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Effects of Oral Catalpol on Alveolar Bone Regeneration in a Rat Model of Alveolar Ridge Preservation

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Abstract

This study investigated the effects of oral catalpol administration on alveolar bone regeneration in a rat model of alveolar ridge preservation (ARP) following tooth extraction. Fifty male Sprague–Dawley rats were randomly assigned to five groups (n = 10/group): Extraction, ARP, ARP + Low-dose Catalpol (25 mg/kg), ARP + Medium-dose Catalpol (50 mg/kg), and ARP + High-dose Catalpol (100 mg/kg). ARP involved Bio-Oss grafting and membrane coverage. Five rats per group were euthanized at 4 and 8 weeks postoperatively. Bone microarchitecture was evaluated using micro-computed tomography, serum bone turnover markers were measured by ELISA, and tissue morphology was examined using Masson's trichrome staining. At 4 weeks, the ARP + High-dose Catalpol group exhibited significantly higher BMD, BV/TV, and Tb.N and lower Tb.Sp than the ARP group. At 8 weeks, BMD and BV/TV remained significantly higher in the ARP + High-dose Catalpol group. Catalpol administration was associated with increased serum BALP and OCN levels and decreased CTX-I levels. Histological observations suggested enhanced bone formation following catalpol treatment. These findings suggest that oral catalpol may promote alveolar bone regeneration following ARP, with the greatest improvements observed at 100 mg/kg.

Introduction

Dental implants are widely regarded as an effective treatment option for edentulous sites [1], and adequate alveolar bone volume and quality are essential for predictable osseointegration and long-term implant stability [2]. However, substantial alveolar bone resorption occurs after tooth extraction, reducing alveolar ridge height and width and potentially compromising subsequent implant placement [3].

To mitigate post-extraction alveolar bone resorption, clinicians have developed and widely investigated approaches such as alveolar ridge preservation (ARP) [4–5]. In ARP, clinicians insert bone substitute materials into extraction sockets and often cover them with barrier membranes. This approach encourages new bone formation and helps preserve alveolar ridge dimensions through the osteoconductive properties of graft materials and the guided bone regeneration principles provided by barrier membranes [6–7]. Numerous clinical and experimental studies have demonstrated that ARP can effectively maintain alveolar ridge contour and reduce post-extraction dimensional changes [8–9]. Bone formation and graft remodeling following ARP remain progressive processes that may continue for several months [10]. In clinical studies, implant placement after ARP is often performed following a healing period of several months [11], which may extend the overall interval between tooth extraction and implant placement. Therefore, although ARP can effectively limit post-extraction alveolar ridge resorption, additional strategies that actively promote and regulate bone regeneration may be needed to further improve the efficiency and predictability of alveolar bone regeneration [12]. Consequently, identifying bioactive agents that actively regulate and promote alveolar bone regeneration represents a potential strategy for improving regenerative outcomes following tooth extraction.

In the search for novel bone regenerative agents, bioactive monomers derived from traditional Chinese medicine have attracted growing attention because of their diverse biological activities, favorable safety profiles, and potential clinical value [13]. Among these compounds, Catalpol, an iridoid glycoside isolated from Rehmannia glutinosa, is recognized as a major quality-control marker of this medicinal herb in the Chinese Pharmacopoeia [14]. Catalpol exhibits a broad spectrum of pharmacological activities, including anti-inflammatory, antioxidant, and tissue-protective effects [15–16]. Increasing evidence suggests that Catalpol may regulate bone metabolism by promoting bone formation and inhibiting bone resorption [17–18]. In vitro studies show that Catalpol enhances osteoblast viability, promotes mesenchymal stem cell proliferation and osteogenic differentiation, and upregulates osteogenesis-related markers, including Runx2, alkaline phosphatase (ALP), and osteocalcin (OCN) [19–20]. Studies involving several cellular and molecular processes support Catalpol's osteogenic effects [21]. Furthermore, Catalpol has also been reported to modulate bone remodeling by promoting an anti-inflammatory microenvironment and reducing osteoclast-mediated bone resorption [22–24]. Consistent with these in vitro findings, in vivo studies have shown that Catalpol promotes bone formation and attenuates bone loss in various bone regeneration and remodeling models, including cranial bone defects, ovariectomy-induced osteoporosis, and orthodontic tooth movement [25–26]. However, existing studies have mainly focused on cranial defects, long bone-related disorders, or osteoporosis models. Alveolar bone is a specialized skeletal tissue that undergoes continuous remodeling in response to local oral and mechanical stimuli; it is unclear whether Catalpol can effectively promote alveolar bone regeneration following tooth extraction, particularly within an alveolar ridge preservation model.

Previous studies have indicated Catalpol's potential in maxillofacial bone regeneration. Catalpol has been shown to promote the proliferation, osteogenic differentiation, and mineralization of jawbone-derived bone marrow mesenchymal stem cells [27–28]. Regarding alveolar bone, although a previous study [29] reported that Catalpol exerted protective effects against alveolar bone injury, that study was based on an inflammatory bone resorption model rather than a post-extraction alveolar bone regeneration model; therefore, the effects of Catalpol on physiological alveolar bone regeneration following tooth extraction remain unclear.

This study aimed to establish an alveolar ridge preservation (ARP) model in SD rats following tooth extraction and evaluate the effects of orally administered Catalpol at different doses on alveolar bone regeneration within extraction sockets. Using micro-CT analysis, serum bone metabolism markers, and Masson's trichrome staining, this study provided multidimensional evidence to assess Catalpol's regenerative effects on alveolar bone and its dose-related responses. These findings may provide preclinical evidence supporting the potential use of Catalpol as an adjunctive strategy for alveolar bone regeneration and provide a basis for further investigation.

Materials and methods

Experimental Animals and Grouping

Fifty adult male Sprague–Dawley (SD) rats (6–8 weeks old, SPF grade, weighing 220–260 g) were obtained from the Animal Center of Hunan University of Chinese Medicine. The Animal Ethics Committee of Hunan University of Chinese Medicine reviewed and approved the experimental protocol (approval no. HNUCM21-2510-25). All animal experimental procedures were performed in accordance with the relevant institutional guidelines and regulations for the care and use of laboratory animals.

The rats were randomly assigned using a random number table to five groups: the Extraction group, the ARP group, the ARP + Low-dose Catalpol group, the ARP + Medium-dose Catalpol group, and the ARP + High-dose Catalpol group, receiving 25, 50, and 100 mg/kg Catalpol, respectively. All animals were housed in the Animal Experiment Center of Hunan University of Chinese Medicine under standard laboratory conditions. The rats were maintained at 24 ± 2°C with 55–60% relative humidity under a 12 h light/12 h dark cycle, with 2–3 rats per cage and free access to food and water. All procedures were initiated after a 1-week acclimatization period.

