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A Randomized, Open-Label, Phase IV Clinical Study to Compare the Safety and Efficacy of the Fixed-Dose Combination of Trypsin, Bromelain, and Rutoside versus Serratiopeptidase in Minor Surgical Wound

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***Abstract*: To compare the safety, tolerability, and efficacy of the enteric-coated tablet containing fixed-dose combination (FDC) of trypsin 48 mg, bromelain 90 mg, and rutoside trihydrate 100 mg versus serratiopeptidase 10 mg for healing wounds after minor surgery. Methods: A prospective, multicenter, open-label, randomized, two-arm, active-controlled, phase IV study was performed. Eligible patients were randomized in a 1:1 ratio to receive either FDC or serratiopeptidase. Patients were evaluated on day 5±2 and day 10±2 along with a telephonic follow-up after seven days of the last dose. Medical examination, vital signs evaluation, and adverse events (AEs) and wound regeneration assessment were performed during follow-up. The global impression of tolerability and efficacy by patients and investigators were analyzed. SAS® version 9.4 was used for statistical analysis. Results: 383 eligible patients were randomized to the treatment groups. The treatments did not affect the laboratory parameters and vital signs significantly. Nine patients experienced AEs. No significant difference was observed by investigator and patients between both the treatments. FDC was significantly ($p<0.05$) high in wound regeneration on day 5±2. However, no significant difference was noted on day 10±2. A significant ($p<0.05$) improvement of total BWAT score in patients who received FDC was observed. The global impression efficacy evaluation by investigators and patients rated that FDC is comparatively efficacious in wound healing. Conclusion: The FDC of trypsin, bromelain, and rutoside trihydrate is equally safe and more efficacious as compared to serratiopeptidase in wound healing.**

***Keywords*: Bromelain, efficacy, rutoside, serratiopeptidase, trypsin, wound**

1. Introduction

Wound healing is crucial as infection and dehiscence are common problems. Therefore, early and better healing can help in reducing the duration of hospitalization [1]. Wound healing consists of four phases which are hemostasis, inflammation, proliferation, and remodeling [2]. Inflammation, a protective measure against injury and infection, is a physiological immune response. Inflammation is a cleaning process against the invading foreign substances leading to homeostasis. Acute inflammation is necessary to maintain physiological homeostasis [3]. Several factors affect the process of wound healing including local factors– oxygenation, infection, foreign body, venous insufficiency, and systemic factors– age, gender, sex hormones, stress, ischemia, alcoholism, smoking, and nutritional status. Systemic factors also include diseases such as diabetes, jaundice, uremia; medicines, such as glucocorticoids; and immunocompromised conditions [2]. Several enzymes are known for their anti-inflammatory actions and are potent substances with a vast range of therapeutic actions; thus, enzyme-based treatment is now an integral part of modern medicine [3, 4]. The therapeutic application of enzymes is vital due to the emerging novel medical conditions and the ineffectiveness of the available conventional therapies [3]. Enzymes can be obtained from animals– trypsin and chymotrypsin; plants– papase and bromelain; bacteria– serratiopeptidase, streptodornase, and streptokinase; and fungi [4].

Serratiopeptidase is produced by Enterobacterium serratia [5]. It is a proteolytic enzyme, has anti-inflammatory, analgesic, and fibrinolytic activities. Anti-inflammatory activity is produced by reducing the amount of fluid in the tissues by thinning the fluid and promoting its drainage and dissolving dead tissue. Analgesic activity is by inhibiting the release of bradykinin from the inflamed tissues. The fibrinolytic activity causes the breakdown of fibrin and other damaged tissue. Serratiopeptidase reduces capillary permeability and promotes

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wound healing [4]. Serratiopeptidase is a protein, and thus, the major challenge is to prevent its degradation while manufacturing, storage, and during digestion and absorption in the gastrointestinal tract. To tackle this problem, serratiopeptidase is formulated as an enteric-coated tablet. [6]. The formation of trypsin occurs in the pancreas in the form of trypsinogen, which is inactive zymogen. Enteropeptidase causes its activation and forms trypsin in the duodenum. The inhibitory action on the vascular permeability and the ability to inhibit the increase in C-reactive protein leads to anti-inflammatory effects [7].

