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Review Article

Trypsin, bromelain and rutoside in dental pathologies-synergistic action and qualitative metanalysis of investigator-initiated studies

Shashank Tiwari[1]*, Bhushan M. Khemnar[2], James John[2], Ganesh H. Divekar[2]

[1]M. V. Hospital and Research Centre, Lucknow, Uttar Pradesh, India
[2]Department of Medical Services, SIRO Clinpharm Ltd., Thane, Maharashtra, India

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***Correspondence:**
Dr. Shashank Tiwari,
E-mail: tdrshashank@gmail.com

ABSTRACT

Inflammation, which manifests as pain and swelling, forms the basis of various dental pathologies like dental caries and pulpitis. Inflammation is also associated with tissue injury in cases of surgical processes like tooth extraction, root canal treatment or third molar extraction. Various drugs which help reducing inflammation are used in the treatment of dental pathologies. In this article, the mechanism of action of trypsin, bromelain and rutoside is mapped to show the clinical benefit. To demonstrate comparative benefit of trypsin, bromelain, and rutoside combination, a literature search was carried out for real-world investigator-initiated studies. A qualitative analysis of these studies was carried out. All 7 studies, identified in literature, controlled clinical studies comparing efficacy and safety of combination of trypsin, bromelain, and rutoside with that of non-steroidal anti-inflammatory drugs like diclofenac, versus other anti-inflammatory enzymes like trypsin-chymotrypsin combination or serratiopeptidase. All studies have demonstrated efficacy benefit of combination of trypsin, bromelain and rutoside. Importance and place of trypsin-bromelain-rutoside combination in therapy of dental pathologies, is further emphasized based on the results of this review.

Keywords: Inflammation, Pain, Swelling, Enzymes, Flavonoids

INTRODUCTION

Dental pathologies include minor pathologies like dental caries to major pathologies like pulpitis or tooth abscess. All these pathologies are associated with inflammation, which is manifested as pain, redness or swelling. Most of dental pathologies are associated with extreme pain and swelling; and controlling them form the basis of the treatment.[1] Certain dental pathologies are treated surgically to help eliminate the underlying root cause. The treatment of these pathologies with or without surgical intervention includes controlling infection and inflammation. The infection is controlled with the help of antibiotics, both covering aerobic and anaerobic bacteria whereas the inflammation is controlled with the help of non-steroidal anti-inflammatory agents (NSAIDs) like ibuprofen or anti-inflammatory enzymes.[1]

The anti-inflammatory enzymes include combination of trypsin, bromelain, rutoside or combination of trypsin-chymotrypsin or serratiopeptidase. These anti-inflammatory enzymes help in reducing pain and swelling by various mechanisms. Though there are limited comparative studies between combination of trypsin, bromelain, rutoside and combination of trypsin-chymotrypsin, limited clinical data demonstrate that the efficacy of serratiopeptidase is least among available anti-inflammatory agents.

Mechanism of action and synergistic effects of trypsin, bromelain, rutoside combination have been described in detail elsewhere; a summary is provided in Table 1.[2] Based on the perceived clinical practice benefit of this combination, searched various real-world investigator-

initiated clinical studies published in India. Descriptive metanalysis of these studies is added in this article.

Synergistic effect of trypsin, bromelain, rutoside combination

Trypsin is serine protease, whereas bromelain is cysteine protease enzyme and rutoside is a bioflavonoid. As trypsin and bromelain have different substrate specificities, the combination of trypsin and bromelain is logical. With different substrate specificities trypsin and bromelain intervene four different processes in an inflammation cascade viz. inflammatory mediators release, adhesion molecules modulation, fibrinolysis activation and enhanced wound healing shown in the Table 1.

Table 1: Mechanisms and clinical benefits of trypsin, bromelain, and rutoside.