Reagents and chemicals

Catalpol (Sichuan Weikeqi Biotechnology Co., Ltd.), Rat Osteocalcin (BGP/OCN) ELISA assay kit (A116478, Shanghai Fusheng Industrial Co., Ltd.), Rat Bone Alkaline Phosphatase (BALP) ELISA assay kit (A108040, Shanghai Fusheng Industrial Co., Ltd.), Rat Type I Collagen C-Terminal Peptide (CTX-I) ELISA assay kit (A108517, Shanghai Fusheng Industrial Co., Ltd.), Universal Tissue Fixative (G1101, Servicebio), Masson's Staining Kit (G1006, Servicebio)

Establishment of the ARP Model and Catalpol Administration

Establishment of an Extraction Socket Preservation Model in SD Rats

Experimental rats were fasted but allowed free access to water for 8 h before surgery. Under aseptic conditions, anesthesia was induced by intraperitoneal injection of tiletamine/zolazepam (50–75 mg/kg).

Rat models of extraction socket and extraction socket preservation were established according to previously reported methods [30–31]. After anesthesia, the rats were placed in the supine position, and local infiltration anesthesia was performed around the right maxillary first molar using 1% lidocaine (0.4 mL/kg). The right maxillary first molar was then extracted, and the interradicular septum was removed using a low-speed tungsten carbide bur (ISO 027, 2.7 mm diameter) under saline irrigation. In the Extraction group, the extraction sockets were directly sutured with 6 − 0 sutures. In the ARP group and ARP + Catalpol groups, the sockets were filled with Bio-Oss bone substitute (Geistlich, Switzerland), covered with a Bio-Gide collagen membrane (Geistlich, Switzerland), and subsequently closed with 6 − 0 sutures.

Catalpol Administration

Catalpol was administered once daily by oral gavage after surgery. The ARP + Low-dose Catalpol group, ARP + Medium-dose Catalpol group, and ARP + High-dose Catalpol group received Catalpol at doses of 25, 50, and 100 mg/kg, respectively. The Extraction group and ARP group received an equivalent volume of solvent by oral gavage.

Sample Collection

Blood samples and maxillary specimens containing the extraction sockets were collected from five rats per group at 4 and 8 weeks after surgery. Rats were anesthetized by intraperitoneal injection of tiletamine/zolazepam (50–75 mg/kg). Before terminal blood collection, anesthetic depth was assessed by the absence of a withdrawal response to toe pinch. The abdominal area was shaved, and terminal blood collection (5–10 mL) was performed via the abdominal aorta under deep anesthesia, with exsanguination resulting in death. Death was confirmed by the cessation of heartbeat and respiration before maxillary specimens containing the extraction sockets were harvested. The specimens were fixed in 4% paraformaldehyde at 4°C for 48 h and subsequently dehydrated in a graded ethanol series before micro-CT and histological analyses.

Micro-CT Analysis

High-resolution micro-computed tomography (micro-CT; VNC-102, Pingsheng Medical Technology Co., Ltd., Kunshan, China) was used to scan the specimens at 12 μm resolution, with operating parameters of 90 kV and 80 μA. The extraction socket region was selected as the region of interest (ROI). Image segmentation and three-dimensional reconstruction were performed using Avatar software, followed by quantitative analysis of bone microarchitectural parameters, including bone mineral density (BMD), bone volume fraction (BV/TV), trabecular number (Tb.N), and trabecular separation (Tb.Sp).

ELISA Analysis of Serum Bone Metabolism Markers

Serum ELISA analysis was performed to determine osteogenesis-related markers (BALP and OCN) and the bone resorption marker CTX-I. Blood samples were centrifuged at 3,000 rpm for 15 min, and serum supernatants were collected, aliquoted, and stored at − 80°C until analysis. We measured BALP, OCN, and CTX-I concentrations using commercial ELISA kits according to the manufacturer's instructions. Absorbance values were measured at 450 nm using a microplate reader, and the concentrations of target markers were calculated according to the corresponding standard curves.

Masson’s trichrome staining

After decalcification and paraffin embedding, sections were deparaffinized and stained according to the manufacturer’s instructions for the Masson’s trichrome staining kit. The stained sections were examined

under a microscope to evaluate new bone formation and collagen fiber organization within the extraction sockets.

Statistical Analysis

All data are presented as mean ± standard deviation (SD). Statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA), and graphs were generated using GraphPad Prism version 10.0. Differences among multiple groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. A value of $P < 0.05$ was considered statistically significant.

Results

Micro-CT Assessment of Bone Healing in Extraction Sockets

Observation via Micro-CT Three-Dimensional Reconstruction

Micro-CT three-dimensional reconstructed images (Figure 1) showed that at 4 weeks after surgery, the extraction sockets in the Extraction group exhibited apparent ridge depression and reduced alveolar ridge height. In contrast, the ARP group and ARP + Catalpol groups showed improved ridge contours compared with the Extraction group. The ARP + Low-dose Catalpol group showed no apparent morphological improvement compared with the ARP group, whereas the ARP + Medium-dose and ARP + High-dose Catalpol groups exhibited increased new bone formation and smoother socket contours.

At 8 weeks after surgery, the alveolar ridge contour of the Extraction group remained irregular with reduced ridge height, whereas the ARP group showed improved morphology. Among the Catalpol-treated groups, the ARP + Low-dose Catalpol group showed limited improvement, while the ARP + Medium-dose Catalpol group exhibited further improvement in ridge contour. The ARP + High-dose Catalpol group showed the most pronounced morphological changes, characterized by a smoother and more continuous ridge contour and more complete filling of the extraction socket.

Analysis of Micro-CT Bone Microstructural Data

Micro-CT analysis of the region of interest (ROI) yielded quantitative bone microstructural parameters (Figure 2). At 4 weeks after surgery, Catalpol treatment showed a dose-related tendency toward enhanced alveolar bone regeneration. The ARP + High-dose Catalpol group showed significantly higher BMD, BV/TV, and Tb.N and lower Tb.Sp compared with the Extraction and ARP groups ($P < 0.05$), showing the greatest improvement among all groups. The ARP + Medium-dose Catalpol group showed significant differences in Tb.N and Tb.Sp compared with the Extraction group ($P < 0.05$). Although the ARP + Low-dose Catalpol group showed higher values for all evaluated parameters compared with the Extraction and ARP groups, no statistically significant differences were observed ($P > 0.05$).

At 8 weeks after surgery, the ARP group showed significantly higher BMD, BV/TV, and Tb.N values and lower Tb.Sp values than the Extraction group ($P < 0.05$). Compared with the ARP group, the ARP + High-

dose Catalpol group exhibited significant increases in BMD and BV/TV ($P < 0.05$), indicating enhanced bone microstructural regeneration. Although the ARP + Medium-dose and ARP + Low-dose Catalpol groups showed higher values than the ARP group for the evaluated parameters, these differences were not statistically significant ($P > 0.05$). Compared with the Extraction group, the ARP + Medium-dose Catalpol group showed significant differences in BV/TV, Tb.N, and Tb.Sp ($P < 0.05$).

