–64.
15. Achudhan D, Liu SC, Lin YY, et al. Antcin K inhibits TNF-alpha, IL-1beta and IL-8 expression in synovial fibroblasts and ameliorates cartilage degradation: implications for the treatment of rheumatoid arthritis. *Front Immunol* 2021;12:790925.
16. Law YY, Tran NB, Song CY, et al. Antcin K inhibits chondrosarcoma motility by reducing MMP 7 expression via down-regulation of the PI3K, Akt, mTOR and NF κB signaling pathway. *Mol Med Rep* 2025;32:180.
17. Achudhan D, Chang SLY, Liu SC, et al. Antcin K inhibits VCAM-1-dependent monocyte adhesion in human rheumatoid arthritis synovial fibroblasts. *Food Nutr Res* 2022;66.
18. Chang TK, Huang LC, Kuo YH, et al. Antcin K ameliorates cardiotoxin-induced skeletal muscle injury and inflammation via IL-10 regulation. *Int J Biol Sci* 2025;21:2493–507.
19. Liu CL, Ho TL, Fang SY, et al. Ugonin L inhibits osteoclast formation and promotes osteoclast apoptosis by inhibiting the MAPK and NF-kappaB pathways. *Biomed Pharmacother* 2023;166:115392.
20. Lee HP, Chen PC, Wang SW, et al. Plumbagin suppresses endothelial progenitor cell-related angiogenesis in vitro and in vivo. *J Funct Foods* 2019;52:537–44.
21. Lee HP, Wang SW, Wu YC, et al. Soya-cerebroside inhibits VEGF-facilitated angiogenesis in endothelial progenitor cells. *Food Agric Immunol* 2020;31:193–204.
22. Su CM, Tsai CH, Chen HT, et al. Melatonin improves muscle injury and differentiation by increasing Pax7 expression. *Int J Biol Sci* 2023;19:1049–62.
23. Wu YH, Ramírez FDM, Lin YY, et al. Betulin inhibits the production of inflammatory cytokines in human gingival fibroblasts and ligature-induced periodontitis. *Int Immunopharmacol* 2025;147:114018.
24. Wu YH, Kuraji R, Taya Y, Ito H, Numabe Y. Effects of theaflavins on tissue inflammation and bone resorption on experimental periodontitis in rats. *J Periodontal Res* 2018;53:1009–19.
25. Wu YH, Taya Y, Kuraji R, et al. Dynamic microstructural changes in alveolar bone in ligature-induced experimental periodontitis. *Odontology* 2020;108:339–49.
26. Liu SC, Hsieh HL, Tsai CH, et al. CCN2 facilitates IL-17 production and osteoclastogenesis in human osteoarthritis synovial fibroblasts by inhibiting miR-655 expression. *J Bone Miner Res* 2022;37:1944–55.
27. Chang JW, Liu SC, Lin YY, et al. Nesfatin-1 stimulates CCL2-dependent monocyte migration and M1 macrophage polarization: implications for rheumatoid arthritis therapy. *Int J Biol Sci* 2023;19:281–93.
28. Liu SC, Tsai CH, Wu TY, et al. Soya-cerebroside reduces IL-1β-induced MMP-1 production in chondrocytes and inhibits cartilage degradation: implications for the treatment of osteoarthritis. *Food Agric Immunol* 2019;30:620–32.
29. Achudhan D, Liu SC, Lin YY, et al. Antcin K inhibits VEGF-dependent angiogenesis in human rheumatoid arthritis synovial fibroblasts. *J Food Biochem* 2022;46:e14022.
30. Scannapieco FA, Gershovich E. The prevention of periodontal disease-An overview. *Periodontol 2000* 2020;84:9–13.
31. Altın A, Korkmaz MZ, Atak M, Mercantepe T, Yılmaz HK. Celastrol restricts experimental periodontitis related alveolar bone loss by suppressing inflammatory cytokine response. *Biomedicine (Taipei)* 2023;13:44–50.
32. Yu W, Zhong L, Yao L, et al. Bone marrow adipogenic lineage precursors promote osteoclastogenesis in bone remodeling and pathologic bone loss. *J Clin Investig* 2021;131:e140214.
33. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature* 2003;423:337–42.
34. Liu PI, Chang AC, Lai JL, et al. Melatonin interrupts osteoclast functioning and suppresses tumor-secreted RANKL expression: implications for bone metastases. *Oncogene* 2021;40:1503–15.
35. Hascoet E, Blanchard F, Blin-Wakkach C, et al. New insights into inflammatory osteoclast precursors as therapeutic targets for rheumatoid arthritis and periodontitis. *Bone Res* 2023;11:26.
36. Xiong J, Cawley K, Piemontese M, et al. Soluble RANKL contributes to osteoclast formation in adult mice but not ovariectomy-induced bone loss. *Nat Commun* 2018;9:2909.

37. Chen PC, Liu JF, Fong YC, et al. CCN3 facilitates runx2 and osterix expression by inhibiting miR-608 through PI3K/Akt signaling in osteoblasts. *Int J Mol Sci* 2019;20:3300.
38. Kuhr A, Popa-Wagner A, Schmoll H, Schwahn C, Kocher T. Observations on experimental marginal periodontitis in rats. *J Periodontal Res* 2004;39:101—6.
39. de Molon RS, de Avila ED, Cirelli JA. Host responses induced by different animal models of periodontal disease: a literature review. *J Investig Clin Dent* 2013;4:211—8.