Postoperative Healing after Surgical Removal of Mandibular Third Molar: A Comparative Study between Two Proteolytic Enzymes

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Objective: The postoperative symptoms after third molar removal surgery were commonly uneventful. The aim of this study was to compare two proteolytic enzymes from different origin and to prove which enzyme provides a faster healing. One of the proteolytic enzymes involves a combination with a flavonoid. This involves trypsin, bromelain, along with a flavonoid rutin. Another proteolytic enzyme is serratiopeptidase that helps in degradation of insoluble proteins causing reduced swelling in the operated site.

Materials and Methods: In this study, voluntary subjects of clinically indicated bilateral lower third impaction were selected. The subjects were recommended to undergo extraction of impacted teeth in two sittings, one extraction per visit. In the first sitting, subjects were prescribed trypsin, bromelain, and rutin combination after removal of 48 teeth and in the second sitting subjects were prescribed serratiopeptidase after removal of 38 teeth. There was a time interval of 2–3 weeks in between the sittings. The post findings such as mouth opening, swelling, and pain scale were noted.

Results: The results showed that the proteolytic enzyme combination of trypsin, bromelain, and rutin is better than serratiopeptidase.

Conclusion: It is recommended that trypsin, bromelain, and rutin combination can be used effectively for postoperative purpose to facilitate wound healing.

Keywords: Bromelain, lyzoheal, rutin, serratiopeptidase, substance P, swelling, trypsin

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INTRODUCTION

Postoperative symptoms after third molar removal surgery were commonly uneventful. The symptoms include swelling, redness, trismus, lymphadenopathy, fever, and masticatory problems. These symptoms may persist for a week or more depending upon the patient’s condition and postoperative care. To overcome these problems, studies have shown that using a proteolytic enzyme aids a better and faster healing when compared to usual anti-inflammatory drugs. In this study, a combination of proteolytic enzyme along with a flavonoid was considered. These include trypsin, bromelain, and rutin. Trypsin, which is an endopeptidase, secreted as trypsinogen in the pancreas then activated in the intestines by endokinase. Bromelain is another enzyme obtained from the stems of ananas (pineapple). Rutin (rutoside) a flavonoid obtained from buckwheat, asparagus, citrus fruits, and berries.

Rutoside is a natural flavone derivative. Rutin has anti-inflammatory, anti-allergy, and immunomodulatory activity. The combination

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of these three compounds forms a natural anti-inflammatory agent. It has been observed that patients taking these enzymes showed significance reduction in pain and inflammation with quicker rates of recovery.

Another proteolytic enzyme called serratiopeptidase was considered as a control group. It is derived from the nonpathogenic Enterococci bacteria Serratia E15 produced in the intestines of silkworms. This enzyme has an inflammatory effect and it is believed to induce degradation of insoluble protein products. It decreases viscosity of the ooze, facilitates draining, and thus alleviates pain by preventing bradykinin release. In maxillary sinus antrotomy, serratiopeptidase is said to significantly reduce buccal swelling. Al-Sandook et al. clinically evaluated the efficacy of orthalforte (prolytic enzymes, trypsin and chymotrypsin) on postoperative sequel following the removal of lower impacted third molar.

The study involves comparison between trypsin, bromelain, and rutin with another proteolytic enzyme serratiopeptidase. The aim of this study was to evaluate the effectiveness of these two enzymes and to prove that which enzyme provides a better and faster healing.

**Materials and Methods**

The study involves by selection of 64 healthy individuals of equal sexes that fall under the following inclusion and exclusion criteria.

The inclusion criteria for selection of subjects were bilateral impacted third molar (mesioangular), subjects between the age of 16–30 years, and female subjects of either luteal or secretory phase of their menstrual periods.

The exclusion criteria involve systemic illness such as diabetes, hypertension, bleeding disorders, immunocompromised patients, pregnant and lactating women, mentally retarded, physically challenged, and uncooperative patients. All the patients were aware of the procedure and informed consent form was obtained from them. Strict instructions were given to them for proper follow-up and postoperative care. They were advised to take a preoperative X-ray (orthopantomogram [OPG]) to rule out any developmental abnormalities, temporomandibular joint problems, or bone abnormalities. The OPG also helped to ensure that the impaction is a mesioangular. Prior to the procedure, the preoperative findings were noted and are tabulated in Table 1.

First, the subjects underwent removal of 48 (right third mandibular molar) teeth under local anesthesia. The wound closure was carried out with 3-0 silk or Vicryl suture material (Ethicon Johnson and Johnson, Cincinnati, OH, USA). Saliva was collected using a collection device such as a Salivette (Nümbrecht, Germany) or equivalent before and after the impaction. The samples were stored at ≤–20°C. The substance P Elisa Kit was used to evaluate the reduction of inflammatory mediators and the results are obtained.