Serum Levels of Bone Metabolism Markers (BALP, OCN, and CTX-I)

At 4 weeks after surgery (Figure 3), serum BALP and OCN levels increased with increasing doses of Catalpol. The ARP + High-dose Catalpol group showed significantly higher BALP and OCN levels than the Extraction group, ARP group, and ARP + Low-dose Catalpol group ($P < 0.05$). The ARP + Medium-dose Catalpol group exhibited significantly higher BALP and OCN levels than the ARP group ($P < 0.05$). In contrast, CTX-I levels were lower in the ARP + Catalpol groups than the Extraction and ARP groups, with significant reductions observed in the ARP + Medium-dose and ARP + High-dose Catalpol groups ($P < 0.05$). No significant differences were observed in the ARP + Low-dose Catalpol group compared with the Extraction or ARP groups ($P > 0.05$).

At 8 weeks after surgery, BALP and OCN levels increased with increasing Catalpol dose, whereas CTX-I levels showed the opposite trend. The ARP + High-dose Catalpol group exhibited significantly higher BALP and OCN levels than the Extraction group, ARP group, and ARP + Low-dose Catalpol group ($P < 0.05$). The ARP + Medium-dose Catalpol group also showed significantly higher BALP and OCN levels than the Extraction and ARP groups ($P < 0.05$). The ARP + Low-dose Catalpol group showed significantly higher BALP and OCN levels than the Extraction group ($P < 0.05$), but no significant differences were observed compared with the ARP group. Regarding CTX-I, the ARP + High-dose Catalpol group showed significantly lower levels than the Extraction group, ARP group, ARP + Low-dose Catalpol group, and ARP + Medium-dose Catalpol group ($P < 0.05$), while the ARP + Medium-dose Catalpol group showed significantly lower CTX-I levels than the Extraction group ($P < 0.05$).

Overall, Catalpol treatment was associated with higher serum BALP and OCN levels and lower CTX-I levels, with more evident changes observed at higher doses. These findings provide serological evidence supporting Catalpol’s regulatory effects on bone metabolism during alveolar bone regeneration.

Histological Evaluation of Extraction Sockets by Masson's Trichrome Staining

At 4 weeks after surgery, Masson's trichrome staining (Figure 4) showed that the Extraction group exhibited limited red-stained bone tissue within the extraction sockets, with sparsely distributed blue-stained collagen fibers arranged in an irregular pattern. Compared with the Extraction group, the ARP group showed an increased area of red-stained bone tissue accompanied by collagen fiber deposition around the newly formed tissue, although the collagen arrangement remained relatively disorganized.

Among the Catalpol-treated groups, the ARP + Low-dose Catalpol group showed a slight increase in red-stained bone tissue compared with the ARP group, while collagen fibers remained loosely arranged. In

contrast, the ARP + Medium-dose and ARP + High-dose Catalpol groups showed more abundant blue-stained collagen fibers surrounding the red-stained bone tissue, with denser, more organized collagen fiber arrangements.

At 8 weeks after surgery, collagen fiber organization was further improved in all groups. The ARP + High-dose Catalpol group showed densely distributed, more regularly arranged collagen fibers within the extraction sockets, with a more organized tissue structure than the other groups. Although collagen deposition increased in the Extraction and ARP + Low-dose Catalpol groups, the collagen fibers remained relatively irregular and less organized than those observed in the ARP + Medium-dose and ARP + High-dose Catalpol groups.

Discussion

The present study provides evidence that Catalpol enhances alveolar bone regeneration when combined with alveolar ridge preservation (ARP) following tooth extraction. Although ARP has been widely applied to reduce post-extraction alveolar ridge dimensional changes, its regenerative outcomes largely depend on the osteoconductive properties of graft materials and the intrinsic healing capacity of host tissues[32–33]. Therefore, introducing bioactive agents that actively regulate bone formation and remodeling may be a promising strategy to further improve the quality and predictability of ARP-mediated regeneration.

In this study, the effects of orally administered Catalpol at different doses were systematically evaluated in an SD rat ARP model using micro-computed tomography (micro-CT), Masson's trichrome staining, and serum biochemical analyses. The results demonstrated that Catalpol, particularly at 100 mg/kg, significantly improved alveolar bone microarchitecture, enhanced mineralized tissue formation, promoted collagen matrix remodeling, increased serum osteogenesis-related markers (BALP and OCN), and reduced the bone resorption marker CTX-I. These findings suggest that Catalpol may serve as a bioactive adjunct to ARP by coordinating bone formation and bone remodeling processes during extraction socket healing.

Previous studies have demonstrated Catalpol's bone-regenerative and bone-protective potential through multiple in vivo and in vitro models, including osteoporotic bone loss, bone defect repair, and periodontal tissue remodeling[34–35]; however, its role in physiological alveolar bone regeneration after tooth extraction remains unclear. In the present study, micro-CT analysis demonstrated that Catalpol supplementation, especially at 100 mg/kg, further enhanced ARP's regenerative effects, as evidenced by improvements in bone microstructural parameters. The increases in bone mineral density (BMD) and bone volume fraction (BV/TV) indicate enhanced mineralized tissue formation within the extraction sockets, while the increased trabecular number (Tb.N) and reduced trabecular separation (Tb.Sp) suggest improved trabecular connectivity and structural organization[36]. These findings suggest that Catalpol not only increases bone volume but may also promote a more organized bone microarchitecture during socket healing. Previous in vitro and in vivo studies have shown that Catalpol enhances osteogenic activity and suppresses excessive bone resorption through multiple biological

processes [37–38]; our previous study also showed that Catalpol promoted osteogenic differentiation and mineralization of jawbone-derived bone marrow mesenchymal stem cells [28]. Combined with the present findings of increased osteogenic markers and decreased bone resorption markers, these results suggest that Catalpol may promote alveolar bone regeneration by enhancing osteogenic activity and maintaining the balance between bone formation and resorption.

Micro-CT analysis confirmed Catalpol's ability to improve alveolar bone microarchitecture; however, changes in bone mass depend on the dynamic balance between bone formation and bone resorption [39]. Therefore, serum bone metabolism markers provide complementary information regarding the systemic regulation of this balance [40]. In the present study, ELISA analysis demonstrated that oral administration of Catalpol increased serum BALP and OCN levels, while reducing CTX-I levels, indicating enhanced osteogenic activity accompanied by suppressed bone resorption.BALP, which is primarily secreted by active osteoblasts, is widely recognized as an early marker of osteogenic differentiation [41]. OCN is produced by mature osteoblasts and is closely associated with extracellular matrix mineralization and late-stage bone formation [42]. The simultaneous elevation of BALP and OCN observed in this study suggests that Catalpol may promote osteogenesis throughout different stages of alveolar bone healing rather than affecting a single phase of bone formation. Conversely, CTX-I, a degradation product of type I collagen, reflects osteoclast-mediated bone resorption activity [43]. The decreased serum CTX-I levels suggest that Catalpol may inhibit excessive bone resorption, thereby contributing to a favorable bone remodeling environment. These findings are consistent with previous reports showing that Catalpol promotes osteogenic activity and reduces excessive osteoclast-mediated bone resorption in different bone-related models [44–45]. Combined with the micro-CT findings in the present study, these serological results provide further evidence that Catalpol may enhance alveolar bone regeneration through coordinated regulation of osteoblast-mediated bone formation and osteoclast-mediated bone resorption. Furthermore, the more pronounced effects at 8 weeks than at 4 weeks suggest that Catalpol’s regulatory influence may continue during the intermediate and later stages of extraction socket healing.