Bromelain is obtained from the stem and fruit of pineapple (Ananas comosus). It is known for its proteolytic activity and belongs to a family of sulfhydryl enzymes. Singh T, et al., in their prospective randomized trial, indicated the effectiveness of oral bromelain therapy in the management of swelling and pain and promoting healing after surgical extraction of third molars. Bromelain also reduces the post-surgical complications by altering bradykinin which is a pain mediator, inhibiting the formation of prostaglandins–primarily PGE2 which are responsible for inflammation, and causing fibrinolysis which promotes the reabsorption of edema in the blood circulation. It is also known to possess antimicrobial properties [8,9]. Some studies also suggest the topical application of formulations of bromelain is also proved to be effective in healing wounds of deep partial and full-thickness. It causes removal of eschar without affecting the healthy and viable tissues, leaving behind a clean dermal and subdermal tissue leading to wound closure [10]. Rutoside is a flavonol obtained from plants such as buckwheat, passionflower, apple, and tea. Rutoside is known to have numerous pharmacological actions including anti-Alzheimer, anti-convulsant, anti-inflammatory, anti-allergic, prevention of neuroinflammation, sedation, anti-platelet aggregatory, analgesic, and anti-arthritic effects. It inhibits the aggregation of platelets and causes a decrease in capillary permeability leading to blood thinning and improvement in blood circulation. It is also known for its protective action on the wound and is useful topically to reduce oxidative stress and combats harmful free radicals promoting the healing of the wound [7, 11-13]. Rutoside also suppresses the inflammatory and proarthritic mediators of macrophages [7]. A combination of trypsin, bromelain, and rutoside, a mixture of enzymes and flavonoid, are known to have anti-inflammatory and analgesic effects and are used in several clinical studies in the form of enteric-coated tablet for the management of wound healing. However, the exact mechanism of the combination is still unclear.7 comparing the effects of different oral enzymes in the management of wound will aid in the selection of the most appropriate oral enzyme for wound healing [1]. In the current clinical study, we aimed to compare the safety and efficacy of the fixed-dose combination of trypsin, bromelain, and rutoside trihydrate enteric-coated tablet versus serratiopeptidase enteric-coated tablet for healing potential in wounds after minor surgery.

2. Materials and Methods

A prospective, open-label, randomized, parallel, two-arm, active-controlled, phase IV clinical study was performed at 12 centers in India from 18th July, 2018, to 21st June, 2019. The study was conducted per the ethical principles that have their origins in the Declaration of Helsinki; International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use–Good Clinical Practice Guidelines (ICH–GCP); Schedule Y and other regulatory provisions under the Drug and Cosmetics Rules; GCP Guidelines issued by Central Drugs Standard Control Organization; "Ethical Guidelines for Biomedical Research on Human Patients" published by Indian Council of Medical Research and per New Drugs and Clinical Trials Rules, 2019, requirement. This study was registered with the Clinical Trial Registry of India (CTRI no. CTRI/2018/04/013151, Registered on: 10th April, 2018) and subjects were insured for financial compensation and medical management as per New Drug and Clinical Rules, 2019.

1) Sample size determination

A sample size of 348 patients (174 per treatment group) was needed to assess the study objective. Considering 10% dropout, total sample size of 383 patients were planned to be enrolled in this study.

2) Selection, screening, and randomization of participants

The study enrolled 397 male or female patients aged 18 to 65 years with surgical wounds after minor surgery. Patients were provided with ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. All questions about the trial were answered to the satisfaction of the patient. All the patients who were ready to provide written informed consent were enrolled in the study. Those who were able to follow the study directions and agreeing to commit to all follow-up visits as per protocol, and willing to accept the restrictions associated with the study procedure were included in the study.

