

Innovative Development and Characterisation of a Hyaluronic Acid Gelatin-based Injectable Hydrogel Infused with Ranitidine for Periodontal Applications: An In-vitro Study

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ABSTRACT

Introduction: Periodontitis, a chronic inflammatory disease of the periodontium, is driven by bacterial biofilms that disrupt host immune responses and accelerate disease progression. While scaling and root planing remain standard treatments, their limitations in preventing bacterial recolonisation highlight the need for adjunctive therapies. Hydrogels offer a promising solution due to their biocompatibility, ease of application, and sustained drug release, enhancing the effectiveness of conventional periodontal treatment.

Aim: To develop and characterise a Hyaluronic Acid (HA) and gelatin-based injectable hydrogel incorporating ranitidine, an H₂ receptor antagonist, for periodontal applications.

Materials and Methods: This in-vitro study was conducted at Saveetha Dental College, Chennai, Tamil Nadu, India, from February 2024 to May 2024. HA, gelatin, and ranitidine were combined to formulate the hydrogel. The formulation was refined and cross-linked before being stored. To assess the hydrogel's safety, haemolytic and biocompatibility assays

were conducted for both the hydrogel group and the ranitidine plus hydrogel group. Biocompatibility was evaluated using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) assay on human periodontal ligament fibroblasts, and haemolysis was detected by spectrophotometry at 545 nm following incubation with Red Blood Cells (RBCs). Fibroblast morphological alterations were observed using phase contrast microscopy. Intergroup comparisons were conducted using ANOVA, and a p-value of ≤ 0.05 was considered statistically significant.

Results: The haemolytic assay indicated minimal haemolysis ($<2\%$) at therapeutic concentrations, comparable to Negative Controls (NC). Biocompatibility tests revealed $>90\%$ cell viability in the ranitidine hydrogel group, with no significant morphological alterations in fibroblasts, indicating low cytotoxicity ($p \leq 0.05$).

Conclusion: The developed ranitidine hydrogel demonstrated excellent biocompatibility and non haemolytic properties, underscoring its potential as a safe and effective intra-pocket delivery system for periodontal therapy.

Keywords: Histamine, Histamine blocker, Hyaluronic acid, Periodontitis

INTRODUCTION

Periodontitis, a chronic inflammatory disorder affecting the investing structures of teeth, leads to the deterioration of the gingiva and periodontal ligament, ultimately resulting in tooth mobility and loss [1]. Periodontitis has been closely associated with several bacterial species in dental plaque. *Porphyromonas gingivalis*, *Treponema denticola*, *Fusobacterium nucleatum*, and *Actinobacillus actinomycetemcomitans* are some of the species playing a pivotal role in the pathogenesis of periodontal disease [2]. This microbial biofilm activates an inflammatory immune response [3].

Timely intervention can arrest the disease and improve the clinical conditions of the supporting structures of the teeth. Scaling and root planing have been established as the gold standard treatment for periodontitis despite certain shortcomings, such as inaccessibility to furcation areas and interdental regions, which provide niches for further bacterial colonisation and, consequently, recurrence of the disease [4,5]. Although systemic antimicrobial therapy has helped to curb this effect by achieving therapeutic crevicular levels and providing systemic action, antibiotic resistance has emerged as a significant issue during the course of treatment [6]. Thus, local drug delivery into the periodontal pocket, without systemic dissipation, has proven to be a promising avenue for overcoming the adverse effects associated with traditional treatments [7,8].

Various treatment modalities have been proposed, among which injectable hydrogels have emerged as a promising material for the management of periodontitis. Literature evidence has demonstrated

that chitosan-based hydrogels provide a solid foundation for the development of multifunctional local drug delivery biomaterials for periodontitis treatment [9]. Similarly, antibiotic loaded and herbal-based hydrogels have also shown proven efficacy [10,11]. These hydrogels offer several advantages, such as minimally invasive administration, tunable mechanical properties, and the ability to encapsulate bioactive molecules for controlled release [12]. Among the different types of injectable hydrogels, those based on HA have received considerable attention due to their biocompatibility and biodegradability [13].