Serological evidence suggests that Catalpol regulates bone remodeling by promoting bone formation and suppressing bone resorption; however, its effects on tissue-level bone maturation require further evaluation. In this study, Masson’s trichrome staining, which visualizes the collagen-rich matrix and mineralized bone tissue[46], showed that at 4 weeks after surgery, the Extraction and ARP groups exhibited limited mineralized tissue formation and sparse collagen fiber deposition within the extraction sockets. The ARP + Low-dose Catalpol group showed limited improvement compared with the ARP group, whereas the ARP + Medium-dose and ARP + High-dose Catalpol groups exhibited increased mineralized tissue formation and denser collagen fiber organization. At 8 weeks after surgery, the groups showed further differences in collagen organization and tissue maturation. In the Extraction and ARP + Low-dose Catalpol groups, collagen fibers remained relatively sparse and irregularly arranged, indicating less organized collagen deposition. In contrast, the ARP + Medium-dose and ARP + High-dose Catalpol groups showed more organized collagen fiber arrangements and architecture, with the ARP + High-dose

Catalpol group showing the most pronounced changes. These findings are consistent with the progressive maturation and remodeling process of newly formed bone tissue described in previous studies [47–48]. Together with the Micro-CT and ELISA findings, these results suggest that Catalpol may therefore contribute not only to increased bone formation but also to improved organization of the regenerated tissue.

In summary, building on previous in vitro evidence showing that Catalpol promotes osteogenic differentiation of maxillary bone marrow mesenchymal stem cells, this study provided in vivo evidence supporting the bone-regenerative potential of orally administered Catalpol in an SD rat extraction socket preservation model. The findings revealed dose-related effects of Catalpol and established a complementary evidence framework integrating imaging, histological, and serological assessments, extending the current understanding of Catalpol-mediated bone regeneration from cellular models to the maxillofacial skeletal environment.

This study has several limitations. First, the observation period was limited, and local Catalpol concentrations in alveolar bone tissue were not evaluated. Second, this study was conducted in a healthy rat ARP model, which may not fully represent the complex clinical conditions associated with implant therapy.Future studies should investigate the mechanisms underlying Catalpol-mediated alveolar bone regeneration, evaluate its effects under pathological conditions such as diabetes and osteoporosis, and validate the efficacy and safety of Catalpol combined with ARP in large-animal models to support clinical translation [49–50].

Declarations

Competing interests

The authors declare that they have no competing interests, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions

Ran Liu: Conceptualization, methodology, investigation, data curation, formal analysis, visualization, and writing—original draft review and editing.

Yudie Peng: Investigation.

Niannian Zhou: Investigation.

Rongsui Tang: Conceptualization and methodology.

Yuxuan Guo: Data curation and formal analysis.

Jing Zhou: Supervision, project administration, and writing—review and editing.

References

1. Dang, A. T. et al. Local E-rhBMP-2/β-TCP application rescues osteocyte dendritic integrity and reduces microstructural damage in alveolar bone post-extraction in MRONJ-like mouse model. *Int. J. Mol. Sci.* **25**, 6648. https://doi.org/10.3390/ijms25126648 (2024).
2. Pilipchuk, S. P. et al. Tissue engineering for bone regeneration and osseointegration in the oral cavity. *Dent. Mater.* **31**, 317–338. https://doi.org/10.1016/j.dental.2015.01.006 (2015).
3. Li, N. et al. Advances in biomaterials for oral-maxillofacial bone regeneration: spotlight on periodontal and alveolar bone strategies. *Regen Biomater.* **11**, rbae078. https://doi.org/10.1093/rb/rbae078 (2024).
4. Kim, S. & Kim, S. G. Advancements in alveolar bone grafting and ridge preservation: a narrative review on materials, techniques, and clinical outcomes. *Maxillofac. Plast. Reconstr. Surg.* **46**, 14. https://doi.org/10.1186/s40902-024-00425-w (2024).
5. Quisiguiña Salem, C., Ruiz Delgado, E., Crespo Reinoso, P. A. & Robalino, J. J. Alveolar ridge preservation: a review of concepts and controversies. *Natl. J. Maxillofac. Surg.* **14**, 167–176. https://doi.org/10.4103/njms.njms_224_22 (2023).
6. Kalsi, A. S., Kalsi, J. S. & Bassi, S. Alveolar ridge preservation: why, when and how. *Br. Dent. J.* **227**, 264–274. https://doi.org/10.1038/s41415-019-0647-2 (2019).
7. Atieh, M. A. et al. Interventions for replacing missing teeth: alveolar ridge preservation techniques for dental implant site development. *Cochrane Database Syst. Rev.* **4**, CD010176. https://doi.org/10.1002/14651858.CD010176.pub3 (2021).
8. Wei, Y. P. et al. Alveolar ridge preservation at molar extraction sites: a systematic review and meta-analysis. *J. Evid. Based Dent. Pract.* **25**, 102074. https://doi.org/10.1016/j.jebdp.2024.102074 (2025).
9. Avila-Ortiz, G., Chambrone, L. & Vignoletti, F. Effect of alveolar ridge preservation interventions following tooth extraction: a systematic review and meta-analysis. *J. Clin. Periodontol*. **46** (Suppl 21),

195–223. https://doi.org/10.1111/jcpe.13057 (2019).