Patients with uncontrolled diabetes mellitus or any other metabolic disorder; patients with known hypersensitivity to any of the study-related drugs; patient with hepatic and/or renal disorder, bleeding disorders, menorrhagia, hematuria, and hematemesis; patients taking medicines such as tetracycline group of drugs, amoxicillin, aspirin, and anticoagulants including clopidogrel; patients who were enrolled in another clinical investigation or had been enrolled in any surgical wound trial within 30 days before enrollment in this study; female patients of childbearing age not using any contraceptive; pregnant or nursing women; or any other patient who did not fulfill the inclusion criteria in the opinion of the investigator were excluded from the study. After the screening procedure, the investigator randomized the eligible patients in 1:1 ratio to receive either enteric-coated tablet containing a FDC–Enzomac (trypsin 48 mg, bromelain 90 mg, and rutoside trihydrate 100 mg)–treatment A or marketed enteric-coated tablet–serratiopeptidase 10 mg–treatment B. The randomization was done via a list of randomly generated numbers on a computer system using the block randomization technique in a statistic program by the study statistician. The FDC in Enzomac was decided based on the previously published study by Kaur R, et al [7]. Dosing pattern in both the arms were same and one tablet was administered thrice daily through the oral route before

meals for 10 days. Patients were asked to visit study sites for follow-up on day 5±2 and day 10±2. Patients were subjected to physical and clinical examination and evaluation of vital signs during all these follow up visits.

3) Safety assessment

The assessment of safety and tolerability were the primary objectives of our study. All the patients who took even a single dose of the therapy, i.e., intent-to-treat (ITT) population were included in the safety analysis. Safety was assessed throughout the study period and telephonic follow-up after seven days from the last dose of study treatment by evaluating the incidences of adverse events (AEs) and/or serious adverse events (SAEs) and their plausible causal relationship with the study drug. Laboratory investigations– hematological and biochemistry changes were also observed on day 10±2 for safety analysis. The global impression of tolerability by patients and investigators was also analyzed as a part of safety assessment at the end of the treatment procedure. Tolerability of study drugs was rated as excellent (no AEs), good (mild AEs or causality as unassessable, unclassified or unlikely related AEs), poor (moderate to severe AEs or serious and possible, probable and certainly causality) on day 10±2.

4) Efficacy assessment

Efficacy assessment was the secondary objective of our clinical study. A minimum of 80% compliance was taken as satisfactory and patients fulfilling this criterion were included for efficacy analysis. Efficacy was assessed by evaluating the number/percentage of patients with complete wound regeneration and improvement in validated Bates-Jensen wound assessment tool (BWAT) score on day 5±2 and day 10±2 [14]. BWAT score included 13 parameters for evaluation which were size, depth, the appearance of edges, undermining, necrotic tissue type, necrotic tissue amount, exudates type, exudates amount, the color of the skin surrounding the wound, peripheral tissue edema, peripheral tissue indurations, granulation tissue, and epithelialization of the wound. The BWAT score of greater than 9 and less than 13 was considered as wound regeneration, while a BWAT score of 9 or less was considered as complete healing of the wound. Efficacy was also assessed by the global impression by patients and investigators in both the treatment groups. Efficacy of study drugs was rated as excellent (complete regeneration of wound), good (partial regeneration of wound), or poor (degeneration of wound) on day 10±2.

5) Statistical analysis

Statistical analysis was performed using SAS® (Statistical Analysis Software) version 9.4.All the analyses were performed using a 2-sided 5% level of significance.

B. Statistical analysis of safety endpoints

Proportional test was used for comparison of the parameter between treatment groups' incidence of AEs and SAEs at the end of the study. Student's't' test was used for comparison between treatment groups and paired't' test was used for comparison within treatment groups– hematology and biochemistry parameters for baseline and day 10±2.

C. Statistical analysis of efficacy endpoints

Proportional test was used for between treatment groups comparison of parameter number/percentage of patients with complete wound regeneration at the end of the study. Student's't' test was used for between treatment groups comparison and paired't' test was used for within treatment groups comparison of parameters for BWAT score (individual and total score data) for baseline and day 10±2. A chi-square test was used for the global efficacy impression for patients and investigators. Statistical data were on ITT for safety and per-protocol (PP) for efficacy. The values of $p < 0.05$ were considered as statistically significant.

3. Results

Out of the 397 patients enrolled, 14 patients were excluded from the study due to screen failure. Thus, 383 eligible patients were allocated to treatment A (n= 192) and treatment B (n= 191) by 1:1 randomization.