Ranitidine, a well-established Histamine H₂ Receptor (H₂R) antagonist, has been in clinical use since 1988, primarily for managing peptic ulcers and various inflammatory disorders of the gastrointestinal tract. Its primary mechanism of action involves effectively blocking H₂ receptors, predominantly located on gastric parietal cells, thereby suppressing excessive gastric acid secretion. Furthermore, ranitidine is frequently coadministered alongside antibiotics and anti-inflammatory drugs to mitigate gastrointestinal adverse effects, making it a staple in gastroenterological practice [14].

Beyond its gastroprotective effects, an expanding body of evidence has highlighted the intricate role of histamine in the pathogenesis of periodontitis. Histamine, through its interaction with H₂ receptors, can potentiate inflammatory responses, exacerbating periodontal tissue destruction. Notably, recent investigations have elucidated ranitidine's immunomodulatory properties, demonstrating its capacity to attenuate inflammatory cascades

[15,16]. These findings, combined with its favorable safety profile, cost-effectiveness, and minimal adverse effects, underscore the rationale for exploring ranitidine's therapeutic potential beyond its conventional applications. In light of this, the present study sought to harness these attributes by formulating an innovative HA and gelatin-based injectable hydrogel incorporating ranitidine. Thus, the development and characterisation of this ranitidine-loaded HA-gelatin injectable hydrogel pave the way for a transformative, patient-centric therapeutic strategy in periodontal care, contributing to the expanding landscape of innovative drug delivery systems in dental research.

MATERIALS AND METHODS

This in-vitro study was conducted at Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India from February 2024 to May 2024.

Study Procedure

Preparation of ranitidine hydrogel: The injectable hydrogel was prepared [17] as follows: 5 mL of HA was dissolved in deionised water at a concentration of 1.5% w/v. Similarly, a 0.5% gelatin solution was prepared. A 1:1 concentration of the prepared HA and gelatin was mixed, and 500 µL of ranitidine was then added dropwise to the mixture under magnetic stirring (Remi Elektrotechnik Limited, Maharashtra, India) at a final concentration of 50 µg/mL. The resulting solution was injected into a cylindrical mold (10 mm diameter) and subjected to sequential immersion in 0.145 M Calcium Chloride (CaCl₂) and 0.087 M Phosphoric Acid (H₃PO₄) solutions for two hours each, followed by immersion in 10% (v/v) Ammonium Hydroxide (NH₄OH) solution for 30 minutes. Finally, the hydrogel was washed with Phosphate Buffered Saline (PBS) to remove any residual components, and the resultant hydrogel was stored at 4°C. All chemicals were purchased from Merck Chemicals, Darmstadt, Germany.

Haemolysis assay: The haemolysis assay was performed with the following groups: the 100 µg/mL hydrogel group (HA+Gelatin), the 100 µg/mL ranitidine hydrogel group (100 µg/mL ranitidine+HA + Gelatin), the Positive Control (PC) group (Triton X-100), and the Negative Control (NC) group (distilled water) [18]. Five mL of blood were drawn from the antecubital vein of a periodontally and systemically healthy individual. A diluted whole blood solution was prepared by diluting 4 mL of fresh anticoagulant {Ethylenediaminetetraacetic acid (EDTA)} whole blood with 5 mL of 0.9 wt% Sodium Chloride (NaCl) solution. Subsequently, 50 µL of the ranitidine hydrogel sample (test group) was added to 950 µL of 1x PBS solution in a 1.5 mL Eppendorf centrifuge tube. The tube was then incubated at 37°C for 30 minutes. Following incubation, 0.2 mL of the diluted whole blood was added to the tube, and the mixture was further incubated at 37°C for one hour to allow for haemolysis to occur. The solution was then centrifuged at 1000 rpm for 10 minutes to separate the intact cells from the supernatant containing the released hemoglobin. The same methodology was applied for the 100 µg/mL hydrogel group (HA+Gelatin), the PC group (Triton X-100), and the NC group (distilled water). The absorbance of the supernatant was measured at 545 nm using an ultraviolet spectrophotometer. The percentage of haemolysis obtained was then compared between the 100 µg/mL hydrogel group (HA+Gelatin), the 100 µg/mL ranitidine hydrogel group (100 µg/mL ranitidine+HA+Gelatin), the PC group (Triton X-100), and the NC group (distilled water).

