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A multicenter, open-label, randomized, phase IV clinical study comparing the safety and effectiveness of the fixed-dose combination of trypsin, bromelain, and rutoside with standard therapy in the management of wound due to minor surgery

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ABSTRACT

Wound healing is a complex process consisting of steps—hemostasis, inflammation, proliferation, and remodeling. The purpose of this multicenter, open-label, randomized, active-controlled, prospective, two-arm, phase IV clinical study was to evaluate and compare the safety, tolerability, and efficacy of fixed-dose combination (FDC) enteric-coated tablets of trypsin 96 mg + bromelain 180 mg + rutoside trihydrate 200 mg—Enzomac Forte with the enteric-coated tablets of trypsin-chymotrypsin 100000 AU—marketed formulation in the complex wound healing process. An open-label, randomized, parallel, active-controlled, two-arm, phase IV clinical study was conducted at 13 centers in India from December 02, 2017, to May 04, 2019. Adult patients aged 18-65 years (both inclusive) fulfilling the eligibility criteria were included. These patients were randomized into two treatment groups—FDC versus trypsin-chymotrypsin. Adverse events (AEs) and serious adverse events (SAEs) were recorded to evaluate and compare the safety and tolerability. The efficacy of both the treatment in the management of wound healing was noted and compared. AEs were reported in five patients; no SAEs were reported. No clinically significant differences in the occurrence of AEs were found; thus, both the treatments were safe and tolerable. The wound regeneration efficacy on day 10±2 was significantly higher with FDC as compared to trypsin-chymotrypsin. A significantly high improvement of total BWAT score with FDC as compared to trypsin-chymotrypsin was observed. FDC used by the authors in this study was found to be equally safe and tolerable and more effective than the marketed formulation used for wound healing after a minor surgical procedure.

Keywords: Bromelain, Chymotrypsin, Healing, Ruto Side, Trypsin, Wound

1. INTRODUCTION

An incision is considered to be a wound or cut due to a surgical procedure, and it should be appropriately managed to promote healing.¹ Wound healing is an essential process which is composed of four complex and continuous processes. These integrated and overlapping wound healing phases are bio-physiologic and cellular in nature; it includes hemostasis, inflammation, proliferation, and remodeling. Hemostasis involves vascular constriction, platelet aggregation, degranulation, and fibrin formation; inflammation involves neutrophil infiltration, monocyte infiltration, and differentiation to macrophages, and lymphocyte infiltration; proliferation involves re-epithelialization, angiogenesis, collagen synthesis, and extracellular matrix formation; and remodeling involves collagen remodeling, and vascular maturation and regression.²

Several local and systemic factors affect the normal wound healing process. The factors which have a direct effect on the nature of wound are called local factors, while systemic factors are those which affect the overall health status of an individual. Oxygenation, infection, foreign body, and venous insufficiency are local factors, whereas age, sex, disease state, alcoholism, smoking, medications, nutritional status, and immunocompromised conditions are systemic factors.² However, World Health Organization has given another type of classification for the factors affecting wound healing which are patient-related, wound-related, and local factors. Patient-related factors include age, underlying conditions, and the effect of injury on healing; wound-related factors include organ or tissue injured, nature and extent of injury, infection, and the time period between injury and initiation of the treatment; local factors are timing of closure of the wound, hemostasis, and debridement.³

Trypsin, bromelain, and rutoside trihydrate are the active ingredients of the Fixed Dose Combination (FDC) enteric coated tablet used for wound healing in this study by the investigators. The production of trypsin occurs in an inactive form called zymogen trypsinogen which gets activated through a series of steps. The enzyme enteropeptidase is responsible for the proteolytic cleavage and activation of this enzyme.⁴ Since several decades, proteolytic enzymes are known to produce anti-inflammatory activity in post-surgical wounds, degenerative joint conditions and acute injuries such as sports injuries.⁴⁻⁶ Bromelain is an extract containing mixture of proteolytic enzymes as well as non-enzymatic substances; it is obtained from pineapple plant. Numerous published clinical studies have indicated the benefits of bromelain in various surgical procedures.⁷⁻¹³ A published literature indicated the effects of enzyme therapy in the prophylaxis and treatment of post-traumatic and post-operative swelling.⁸ A randomized double-blind study indicated effectiveness of oral bromelain to reduce pain and swelling and promote healing after episiotomy.⁹ Other clinical studies reported clinical efficacy, safety, and tolerability of enzymatic therapy in the management of pain and wound healing after orthopedic surgery, obstetric procedure, rhinoplasty, and minor surgical procedures associated with tooth and eyes. Enzymes are also useful in the management of blunt trauma injuries due to its analgesic properties and its abilities to reduce swelling and tenderness at the site of injury.^{7,10-13} Rutoside is obtained from natural origin and is a flavone derivative. It is useful in treating inflammation and has immunomodulatory and anti-allergic properties. It improves the circulation of blood by inhibiting the aggregation of platelets and decreasing capillary permeability. It also has anti-oxidant property which aids in combating the harmful free radical produced during the inflammatory process.⁴

In the current clinical study, the effects of the FDC enteric coated tablet–Enzomac Forte (Manufactured by: Macleods Pharmaceuticals Ltd., India) is compared with trypsin-chymotrypsin enteric coated tablet. Trypsin-chymotrypsin, a widely used oral combination for healing of wounds due to surgical procedures, orthopedic issues and trauma has anti-inflammatory, fibrinolytic, anti-edematous, antioxidant and anti-infective properties. In addition to these properties, this proteolytic enzyme combination is efficacious in wound repair because of the high bioavailability. It also relieves pain associated with the wound.¹⁴

Therefore, the objective of this study was to evaluate and compare the safety, tolerability, and efficacy of FDC of three agents–trypsin, bromelain, and rutoside trihydrate with a standard marketed product– trypsin-chymotrypsin in wound healing in patients with wounds due to minor surgical procedure.