1) Baseline characteristics and disposition of patients

Baseline characteristics of the patients in treatment A and treatment B were analyzed and are given in table 1. It indicates that the population in treatment A and treatment B had similar values of baseline characteristics. The consort chart in figure 1 represents the data related to the disposition of patients in our clinical study.

2) Safety and tolerability outcome

Safety and tolerability were analyzed in the ITT population. Vital signs were analyzed at baseline, day5±2, and day 10±2. Table 2 indicates that there was no significant difference in the vital signs between both the treatment groups. Also, both treatment A and treatment B did not affect the vital signs significantly except three patients who were reported to have a fever. Laboratory parameters including hematological and biochemistry parameters were evaluated at baseline and day 10±2. The results of these parameters indicated that there was no clinically significant difference between the baseline and day 10±2. However, the difference in hemoglobin levels in treatment A was significant ($p<0.05$) from treatment B on day 10±2. Moreover, there were no clinically significant differences observed in the physical parameters throughout the study period.

Baseline to day 5±2: Headache in one patient and back pain in one patient was reported in treatment a group; headache was reported in one patient of the treatment B group. All the AEs from baseline to day5±2 were of mild severity. There was no significant difference in AE occurrence between both the treatments during this study period. Day 5±2to day 10±2: Thrombocytosis in two patients and fever in one patient was reported in treatment A group; fever in one patient and cold in one patient was reported in the treatment B group. The AEs in treatment A group were mild, while the AEs in the treatment B group were moderate. During telephonic follow-up, common cold was reported in one patient of treatment B. There was no significant difference in AE occurrence between both treatments during the study period. Therefore, nine patients experienced one AE each. There was no significant difference

in AE occurrence between both the treatments. A total of 7 AEs had mild severity and 2 AEs were moderate in nature. Out of nine AEs, eight AEs were judged possibly related to study drug; one AE which was recorded during the telephonic follow-up was not related to the study drug. No SAE or death was reported during the study and follow-up period. Patients and investigators also rated both the treatments as safe and tolerable throughout the study as indicated in table 3. There was no significant difference observed in safety evaluation by investigator as well as patients between both the treatments.

3) Efficacy outcome

Efficacy was assessed in the per-protocol population.

B. Wound regeneration

The wound regeneration status on day 5±2 and day 10±2 in both the treatment group is indicated in figure 2. The figure also shows that treatment A was significantly ($p<0.05$) high in wound regeneration compared to treatment B on day 5±2. However, there was no significant difference observed in both treatment groups on day 10±2.

C. Changes in BWAT score

Efficacy results revealed that there was a significant ($p<0.05$) improvement of total BWAT score in patients who received treatment A compared to treatment B. Out of the 13 BWAT wound characteristics, three characteristics including edges, exudates type, and granulation tissue showed significant improvement ($p<0.05$) at the end of treatment from baseline with both treatments. However, treatment A was significantly ($p<0.05$) high at the end of the treatment in improving BWAT score compared to treatment B except for necrotic tissue amount, which showed treatment B significantly ($p<0.05$) better as compared to treatment A. Remaining BWAT parameters including size, depth, undermining, necrotic tissue type, exudates amount, skin color surrounding the wound, peripheral tissue edema, peripheral tissue indurations, and epithelialization also improved significantly in both the treatment groups. At baseline, total mean BWAT scores were 27.13 and 26.85 in treatment A and treatment B respectively. There was a significant reduction ($p<0.05$) in total mean BWAT score on day 5±2 and day 10±2 in both the treatment groups from baseline. The mean reduction in BWAT score in treatment A was 9.31 (34.33%) and 13.39 (49.35%) on day 5±2 and day 10±2, respectively. Similarly, the mean reduction in BWAT score in treatment B was 8.42 (31.35%) and 12.56 (46.78%) on day 5±2 and day 10±2 respectively. On day 5±2, there was no significant ($p>0.05$) difference between both the treatment groups. However, treatment A significantly improved ($p<0.05$) total BWAT score on day 10±2 compared to treatment B.

D. Global impression efficacy evaluation by investigators and patients

The global impression efficacy evaluation by investigators and patients rated that treatment A is comparatively efficacious to treatment B in treating wound condition and the details are stated in table 4.