Biocompatibility assay: The biocompatibility of the hydrogel group and the ranitidine hydrogel group, along with the control (placebo gel), treated on human periodontal ligament tissue-derived primary fibroblasts, was determined via the MTT assay [19]. Briefly, fibroblast cells were seeded onto a 96-well culture plate and incubated with the hydrogel for 24, 48, and 72 hours. After the incubation period, the cells were treated with 10 µL of stock MTT dye (10 mg/mL)

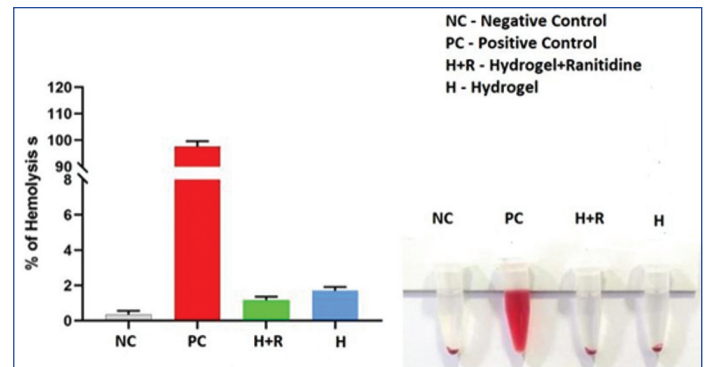
and further incubated at 37°C for four hours. The formazan crystals formed by viable cells were dissolved by replacing the medium with 100 µL of Dimethyl Sulfoxide (DMSO) in each well. The absorbance was measured at 570 nm using a Synergy H1 Hybrid Multi-Mode Reader (BioTek, Winooski, VT, US). This assay was performed in triplicate. Additionally, the morphology of viable cells was viewed using a phase contrast and fluorescence microscope (Carl Zeiss AG, Oberkochen, Germany) at a magnification of 20X.

STATISTICAL ANALYSIS

Data analysis was performed using the Statistical Package for Social Sciences (SPSS, Version 23.0). Intergroup differences were analysed using ANOVA. Pairwise comparisons were performed using Tukey's post-hoc test. A p-value of ≤0.05 was considered statistically significant.

RESULTS

Haemolysis Assay: The lysis of RBCs was less than 2% at a concentration of 100 µg/mL of ranitidine hydrogel, which was significantly lower compared to the PC group, with a p-value of ≤0.05 [Table/Fig-1]. This suggests that ranitidine hydrogel can be used without causing haemolytic destruction to the RBCs.



[Table/Fig-1]: Comparison of haemolysis among groups: Negative Control (NC), Positive Control (PC), 100 µg/mL ranitidine hydrogel (Ranitidine+HA+Gelatin), and 100 µg/mL hydrogel (HA+Gelatin).

Biocompatibility assay: [Table/Fig-2] demonstrated a significant difference in fibroblast viability among the groups at all three time points (p≤0.05). At 24 hours, the control group exhibited the highest viability (3.14±0.00), followed by the hydrogel+ranitidine group (2.97±0.14), while the hydrogel group showed the lowest viability (2.19±0.07). A similar pattern was evident at 48 hours and continued through 72 hours, with the hydrogel+ranitidine group maintaining higher viability compared to the hydrogel group. This consistent finding highlighted the enhanced biocompatibility of the hydrogel+ranitidine formulation. [Table/Fig-3] further confirmed that the hydrogel+ranitidine group maintained significantly better fibroblast viability than the hydrogel group at all time points (p≤0.05). While the control group exhibited the highest viability, the hydrogel+ranitidine formulation was significantly less cytotoxic compared to the hydrogel alone. The significant differences observed at 72 hours suggest a sustained protective effect of ranitidine on fibroblasts. These findings support the hypothesis that the hydrogel+ranitidine combination is the most biocompatible formulation, making it a promising candidate for periodontal therapy.

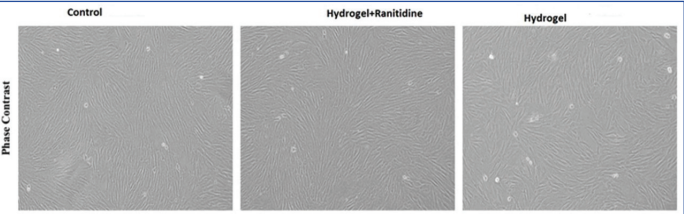
Optical Density (OD) reading at different durations (hrs)	Control group	Hydrogel+ Ranitidine	Hydrogel	ANOVA (p-value)
24	3.14±0.00	2.97±0.14	2.19±0.07	0.007*
48	3.13±0.01	2.56±0.66	2.01±0.35	0.005*
72	3.12±0.02	2.49±0.63	1.93±0.67	0.007*