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Then the subjects were prescribed trypsin, bromelain, and rutin; subsequently, the postoperative symptoms were noted: mouth opening, pain, and infection in the operated site, lymphadenopathy, and swelling. Each of the aforementioned parameters was observed for a week. The findings were noted on alternative postoperative days 1, 3, 5, and 7. The mouth opening was measured by the interincisal distance and examined for enlargement of certain regional lymph nodes. The subjects were asked to rate pain from 0 to 10 (visual analog scale) VAS pain rating scale. The swelling was examined by facial symmetry and tape measuring method considering three points. Tragus is considered as a common point adjoining the angle of mouth and soft-tissue pogonion. Another line adjoining from the outer canthus of the eye to the angle of the mandible bisecting the other two lines is shown in Figure 1. Here the tragus is considered as a common point (A). The other point (B) is considered from the intersection of tragus–angle of mouth line to the line joining outer canthus of eye and angle of the mandible. Similarly another point (C) is considered from the intersection of tragus–pogonion line to the line joining outer canthus of eye and angle of the mandible. The swelling region is considered and calculated as the overall area adjoining these three points.

Once the right side was performed completely, their findings were tabulated as given in Table 1. After a period of

| Table 1: Mean facial swelling |
|-----------------------------|
| **Days** | **Distance from A and B** | **Distance from A and C** | **Distance from B and C** | **Mean value** |
| Preop  | 4.5 | 4.5 | 1.5 | 3.327794878 |
| POD 1  | 4.8 | 4.7 | 1.7 | 3.96540794 |
| POD 3  | 4.8 | 4.7 | 1.7 | 3.965451 |
| POD 7  | 4.5 | 4.5 | 1.5 | 3.327795 |

Preop = preoperative, POD = postoperative day
of 15 days, the subjects were recalled for follow-up after satisfactory healing was achieved; the same procedure was carried out in the left side involving removal of 38 (left third mandibular molar) teeth.

It was a prospective study including patients who required removal of impacted mandibular molar tooth. This study was approved by the local institutional review board. The operation was performed by the same surgeon. In this study, descriptive and inferential statistical analyses are used wherever necessary. Results on continuous scale measurement are given as mean ± standard deviation and results on categorical scale measurement are given in percentage. Level of significance was assessed at 5%. Assumptions made for the analysis of variance test (ANOVA) were dependent variable is normally distributed and the samples were drawn at random. ANOVA was carried to rule out any significant differences in the group means between the patient groups. Student's t test used to find significant differences of study parameters on continuous scale between two groups.

**Trypsin, bromelain, and rutin**

*Facial swelling measurement*

Table 1 shows the mean facial swelling measurement.

**Comparative table**

Table 2 compares the two treatment mean distance as calculated over a period of 7 days and Table 3 shows the comparison of visual analog scale measurement for a sample patient.

**RESULTS AND DISCUSSION**

The following inferences were made from the analysis of dataset:

1. Lower the mean, better the treatment. Hence, treatment T1 is better than T2. The same can also be witnessed from Tables 4 and 5, respectively.
2. Covariance is used to study the consistency of the dataset. Higher the covariance, better the treatment.

![Figure 1: Representation of swelling region](image-url)
Tharani Kumar, et al.: Comparison of effectiveness of the proteolytic enzymes

Hence, treatment T1 is better than T2 as seen from Figure 2.

3. Results of one-tailed test and two-tailed test are reported in Table 6.

4. It is evident from Figure 3 that T2’s VAS is higher than T1’s VAS for all the treatment samples considered for the study, which proves that T1 is better than T2.

5. From statistical inference of Table 7, it is evident that there is significant difference between the treatments given to male and female patients.

The following inferences were made from the analysis of dataset:

On oral administration, bromelain is well absorbed in the gastrointestinal tract with maximum level in the plasma within 1 h and half-life of 6–9 h.

The common problems that patients complain of after the surgical removal of impacted teeth are pain, swelling, and trismus. Various measures such as application of ice, analgesics, anti-inflammatory agents, and proteolytic enzymes have been practiced to minimize these complications.[14,15]

According to Ahmad,[16] salivary substance P is higher in inflammatory condition that can potentially impact dental pain. The salivary substance P level is more in the female patient with inflammatory condition rather than male patient. Gaspani et al.[17] showed bromelain after oral administration, the reduction in prostaglandin E2 (PGE2), and substance P concentration in the exudate.

Kumakura et al.[18] showed that exudates from plasma at the inflammatory site are reduced by inhibiting the generation of bradykinin by depletion of plasma kallikrein.