4. Discussion

The primary objective of our study was to assess and compare the safety and tolerability of FDC of trypsin 48 mg, bromelain 90 mg, rutoside trihydrate 100 mg enteric-coated tablet– treatment A with serratiopeptidase 10 mg enteric-coated tablet– treatment B for wound healing in patients with wounds due to minor surgical procedures. The results indicated that nine patients experienced one AE each during the study period with no significant difference in AE occurrence between both the treatments. Five AEs in treatment A group and three AEs in treatment B group were reported to be possibly associated with the study drug; the remaining one AE of treatment B group was reported to be unlikely related to the study drug. No SAE was reported during the study and follow-up period in any of the treatment groups. Laboratory investigations during the study showed no impact on patients' safety and tolerability during the treatment period. Thrombocytosis was reported in two patients of treatment a group. However, the majority of the patients of treatment a group did not report any AE related to hematology and biochemistry parameters. Though these AEs were found to be possibly related, no conclusive evidence could be established as AEs were reported in only 1.31% patients of treatment A. No significant abnormality during the study period was noted during physical and vital signs examinations; except the reporting of study indication.

The secondary objective was to evaluate the efficacy of treatment A versus treatment B in patients for their healing potential after minor surgery. The proportion of patients with wound regeneration was significantly ($p<0.05$) high in treatment A group compared to treatment B group on day 5±2 while there was no significant difference in wound regeneration between both the treatments on day 10±2. From the 13 parameters of BWAT score which significantly improved at the end of study from baseline in both study treatment groups; three parameters including edges, exudates type, and granulation tissue significantly improved at the end of treatment (day 10±2) from baseline in treatment A as compared to treatment B. The total BWAT score was also significantly high ($p<0.05$) in treatment A group compared to treatment B group.

Several previously published literatures indicating the safety, tolerability, and efficacy of the FDC used in treatment A supports the current clinical study [1, 7-9, 11, 15]. Biziulevičius indicates that proteolytic enzymes are involved in the wound healing process through the induction of tissue morphogenesis, angiogenesis, and tissue modulation. He explains that these enzymes accelerate the healing process, and thus, they reduce the time for wound regeneration [15]. Chandanwale A, et al. conducted a randomized clinical trial to evaluate and compare the efficacy, safety, and tolerability of trypsin:chymotrypsin (6:1) with serratiopeptidase 5 mg and with a FDC of trypsin 48 mg, bromelain 90 mg and rutoside 100 mg in wound management. A significant reduction in erythema scores, local irritation, edema scores, wound indurations and tenderness was noted with all the three treatments suggesting the efficacy of each treatment. Mean scores for pain were also found to be reduced with these three treatments. Although the results of this study indicated that all three treatments were safe and tolerable

Table 1
Baseline characteristics

Characteristics	Treatment A	Treatment B
Age (years) (mean±SD)	37.41±12.59	37.93±13.01
Height (cm) (mean±SD)	161.71±7.46	161.02±7.16
Weight (kg) (mean±SD)	62.98±9.09	62.47±11.16
Sex (n) (male;female)	123;69	108;83

SD: standard deviation; n: number of patients; Treatment A: trypsin 48 mg, bromelain 90 mg, and rutoside trihydrate 100 mg; Treatment B: serratiopeptidase 10 mg

Table 2
Summary of vital signs

Vital signs (mean± SD)	Treatment A			Treatment B		
	Baseline	Day 5±2	Day 10±2	Baseline	Day 5±2	Day 10±2
Body temperature (ºF)	98.23±0.54	98.19±0.60	98.22±0.53	98.25±0.58	98.26±0.57	98.26±0.53
Pulse rate (beats/min)	79.32±5.31	78.73±4.95	78.36±4.65	77.66±5.60	78.33±4.97	77.81±4.98
Respiratory rate (breaths/min)	17.04±1.92	17.22±1.96	17.23±2.00	17.09±1.98	17.18±2.00	17.31±1.80
SBP (mm/Hg)	122.26±7.12	121.61±6.88	121.26±6.33	121.47±7.09	120.72±6.63	121.27±6.34
DBP (mm/Hg)	78.48±5.26	78.68±5.71	78.14±5.15	77.85±5.07	78.36±5.08	78.45±5.60