10. Zellner, J. W., Allen, H. T., Kotsakis, G. A. & Mealey, B. L. Wound healing after ridge preservation: a randomized controlled trial on short-term (4 months) versus long-term (12 months) histologic outcomes. *J. Periodontol*. **94**, 622–629. https://doi.org/10.1002/JPER.22-0187 (2023).
11. Cardaropoli, D., Bellomo, M., Tamagnone, L. & Leonardi, R. Bone and soft tissue modifications in immediate implants versus delayed implants inserted following alveolar ridge preservation: a randomized controlled clinical trial. Part II: radiographic outcomes. *Int. J. Periodontics Restor. Dent.* **42**, 487–494. https://doi.org/10.11607/prd.5567 (2022).
12. Jung, R. E., Ioannidis, A., Hämmerle, C. H. F. & Thoma, D. S. Alveolar ridge preservation in the esthetic zone. *Periodontol 2000*. **77**, 165–175. https://doi.org/10.1111/prd.12209 (2018).
13. Liu, B., Mao, X., Gao, Z. J. & Wang, H. Natural traditional Chinese medicine products: emerging therapeutic targets for the treatment of osteoporosis. *J. Orthop. Surg. Res.* **20**, 469. https://doi.org/10.1186/s13018-025-05879-w (2025).
14. Li, M. et al. A systematic review on botany, processing, application, phytochemistry and pharmacological action of Radix Rehmanniae. *J. Ethnopharmacol.* **285**, 114820. https://doi.org/10.1016/j.jep.2021.114820 (2022).
15. Tao, J. H. et al. UPLC-Q-TOF/MS-based metabolic profiling comparison of four major bioactive components in normal and CKD rat plasma, urine and feces following oral administration of Cornus officinalis Sieb and Rehmannia glutinosa Libosch herb couple extract. *J. Pharm. Biomed. Anal.* **161**, 254–261. https://doi.org/10.1016/j.jpba.2018.08.051 (2018).
16. Hassan, M. M. et al. Multifaceted therapeutic potentials of catalpol, an iridoid glycoside: an updated comprehensive review. *Inflammopharmacology*, 1–27. https://doi.org/10.1007/s10787-025-01694-1(2025).
17. Bhattamisra, S. K., Yap, K. H., Rao, V. & Choudhury, H. Multiple biological effects of an iridoid glucoside, catalpol and its underlying molecular mechanisms. *Biomolecules* **10**, 32. https://doi.org/10.3390/biom10010032 (2020).
18. Zhang, J. N. & Liu, K. X. Research progress of catalpol. *Drug Eval Res.* **42**, 1680–1684. https://doi.org/10.7501/j.issn.1674-6376.2019.08.035 (2019).
19. Cheng, J. et al. Catalpol promotes the proliferation and differentiation of osteoblasts induced by high glucose by inhibiting KDM7A. *Diabetes Metab. Syndr. Obes.* **13**, 705–712. https://doi.org/10.2147/DMSO.S246433 (2020).
20. Bian, Y. et al. Catalpol attenuates postmenopausal osteoporosis by activating SIRT1-mediated P53 deacetylation and inhibiting CASPASE-3-driven osteoblast apoptosis. *Int. Immunopharmacol.* 168(Pt 1), 115820. (2026). https://doi.org/10.1016/j.intimp.2025.115820
21. Fu, S. P. & Zhang, R. H. Effects of different concentrations of catalpol on proliferation and osteogenic differentiation of bone marrow mesenchymal stem cells in SD rats. *Lishizhen Med. Mater. Med. Res.* **23**, 2398–2400. https://doi.org/10.3969/j.issn.1008-0805.2012.10.007 (2012).

22. Zhang, P., Feng, Q., Chen, W. & Bai, X. Catalpol antagonizes LPS-mediated inflammation and promotes osteoblast differentiation through the miR-124-3p/DNMT3b/TRAF6 axis. *Acta Histochem.* **126**, 152118. https://doi.org/10.1016/j.acthis.2023.152118 (2024).
23. Chen, L. et al. STAT3 activation by catalpol promotes osteogenesis-angiogenesis coupling, thus accelerating osteoporotic bone repair. *Stem Cell. Res. Ther.* **12**, 108. https://doi.org/10.1186/s13287-021-02178-z (2021).
24. Zhang, Y. et al. Catalpol modulates the crosstalk between mesenchymal stromal cells and macrophages via paracrine signaling to enhance angiogenesis and osteogenesis. *Exp. Cell. Res.* **418**, 113269. https://doi.org/10.1016/j.yexcr.2022.113269 (2022).
25. Lai, N. et al. Regulatory effect of catalpol on Th1/Th2 cells in mice with bone loss induced by estrogen deficiency. *Am. J. Reprod. Immunol.* **74**, 487–498. https://doi.org/10.1111/aji.12423 (2015).
26. Hu, J. et al. Catalpol enhances osteogenic differentiation of human periodontal stem cells and modulates periodontal tissue remodeling in an orthodontic tooth movement rat model. *Drug Des. Devel Ther.* **18**, 4943–4960. https://doi.org/10.2147/DDDT.S482969 (2024).
27. Tang, R. S. & Zhou, J. Research progress on traditional Chinese medicine monomers in promoting bone defect repair and regeneration. *J. Oper. Dent. Endod Periodontol*. **29**, 417–421. https://doi.org/10 (2024). 15956/j.cnki.chin.j.conserv.dent.2024.07.009
28. Tang, R. et al. The impact of catalpol on the osteogenic differentiation of jaw bone marrow mesenchymal stem cells based on network pharmacology, molecular docking, and experimental validation. *BMC Oral Health*. **25**, 1613. https://doi.org/10.1186/s12903-025-06901-7 (2025).
29. Li, Y., Jin, X. & Mao, L. Protective effect of catalpol on nicotine-induced alveolar bone injury and associated underlying mechanisms. *Mol. Med. Rep.* **16**, 8345–8350. https://doi.org/10.3892/mmr.2017.7604 (2017).
30. Jiang, J., Mou, J., Wang, S. & Liu, J. Protocol for establishing a mouse model of bilateral maxillary first molar extraction to study alveolar bone healing. *STAR. Protoc.* **7**, 104366. https://doi.org/10.1016/j.xpro.2026.104366 (2026).
31. Willett, E. S. et al. Standardized rat model testing effects of inflammation and grafting on extraction healing. *J. Periodontol*. **88**, 799–807. https://doi.org/10.1902/jop.2017.160771 (2017).
32. Avila-Ortiz, G., Elangovan, S., Kramer, K. W., Blanchette, D. & Dawson, D. V. Effect of alveolar ridge preservation after tooth extraction: a systematic review and meta-analysis. *J. Dent. Res.* **93**, 950–958. https://doi.org/10.1177/0022034514541127 (2014).
33. De Risi, V., Clementini, M., Vittorini, G., Mannocci, A. & De Sanctis, M. Alveolar ridge preservation techniques: a systematic review and meta-analysis of histological and histomorphometrical data. *Clin. Oral Implants Res.* **26**, 50–68. https://doi.org/10.1111/clr.12288 (2015).
34. Xu, L. et al. Catalpol ameliorates dexamethasone-induced osteoporosis by promoting osteogenic differentiation of bone marrow mesenchymal stem cells via the activation of PKD1 promoter. *J. Pharmacol. Sci.* **153**, 221–231. https://doi.org/10.1016/j.jphs.2023.10.002 (2023).