[Table/Fig-2]: Comparison of viability of fibroblasts at 24 hours, 48 hours and 72 hours between control, hydrogel + ranitidine and hydrogel groups.
*Statistically Significant

Time (hrs)	Group comparison	Mean difference	p-value
24	Control vs Hydrogel + Ranitidine	0.17	0.04*
	Control vs Hydrogel	0.95	0.001*
	Hydrogel + Ranitidine vs Hydrogel	0.78	0.001*
48	Control vs Hydrogel + Ranitidine	0.57	0.05*
	Control vs Hydrogel	1.12	0.001*
	Hydrogel + Ranitidine vs Hydrogel	0.55	0.05*
72	Control vs Hydrogel + Ranitidine	0.63	0.04*
	Control vs Hydrogel	1.19	0.001*
	Hydrogel + Ranitidine vs Hydrogel	0.56	0.05*

[Table/Fig-3]: Tukey's post-hoc pairwise comparisons for fibroblast viability.
*Statistically Significant

Morphology of fibroblast: Assessing the morphology of fibroblasts under phase contrast microscopy revealed that there were no changes in cell morphology in either the hydrogel+ranitidine or hydrogel groups when compared with the control group. This suggests that the test groups do not induce any morphological changes in fibroblasts and maintain cell viability, thus making them favorable for the intended purpose [Table/Fig-4].



[Table/Fig-4]: Morphology of fibroblasts of control, hydrogel + ranitidine, and hydrogel groups.

DISCUSSION

Histamine appears to prevent the release of lysozyme and β -glucuronidase by binding to H2 receptors on the surface of neutrophils, monocytes, and macrophages. This leads to elevated production of cyclooxygenase-2 and Interleukin-6 (IL-6), as well as prostaglandin E2 secreted by periodontal ligament fibroblast cells. Furthermore, natural killer cells and cytotoxic T-lymphocytes are inhibited by histamine [20]. Periodontal disease-related histamine production appears to encourage the growth of T suppressor cells, which in turn suppress cellular immunity. Additionally, it has been suggested that histamine, through H2 receptors, may impede the humoral immune response by preventing B-cells from releasing Immunoglobulins (Igs) such as IgG and IgM. Thus, the evidence suggests that histamine prevents host defense mechanisms by interfering with humoral and cellular immunity, thereby deteriorating periodontal health [21,22].

Treatment strategies for periodontal disease: Treatment strategies for periodontal disease can be categorised into surgical, non-surgical, or a combination of both. Non surgical treatments are designed to eradicate bacterial load and improve the immune response. However, it should be noted that the improvements achieved with non-surgical treatment have been observed to be temporary due to the virulent nature of the pathogenic organisms causing the disease and the altered host immune response. Therefore, most dental professionals concur on the use of adjuncts along with mechanical debridement [23,24].

The results of the present study indicate that ranitidine hydrogel presents a biocompatible solution for treating periodontal disease non invasively. Ranitidine hydrogel has shown the ability to preserve the viability and morphology of healthy periodontal ligament fibroblasts and has been found not to induce haemolysis, making it a favorable option for periodontal disease treatment. This result aligns with a previous study conducted by Wolak M et al., which found that ranitidine did not affect the viability of fibroblasts in a rat model [25].

Another animal study documented the role of histamine in osteoclastogenesis, where histamine treated mandibles of rats showed an increase in osteoclasts and their precursors. In contrast, treatment with an H2R blocker decreased the precursor osteoclast cells and helped reduce bone loss [16]. Additionally, another investigation explored the clinical and histopathological alterations linked to experimental periodontitis in rabbits following the topical application of an H2R blocker. The H2R blocker, applied across various tested concentrations, demonstrated remarkable efficacy in curbing inflammation and bone loss by over 90%. Histopathological analyses revealed a substantial decrease in bone levels and a notable reduction in inflammatory responses. The study provided both morphological and histological evidence underscoring the potency of the topically active H2R blocker as a suppressor of *Porphyromonas gingivalis*-induced periodontal inflammation, effectively halting or mitigating tissue damage while modulating the cellular composition of the inflammatory infiltrate. Moreover, a pronounced reduction in the number of osteoclasts, accompanied by an increased prevalence of apoptotic osteoclasts, was observed upon exposure to the H2R blocker. A notable decrease in the density of Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL)-positive cells was also documented. The diminished osteoclast population is likely attributed to the H2R blocker's induction of osteoclast apoptosis [26]. de Oliveira PA et al., proposed that exposure to an H2R blocker in rats with induced periodontitis led to a decrease in bone loss, attributed to a reduction in osteoclast numbers and diminished immunoexpression of IL-6, Matrix Metalloproteinase-1 (MMP-1), and MMP-9. These findings further support the notion that H2R blockers exert a protective effect in managing periodontal disease [27].