Ingredients	Pharmacodynamic effects	Mechanism
Trypsin (Serine protease)	Fibrinolysis, leading to breakdown of thrombi	Displacement of 'bound plasmin' from plasma proteins (e.g., α2-antitrypsin); free plasmin mediates fibrinolysis
	Reduces the release of inflammatory mediators like Interferon gamma (IFNγ, thus limiting the further activation of macrophages.)	Cleavage of molecules on antigen presenting cells (APCs), thereby increasing the activation threshold of T cells
	Wound healing and tissue repair	Promotion of macrophage differentiation to a more healing/repairing profile (M2), rather than inflammatory profile (M1) [Mediated by trypsin activation of PAR1 and PAR2 receptors on macrophages]
Bromelain (Cysteine protease)	Reduces vascular permeability	Depletion of kininogen, which is required for producing bradykinin (an important mediator in causing vasodilation and increased vascular permeability)
	Fibrinolysis, leading to breakdown of thrombi	Proteolytic conversion of plasminogen to plasmin
	Inhibition of platelet aggregation, preventing formation of thrombi	Inhibition of adhesion molecules on the platelet surface
	Inhibition of leucocyte migration to site of inflammation (especially neutrophils)	Alteration of cell surface molecules that are involved in leucocyte cellular adhesion
	Reduced inflammatory cytokine production by macrophages	Alteration of cell surface molecules that are involved in leucocyte activation
Rutoside (Bioflavonoid)	Inhibition of platelet aggregation; preventing formation of thrombi	Inhibition of intracellular calcium [Ca^{2+}] mobilization in platelets
	Reduced inflammatory cytokine production by macrophages	Inhibition of transcription of genes for proinflammatory cytokines
	Reduced tissue damage by reactive oxygen species/ free radicals (Antioxidant effect)	Scavenging of free radicals, inhibition of generation of reactive oxygen and nitrogen species
	Reduces vascular permeability	Attenuation of the effects of histamine, bradykinin, as well as fibrin degradation products and by inhibiting the production of the nitric oxide

LITERATURE SEARCH

Databases searched

The literature search strategy utilised to search investigator-initiated studies with combination of trypsin, bromelain, rutoside in dental pathologies was defined. The database searched included Google scholar, PubMed and PubMed central. As the preferred journals for the publication of the investigator-initiated studies include the non-indexed journal google scholar database was used in the addition to the standard biomedical research journals.

Search string used

To search non-sponsored, investigator-initiated studies following search string was used: trypsin, bromelain, rutoside, efficacy and safety, tooth extraction, third molar extraction, dental pathologies.

RESULTS

Total 14 articles were reviewed based on initial result of the search. Out of these 14 articles, 3 articles were sponsored studies and hence removed from further analysis. Out of remaining 11 articles, 2 articles were

related to osteoarthritis of temporomandibular joint (TMJ) and 2 articles were with single agent bromelain. As the pathophysiology of TMJ osteoarthritis is different than other dental pathologies or post-surgical intervention for dental pathologies, hence these 2 articles were not taken ahead for meta-analysis. Two articles with single agent Bromelain were also removed as the objective is to have efficacy of combination of trypsin, bromelain and rutoside. Remaining 7 articles were taken ahead for descriptive metanalysis. The summary data from these studies are presented in Table 2.[3-9]

Table 2: Literature references and summary results.

Author name and years	Study design and number of subjects	Treatment arms and treatment	Parameters evaluated	Observations/ results
Wala LJ, 2020[3]	Randomized controlled study; 230 patients undergoing third molar extraction (115 patients in each arm)	Group 1: combination of trypsin, bromelain, rutoside. Group 2: Ibuprofen 400 mg, trypsin-chymotrypsin	Measurement of facial swelling, maximal mouth opening. Pain using visual analogue scale (VAS)	Group 1 with bromelain, trypsin and rutoside combination showed a significant reduction in pain score when compared with group 2 at all-time intervals
Menon VD, 2021[4]	Randomized controlled study; 60 patients undergoing extraction of impacted mandibular third molar (30 patients in each arm)	Group 1: combination of trypsin, bromelain, rutoside. Group 2: Diclofenac with serratiopeptidase	Measurement of facial swelling Pain using visual analogue scale (VAS)	Group 1 reduced facial swelling significantly whereas group 2 reduced pain significantly
Kumar ST, 2020[5]	Intra-subject, controlled, comparative study; extraction of bilateral impacted third molar in 48 patients	Patients received combination of trypsin, bromelain, rutoside post first extraction (n=48) and serratiopeptidase post second extraction, (n=38)	Mouth opening, swelling, and pain	All clinical parameters were better with combination of trypsin, bromelain, rutoside as compared to serratiopeptidase group
Islam MA, 2020[6]	Randomized controlled study; 50 patients post-removal of impacted third molar	Group 1: Combination of trypsin, bromelain, rutoside. Group 2: Diclofenac.	Mouth opening, swelling, and pain	Mouth opening and swelling reduction after 3 and 7 days statistically. Significant in favour of combination of trypsin, bromelain, rutoside
Chanu KS, 2017[7]	Randomized controlled, double-blind study; 90 patients undergoing extraction of impacted mandibular third molar.	Group A: Placebo, Group B: Combination of trypsin, bromelain, rutoside, Group C: Diclofenac	Mouth opening, swelling, and pain	Combination of trypsin, bromelain, rutoside more effective for said parameters when compared to use of diclofenac sodium/placebo
Gokkula-krishnan S, 2016[8]	Randomized controlled, study; 30 patients undergoing extraction of moderately difficult impacted mandibular third molar divided into 3 groups	Group I: Diclofenac, Group II: Combination of trypsin, bromelain, rutoside and diclofenac, Group III: combination of serratiopeptidase and diclofenac	Pain, swelling, trismus and quality of life post op day 1, 3, 5 and 7	Pain and swelling reduction was more in group II as compared to group I and group III. The quality of life was also better in group II.
Vishal KT, 2021[9]	Intra-individual, randomized, double-blind, crossover study; 18 patients undergoing extraction of third molar	1st extraction: amoxicillin, aceclofenac, paracetamol, 2nd extraction: Combination of TBR in addition to first extraction treatment	Pain, swelling and trismus on day 3, 5, and 7	Reduction in mean pain intensity and mandibular swelling was significantly more post second extraction