35. Zhao, L. et al. Catalpol protects against high glucose-induced bone loss by regulating osteoblast function. *Front. Pharmacol.* **12**, 626621. https://doi.org/10.3389/fphar.2021.626621 (2021).
36. Bouxsein, M. L. et al. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *J. Bone Min. Res.* **25**, 1468–1486. https://doi.org/10.1002/jbmr.141 (2010).
37. Zhu, Y., Wang, Y. M., Jia, Y. C., Xu, J. & Chai, Y. Catalpol promotes the osteogenic differentiation of bone marrow mesenchymal stem cells via the Wnt/β-catenin pathway. *Stem Cell. Res. Ther.* **10**, 37. https://doi.org/10.1186/s13287-019-1143-y (2019).
38. Gong, W. et al. Rehmannia glutinosa Libosch extracts prevent bone loss and architectural deterioration and enhance osteoblastic bone formation by regulating the IGF-1/PI3K/mTOR pathway in streptozotocin-induced diabetic rats. *Int. J. Mol. Sci.* **20**, 3964. https://doi.org/10.3390/ijms20163964 (2019).
39. Corral, D. A. et al. Dissociation between bone resorption and bone formation in osteopenic transgenic mice. *Proc. Natl. Acad. Sci. U. S. A.* 95, 13835–13840. (1998). https://doi.org/10.1073/pnas.95.23.13835
40. Tian, L. L. & Feng, Z. P. Recent advances in the study of biochemical markers of bone turnover. *Chin. J. Osteoporos.* **28**, 755–759. https://doi.org/10.3969/j.issn.1006-7108.2022.05.025 (2022).
41. Kuo, T. R. & Chen, C. H. Bone biomarkers for the clinical assessment of osteoporosis: recent developments and future perspectives. *Biomark. Res.* **5**, 18. https://doi.org/10.1186/s40364-017-0097-4 (2017).
42. Karsenty, G. Osteocalcin: a multifaceted bone-derived hormone. *Annu. Rev. Nutr.* **43**, 55–71. https://doi.org/10.1146/annurev-nutr-061121-091348 (2023).
43. Yang, Y. H. et al. Clinical application of bone biomarkers OC, CTX-I, BAP, and tP1NP in osteoporosis. *Chin. J. Med. Res.* **13**, 162–164. https://doi.org/10.14033/j.cnki.cfmr.2015.27.082 (2015).
44. Chen, S. et al. Catalpol attenuates osteoporosis in ovariectomized rats through promoting osteoclast apoptosis via the Sirt6-ERα-FasL axis. *Phytomedicine* **123**, 155262. https://doi.org/10.1016/j.phymed.2023.155262 (2024).
45. Meng, J. et al. Catalpol suppresses osteoclastogenesis and attenuates osteoclast-derived bone resorption by modulating PTEN activity. *Biochem. Pharmacol.* **171**, 113715. https://doi.org/10.1016/j.bcp.2019.113715 (2020).
46. Gaytan, F., Morales, C., Reymundo, C. & Tena-Sempere, M. A novel RGB-trichrome staining method for routine histological analysis of musculoskeletal tissues. *Sci. Rep.* **10**, 16659. https://doi.org/10.1038/s41598-020-74031-x (2020).
47. Shapiro, F. & Wu, J. Y. Overview of woven bone: structural classification based on its integral role in developmental, repair, and pathological bone formation across vertebrate groups. *Eur. Cell. Mater.* **38**, 137–167. https://doi.org/10.22203/eCM.v038a11 (2019).
48. Florencio-Silva, R., Sasso, G. R., Sasso-Cerri, E., Simões, M. J. & Cerri, P. S. Biology of bone tissue: structure, function, and factors that influence bone cells. *Biomed. Res. Int.* 421746. (2015). https://doi.org/10.1155/2015/421746 (2015).

49. Zhang, Y., Zhang, J., Sun, B., Ma, L. & Ma, Y. Catalpol promotes osseointegration of titanium implants under conditions of type 2 diabetes via AKT/GSK3β/FYN pathway-mediated NRF2 activation. *ACS Omega*. **9**, 5761–5771. https://doi.org/10.1021/acsomega.3c08518 (2024).
50. Yang, J. et al. Enhancement of local osseointegration and implant stability of titanium implants in osteoporotic rats by biomimetic multilayered structures containing catalpol. *ACS Omega*. **9**, 29544–29556. https://doi.org/10.1021/acsomega.4c02322 (2024).

Figures

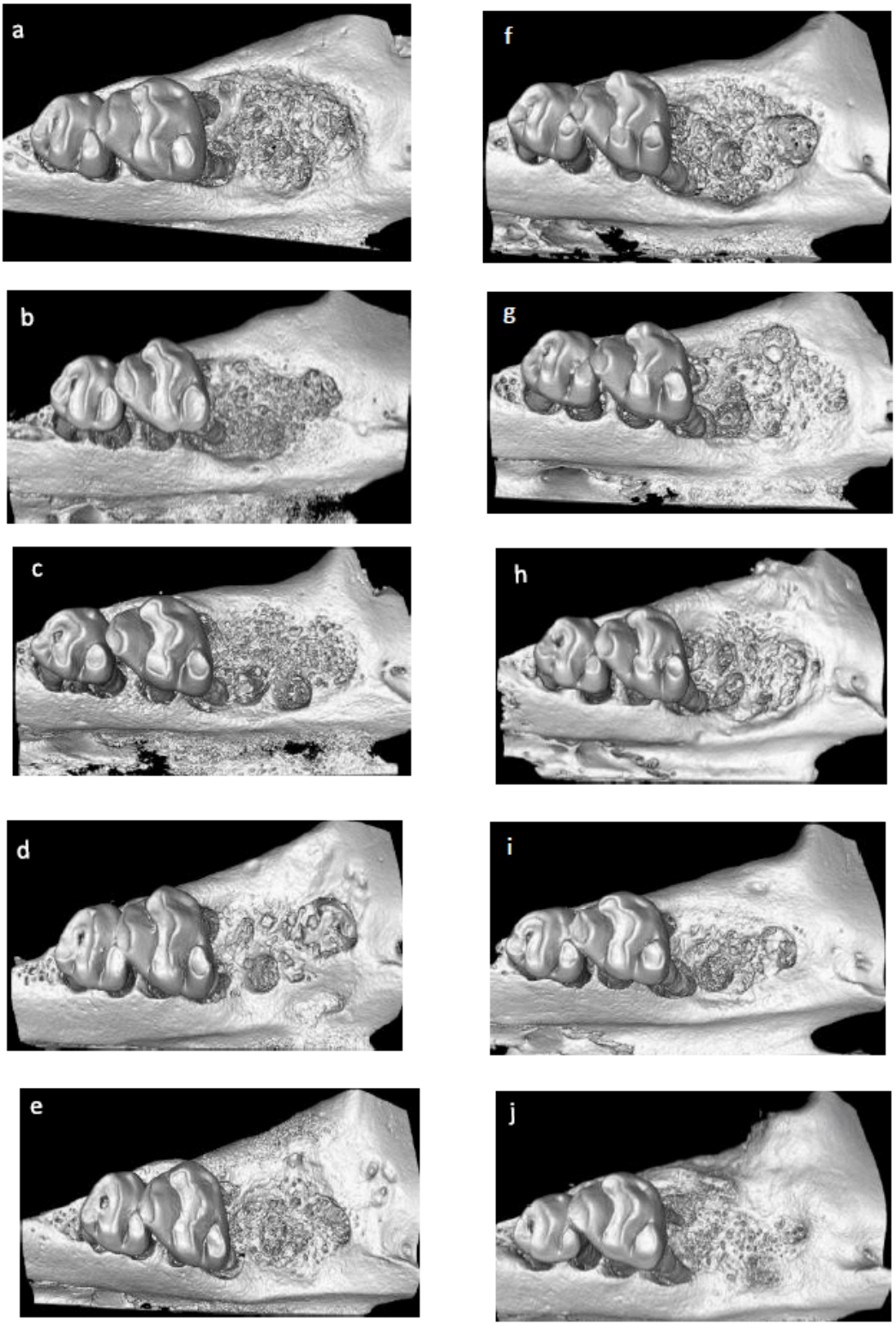


Figure 1

Three-dimensional micro-CT reconstruction of extraction sockets after surgery

Representative three-dimensional reconstruction images of the extraction sockets at 4 and 8 weeks after surgery. At 4 weeks, panels (a–e) represent the Extraction, ARP, ARP + Low-dose Catalpol, ARP + Medium-dose Catalpol, and ARP + High-dose Catalpol groups, respectively. Panels (f–j) show the

corresponding groups at 8 weeks in the same order. ARP, alveolar ridge preservation; micro-CT, micro-computed tomography.