Overall, the findings from previous studies point to the beneficial effects of H2R blockers in periodontal disease. Our findings align with those of previous studies [25-27]. This in-vitro study paves the way for the development of a novel H2R blocker as a local drug in the management of periodontal disease.

Limitation(s)

While this study provides compelling in-vitro evidence of the ranitidine-loaded hydrogel's biocompatibility, non haemolytic nature, and potential for intra-pocket delivery, it is not without limitations. First, the findings are confined to laboratory conditions, which may not fully replicate the complex and dynamic environment of the periodontal pocket in vivo. Factors such as salivary flow, mechanical forces, and the presence of a diverse microbial biofilm could influence the hydrogel's stability, drug release profile, and therapeutic efficacy. Additionally, long-term safety, biodegradability, and the hydrogel's ability to sustain its pharmacological action over time remain unexplored. Therefore, extensive in vivo and clinical studies are essential to validate these preliminary results and establish the hydrogel's practical applicability in periodontal therapy.

CONCLUSION(S)

The study findings suggest that the developed ranitidine hydrogel is biocompatible and non haemolytic to RBCs. Furthermore, exposure to fibroblasts caused no morphological alterations, with cells remaining viable even at 72 hours, indicating minimal cytotoxicity. These promising results imply its potential application in periodontal therapy, warranting further validation through large-scale clinical trials.

REFERENCES

[1] Slots J. Periodontitis: facts, fallacies and the future. Periodontol 2000. 2017;75(1):7-23.
[2] Rajasekar A, Varghese SS. Microbiological profile in periodontitis and peri-implantitis: A systematic review. J Long-Term Eff Med Implants. 2022;32(4):83-94.
[3] Ebersole JL, Dawson III D, Emecen Huja P, Nagarajan R, Howard K, Grady ME, et al. The periodontal war: Microbes and immunity. Periodontol 2000. 2017;75(1):52-115.