All 7 studies are comparative and controlled studies. A total of 526 patients were studied across these 7 studies. The combination of trypsin, bromelain and rutoside is compared with various other anti-inflammatory enzymes or against NSAIDs. In all 7 studies, the combination of trypsin, bromelain and rutoside has shown comparatively higher reduction in swelling. The mechanism of action of trypsin, bromelain and rutoside stated in Table 1 forms the basis of this higher efficacy.

DISCUSSION

The clinical benefit of trypsin, bromelain, rutoside combination is seen in all selected studies. The combination has shown better efficacy in terms of anti-inflammatory effects like reduction of pain and swelling. Once clinical study compared combination of trypsin, bromelain, rutoside vs combination of trypsin and chymotrypsin wherein significant swelling reduction was seen in combination of trypsin, bromelain, rutoside. The rationale of combining trypsin, bromelain and rutoside helps to provide justification for these results. The pharmacodynamic effects of combination of trypsin, bromelain, rutoside are pictorially depicted by Daftary et al which will further help in understanding the superior efficacy of combination of trypsin, bromelain, rutoside vs combination of trypsin and chymotrypsin.[2]

In another clinical study with comparison of combination of trypsin, bromelain, rutoside and serratiopeptidase-diclofenac combination was studied in patients undergoing third molar extraction. Though the sample size is limited in this study, it showed better reduction of swelling in combination of trypsin, bromelain, rutoside group as compared to serratiopeptidase-diclofenac combination. Anti-inflammatory enzymes reduce edema more than pain which was clearly demonstrated in this study. NSAIDs which mainly act on cyclooxygenase pathway reduce pain more and have limited action on swelling.

Long-term use of NSAIDs have marked negative impact on wound healing. NSAIDs have anti-proliferative effect on blood vessels and skin, thereby delaying healing rate.[10] NSAIDs also diminish response of the mesenchymal stem cells contributing to delay in healing.[11] If NSAIDs are used during the proliferative phase of healing, they may increase scar formation, by inhibiting PGE2 production.[12] Due to these efficacy issues with NSAIDs, using combination of trypsin, bromelain and rutoside for controlling inflammation would be more helpful.

The combination of trypsin, bromelain and rutoside has been shown to increase the absorption of antibiotics; this action is primarily driven by the bromelain component. Hence, using the combination of trypsin, bromelain and rutoside is further helpful in controlling the infection too.[13,14]

The multi-pronged mechanism of action of trypsin, bromelain and rutoside, which controls myriad pathways of inflammation, including cell migration, T cell activation, inflammatory mediators and free radical release and clot formation, provides the basis for superior efficacy of this combination, as documented in the seven different investigator-initiated studies.

CONCLUSION

The findings support the use of this enzyme-flavonoid combination in managing post-operative pain and swelling and speeding up the healing in dental pathologies and surgeries.

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