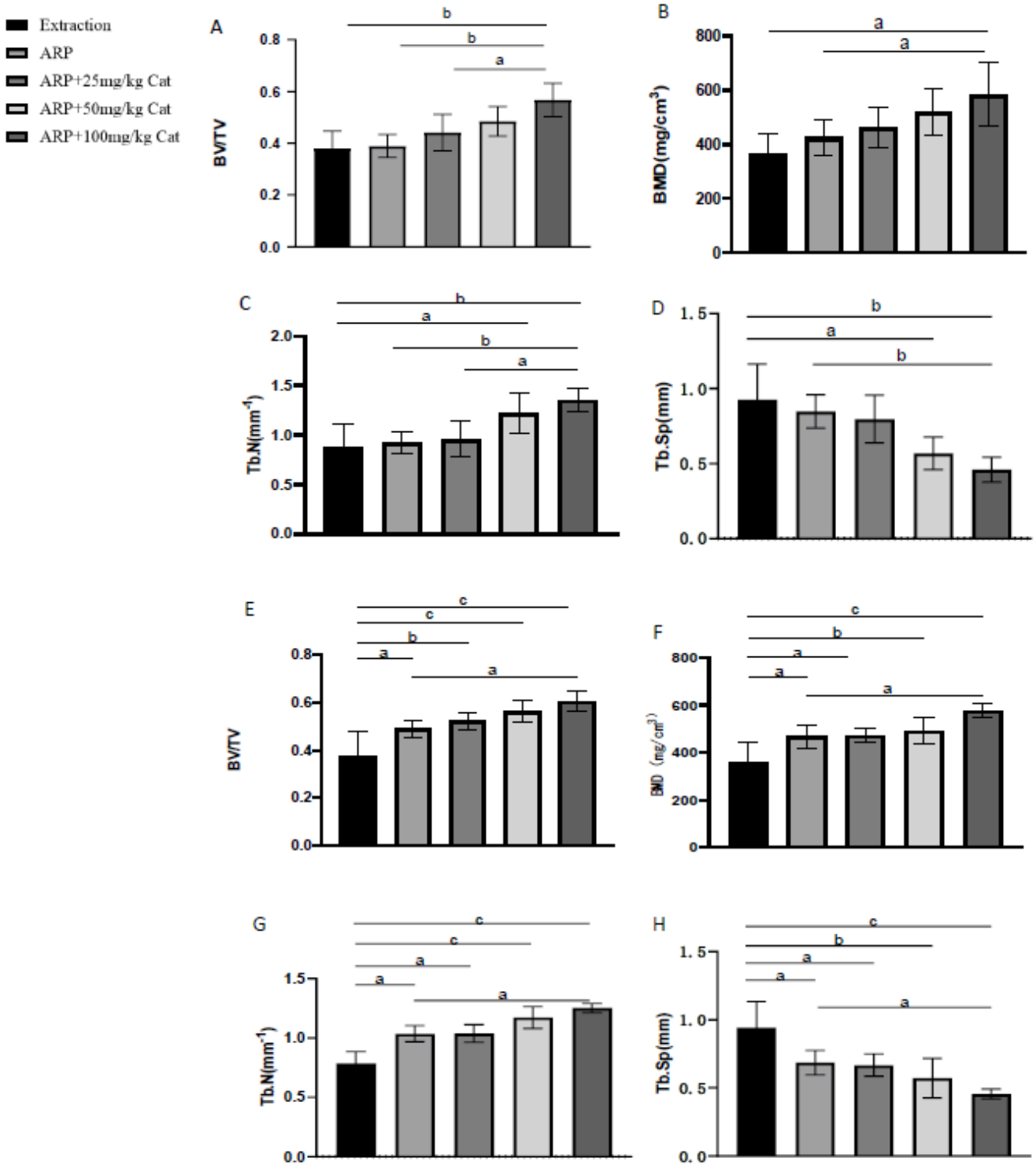


Figure 2

Micro-CT analysis of alveolar bone microstructural parameters

Quantitative analysis of alveolar bone microstructural parameters in the extraction socket region at 4 and 8 weeks after surgery. Panels (A–D) show BV/TV, BMD, Tb.Sp, and Tb.N at 4 weeks, respectively, and panels (E–H) show the corresponding parameters at 8 weeks in the same order. Data are presented as mean ± SD. Superscript letters indicate statistical significance: $P < 0.05$, $P < 0.01$, and $P < 0.001$. BV/TV, bone volume fraction; BMD, bone mineral density; Tb.Sp, trabecular separation; Tb.N, trabecular number; micro-CT, micro-computed tomography.