The effectiveness of bromelain in reducing swelling and pain after surgical removal of third molars was assessed in Tachibana et al.[10] and it was concluded that 28 patients (70%) had reduced swelling and pain after consuming bromelain. Singh et al.[9] studied the ability of serrapeptase to decrease the postoperative swelling, trismus, and pain after third molar impaction surgery. But, our paper has compared two proteolytic enzymes[9,10] from different origin, and to prove which enzyme provides a faster healing. One of the proteolytic enzymes involves a combination with a flavonoid. This involves trypsin, bromelain, along with a flavonoid rutin. Another proteolytic enzyme is serratiopeptidase that helps in degradation of insoluble proteins causing reduced swelling in the operated site. It is concluded that the first combination was better by 9.7% on the mean of postoperative swelling.

### Table 3: Visual analog scale measurement for a sample patient

| Sr. no. | Patient name | Sex | Age | VAS—trypsin, bromelain, and rutin | VAS—serratiopeptidase |
|---------|--------------|-----|-----|----------------------------------|-----------------------|
| 1       | XYZ          | M   | 18  | 0 8 9 1                          | 0 9 10 2              |

VAS = visual analog scale, Preop = preoperative

### Table 4: Statistical computations for treatment—T1

| Treatment 1 | Preop | Day 1 | Day 3 | Day 7 | Mean |
|-------------|-------|-------|-------|-------|------|
| Mean of swelling (mm²) | 4.209817945 | 4.63165282 | 5.031653 | 4.209818 | 4.520735382 |
| Mean of substance P (pg) | 30.8 | 24.0 | – | 14.0 | 22.93 |
| SD (mm²) | 0.493473033 | 0.56776345 | 0.567763 | 0.493473 | 0.517613327 |
| Covariance | 11.72195662 | 12.2583336 | 11.28384 | 11.72196 | 11.44975945 |

Preop = preoperative, SD = standard deviation

### Table 5: Statistical computations for treatment—T2

| Treatment 2 | Preop | Day 1 | Day 3 | Day 7 | Mean |
|-------------|-------|-------|-------|-------|------|
| Mean of swelling (mm²) | 4.4068495 | 5.206849 | 6.00684948 | 4.406849 | 5.006849 |
| Mean of substance P (pg) | 30.9 | 28 | – | 17.0 | 25.30 |
| SD (mm²) | 0.5230445 | 0.523044 | 0.52304449 | 0.523044 | 0.523044 |
| Covariance | 11.868898 | 10.04532 | 8.70746782 | 11.8689 | 10.44658 |

Preop = preoperative, SD = standard deviation

Figure 2: Covariance comparisons for the two treatments
**Table 6: Statistical inferences**

| Statistical inference | Tailed test | Two-tailed test |
|-----------------------|-------------|-----------------|
| \(\chi^2\)            | 0.031319    | 0               |
| Degrees of freedom    | 1           | 1               |
| \(P\) Value           | 0.4298      | 1               |
| 95 % CI               | [-0.1295833 1.0000000] | [-0.1730264 0.2042764] |
| Hypothesis            | H0: T1 is better than T2 | H0: T1 is equal to T2 |
|                       | H1: T2 is better than T1 | H1: T1 is not equal to T2 |
| Inference             | As \(P\) value is within the CI, H0 is accepted | As \(P\) value = 1 and does not lie within the CI, null hypothesis is rejected and alternate hypothesis is accepted with 95% CI |

CI = confidence interval

**Figure 3:** Comparison of visual analog scale (VAS) for both the treatments

**Table 7: Test for significant difference between the treatments among different genders**

| Statistical inference | Tailed test |
|-----------------------|-------------|
| \(\chi^2\)            | 0.13573     |
| Degrees of freedom    | 1           |
| \(P\) value           | 0.7126      |
| Hypothesis            | H0: There is no significant difference between male and female for the treatments given |
|                       | H1: There is significant difference between male and female for the treatments given |
| Inference             | There is significant difference between the treatments given to male and female patients |

**Conclusion**

Serratiopeptidase caused allergic reaction in some individuals because of proteolytic nature of the drug\(^{[9]}\) and causes increase in eosinophils. Due to this reaction, the bronchial asthmatic patients is contraindicative for the use of the same. However, the trypsin, bromelain, and rutin combination showed better results than that of serratiopeptidase. It was revealed that the trypsin, bromelain, and rutin combination had decrease in the inflammatory mediators such as PGE2 and substance P in the oral fluid and clinically the size of the swelling, mouth opening, and exudates from the surgical site was found reduced postoperatively, thus facilitating wound healing.

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**Conflicts of interest**

There are no conflicts of interest.

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