- [4] Mombelli A. Microbial colonization of the periodontal pocket and its significance for periodontal therapy. *Periodontol*. 2000; 2018;76(1):85-96.
- [5] Cobb CM. Clinical significance of non-surgical periodontal therapy: An evidence based perspective of scaling and root planing. *J Clin Periodontol*. 2002;29:22-32.
- [6] Ilyes I, Boariu M, Rusu D, Iorio-Siciliano V, Vela O, Boia S, et al. Comparative Study of systemic vs. local antibiotics with subgingival instrumentation in stage III–IV periodontitis: A retrospective analysis. *Antibiotics*. 2024;13(5):430.
- [7] Meenakshi PS, Malaippan S. Additive effect of brown flaxseed extract gel on postsurgical periodontal therapy in periodontitis patients: A randomised controlled trial. *J Clin Diagn Res*. 2024;18(7):ZC16-ZC20.
- [8] Bajpai D, Malaippan S. Role of mustard seed extract based hydrogel as an adjunct to non-surgical periodontal therapy in chronic periodontitis patients: A pilot interventional study. *J Clin Diagn Res*. 2024;18(6):ZC21-ZC24.
- [9] Ma S, Lu X, Yu X, Du Y, Xu S, Li M, et al. An injectable multifunctional thermo-sensitive chitosan-based hydrogel for periodontitis therapy. *Biomaterials Advances*. 2022;142:213158.
- [10] Valan AS, Kolli S, Eswaramoorthy R, Krithikadatta J, Sureshbabu NM. Comparison of antibacterial efficacy of triple antibiotic-loaded hydrogel versus modified triple antibiotic-loaded hydrogel as intracanal medicament against *Enterococcus faecalis*: An in vitro study. *Eur Endod J*. 2024;9(2):154-60.
- [11] Dharini S, Pandiar D, Rajeshkumar S, Krishnan RP. Evaluation of anti-inflammatory and antioxidant properties of *persea americana* and *syzygium aromaticum*-based herbal mouthwash formulation: An in-vitro study. *J Clin Diagn Res*. 2024;18(10):ZF01-ZF05.
- [12] Omidian H, Chowdhury SD. Advancements and applications of injectable hydrogel composites in biomedical research and therapy. *Gels*. 2023;9(7):533.
- [13] Trombino S, Servidio C, Curcio F, Cassano R. Strategies for hyaluronic acid-based hydrogel design in drug delivery. *Pharmaceutics*. 2019;11(8):407.
- [14] Clark K, Lam LT, Gibson S, Currow D. The effect of ranitidine versus proton pump inhibitors on gastric secretions: A meta-analysis of randomised control trials. *Anaesthesia*. 2009;64(6):652-57.
- [15] Minami T, Kuroishi T, Ozawa A, Shimauchi H, Endo Y, Sugawara S. Histamine amplifies immune response of gingival fibroblasts. *J Dent Res*. 2007;86(11):1083-88.
- [16] Blosse-Duplan M, Baroukh B, Dy M, de Vernejoul MC, Saffar JL. Histamine promotes osteoclastogenesis through the differential expression of histamine receptors on osteoclasts and osteoblasts. *Am J Pathol*. 2009;174(4):1426-34.
- [17] Banigo AT, Konings IBM, Nauta L, Zoetebier B, Karperien M. Synthesis and engineering of hyaluronic acid-gelatin hydrogels with improved cellular attachment and growth. *Polymers (Basel)*. 2024;16(23):3410.
- [18] Sæbø IP, Bjørås M, Franzky H, Helgesen E, Booth JA. Optimization of the hemolysis assay for the assessment of cytotoxicity. *Int J Mol Sci*. 2023;24(3):2914.
- [19] Koka P, Mundre RS, Rangarajan R, Chandramohan Y, Subramanian RK, Dhanasekaran A. Uncoupling warburg effect and stemness in CD133+ ve cancer stem cells from Saos-2 (osteosarcoma) cell line under hypoxia. *Mol Biol Rep*. 2018;45(6):1653-62.
- [20] Azuma Y, Shinohara M, Wang PL, Hidaka A, Ohura K. Histamine inhibits chemotaxis, phagocytosis, superoxide anion production, and the production of TNF α and IL-12 by macrophages via H2-receptors. *Int Immunopharmacol*. 2001;1(9-10):1867-75.
- [21] Kmiecik T, Otocka-Kmiecik A, Górka-Ciebiada M, Ciebiada M. State of the art paper T lymphocytes as a target of histamine action. *Arch Med Sci*. 2012;8(1):154-61.
- [22] Steinsvoll S, Helgeland K, Schenck K. Mast cells- A role in periodontal diseases? *J Clin Periodontol*. 2004;31:413-19.
- [23] Syed VA, Pavithra A, Rajasekar A, Shanmugam R. Preparation of nutmeg gel and evaluation of its efficacy as local drug delivery in Stage II Grade A periodontitis patients: A prospective interventional study. *J Clin Diagn Res*. 2021;19(2):ZC01-ZC05.
- [24] Chatterjee S, Rajasekar A. Preparation and characterization of ferulic acid hydrogel and its application as a local drug delivery agent in periodontitis. *Cureus*. 2024;16(5):e60534.
- [25] Wolak M, Bojanowska E, Staszewska T, Ciosek J, Juszcak M, Drobnik J. The role of histamine in the regulation of the viability, proliferation and transforming growth factor β 1 secretion of rat wound fibroblasts. *Pharmacol Rep*. 2017;69(2):314-21.
- [26] Longhini R, Aparecida de Oliveira P, Sasso Cerri E, Cerri PS. Cimetidine reduces alveolar bone loss in induced periodontitis in rat molars. *J Periodontol*. 2014;85(8):1115-25.
- [27] de Oliveira PA, de Pizzol Júnior JP, Longhini R, Sasso Cerri E, Cerri PS. Cimetidine reduces interleukin 6, matrix metalloproteinases 1 and 9 immunoreexpression in the gingival mucosa of rat molars with induced periodontal disease. *J Periodontol*. 2017;88(1):100-11.

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