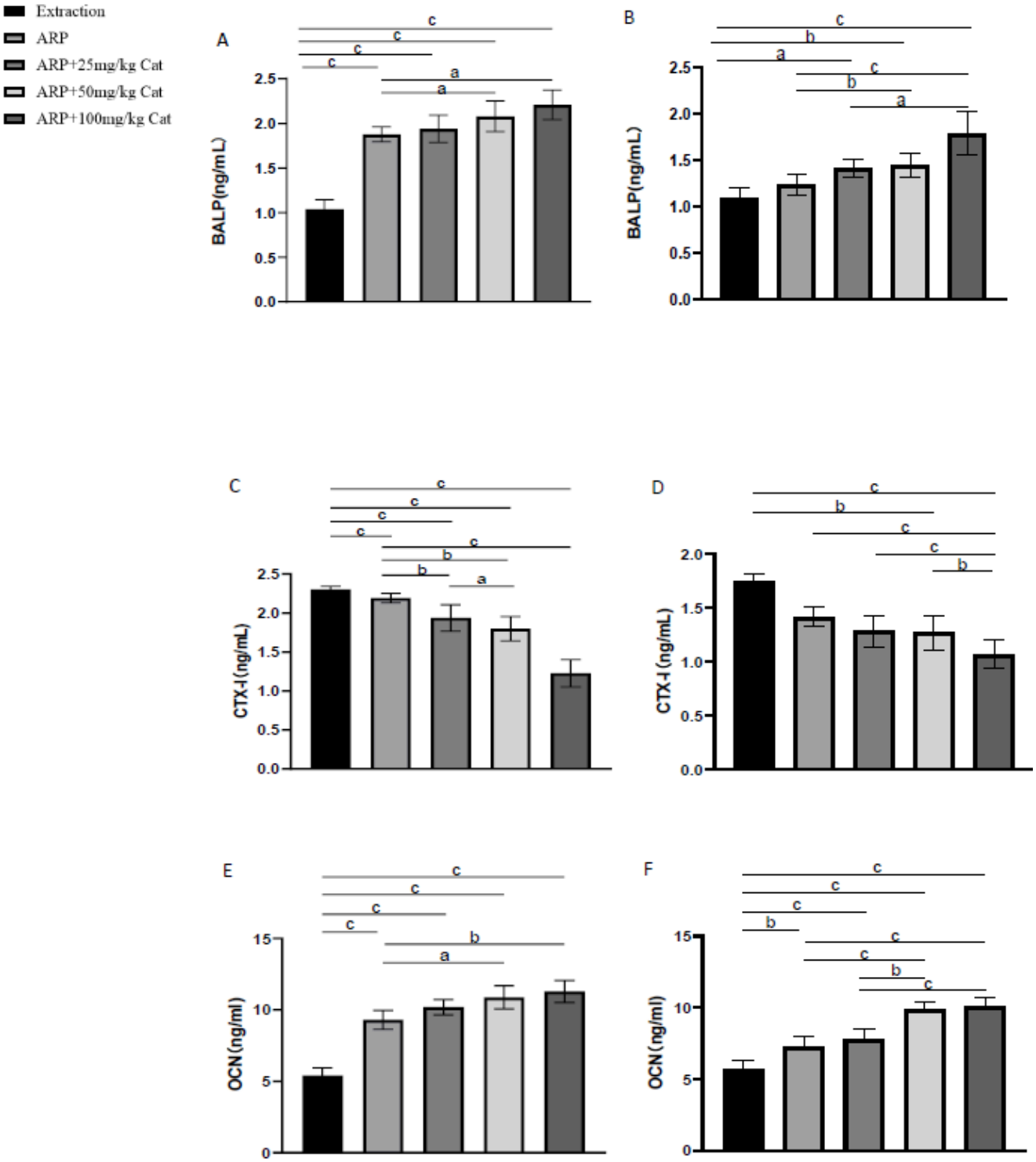


Figure 3

Effects of Catalpol on serum bone metabolism markers

Serum levels of bone alkaline phosphatase (BALP), C-terminal telopeptide of type I collagen (CTX-I), and osteocalcin (OCN) were measured by ELISA at 4 and 8 weeks after surgery. Panels (A, B) show BALP levels at 4 and 8 weeks, respectively; panels (C, D) show CTX-I levels at 4 and 8 weeks, respectively; and panels (E, F) show OCN levels at 4 and 8 weeks, respectively. Data are presented as mean ± SD. Superscript letters indicate statistical significance: $^{a}P< 0.05$, $^{b}P < 0.01$, and $^{c}P < 0.001$. BALP, bone alkaline phosphatase; CTX-I, C-terminal telopeptide of type I collagen; OCN, osteocalcin; ELISA, enzyme-linked immunosorbent assay.

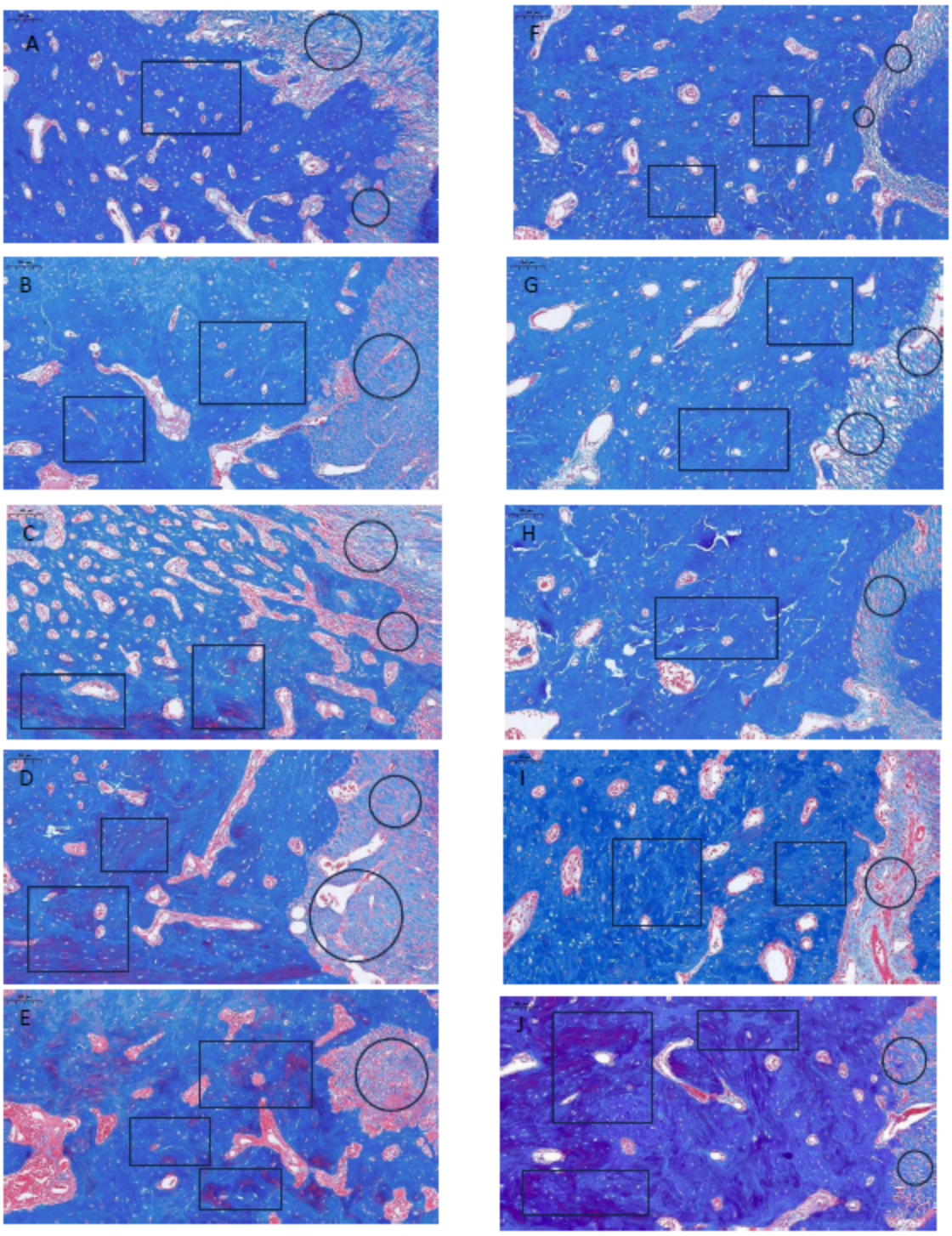


Figure 4

Histological evaluation of extraction socket healing by Masson's trichrome staining

Representative Masson's trichrome-stained images of the extraction sockets at 4 and 8 weeks after surgery. At 4 weeks, panels (A–E) represent the Extraction, ARP, ARP + Low-dose Catalpol, ARP + Medium-dose Catalpol, and ARP + High-dose Catalpol groups, respectively. Panels (F–J) show the corresponding groups at 8 weeks in the same order. Rectangular boxes indicate areas of new bone

formation, whereas circular boxes indicate regions of connective tissue. ARP: alveolar ridge preservation.