DBP: Diastolic blood pressure; SBP: Systolic blood pressure; SD: standard deviation; Treatment A: Trypsin 48 mg, bromelain 90 mg, and rutoside trihydrate 100 mg; Treatment B: Serratiopeptidase 10 mg

Table 3
Global impression of tolerability by investigator and patients

Global impression: tolerability (n[%])	By investigator		By patients	
	Treatment A	Treatment B	Treatment A	Treatment B
Excellent	177 (97.25)	177 (97.79)	177 (97.25)	177 (97.79)
Good	5 (2.75)	4 (2.21)	5 (2.75)	4 (2.21)
Poor	0	0	0	0

n: Number of patients; Treatment A: Trypsin 48 mg, bromelain 90 mg, and rutoside trihydrate 100 mg; Treatment B: Serratiopeptidase 10 mg

Table 4
Global impression of efficacy by investigator and patients

Global impression: efficacy (n[%])	By investigator		By patients	
	Treatment A	Treatment B	Treatment A	Treatment B
Excellent	110(60.44)*	91 (50.28)	112 (61.54)	92 (50.83)
Good	72 (39.56)	87 (48.07)	70 (38.46)	87 (48.07)
Poor	0	3 (1.66)	0	2 (1.10)

and trypsin: chymotrypsin was superior to the other two treatments, the small sample size of 25 patients in each group makes it important to conduct further studies to establish a firm conclusion based on these results [1]. A review published by Kaur R, et al. also indicated the safety and effectiveness of the FDC used in our study in the management of inflammation [7]. Another prospective randomized clinical study by Singh T, et al. evaluated the effect of bromelain in the management of swelling and pain due to the removal of third molars. Bromelain was found to be effective in 70% of the patients. The study concluded that bromelain is a successful enzyme therapy that can be taken orally to reduce pain and swelling post-oral surgery [8]. A review on bromelain published in 2016also indicated the multiple therapeutic effects of bromelain including anti-inflammatory and antimicrobial effects [9]. A review entitled “the pharmacological potential of rutin” published in 2017 indicated that rutin is a safe and effective therapy and has protective effects on wound [11]. Thus, in support of the previous studies, our study proved the safety, tolerability and effectiveness of FDC–trypsin, bromelain and rutoside trihydrate. In addition to the previous studies, we also found that this FDC is more effective than serratiopeptidase10 mg in wound management.

5. Clinical Trial Limitations

This clinical study included participants of Indian origin only; thus, the variation in the safety and efficacy based on different race and geographical origin could not be identified. Also, children, elderly, and pregnant women were excluded from this study; thus, data pertaining to this population could not be extrapolated.

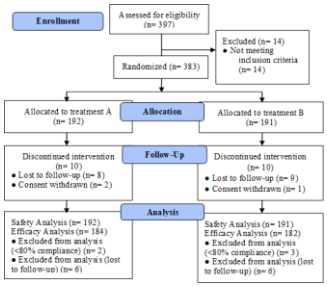

Fig. 1. Disposition of patients

Treatment A: Trypsin 48 mg, bromelain 90 mg, and rutoside trihydrate 100 mg; Treatment B: Serratiopeptidase 10 mg

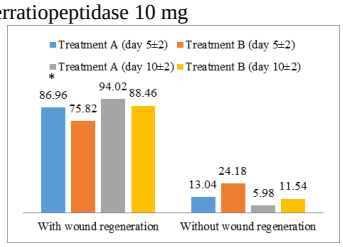

Fig. 2. Percentage of patients with and without wound regeneration

Treatment A: Trypsin 48 mg, bromelain 90 mg, and rutoside trihydrate 100 mg; Treatment B: Serratiopeptidase 10 mg; *Significant ($p<0.05$) difference from treatment B on day 5±2

6. Conclusion

Adequate care and therapeutic measures are needed to promote wound healing post-surgery. The FDC of enzymes–trypsin and bromelain and flavonoid– rutin is found to be safe and effective in wound management. The improvement in the BWAT score is also higher in with FDC as compared to the serratiopeptidase group. Therefore, the combination of trypsin, bromelain, and rutoside trihydrate is safe, tolerable, and more effective than serratiopeptidase in the management of wounds due to minor surgical procedures.

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