2. METHODS

An open-label, randomized, parallel, active-controlled, prospective, two-arm, phase IV clinical study was conducted at 13 centers in India from December 02, 2017, to May 04, 2019. All study-related documents were approved by the institutional ethics committee and the study was conducted in accordance with the ethical guidelines as per 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 in accordance with New Drugs and Clinical Trials Rules, 2019 requirement. This study was registered with the Clinical Trial Registry of India (CTRI no. CTRI/2017/10/010004, Registered on: 04/10/2017), and subjects were insured for financial compensation and medical management as per New Drug and Clinical Trials Rules, 2019.

2.1 Selection, Screening and Randomization of Participants

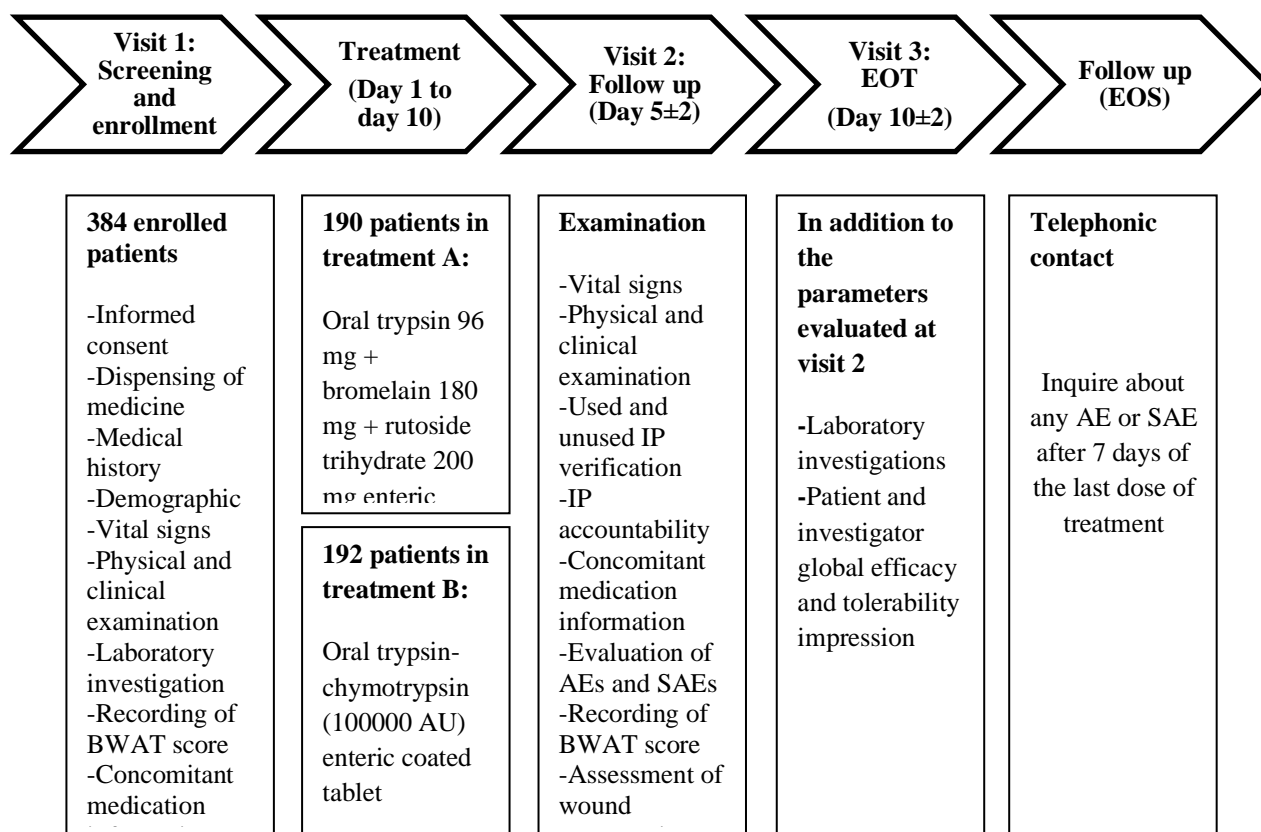


Fig. 1: Study Design

EOT: End of treatment, EOS: End of study, BWAT: Bates-Jensen wound assessment tool, AU: Armour units, AE: Adverse event, SAE: Serious AE

A total of 384 male and female patients aged 18-65 years with wounds from minor surgical procedure and who consented to participate in the study were enrolled after obtaining signed informed consent and checking inclusion and exclusion criteria. Patients able to follow all study directions and commit to all follow-up visits as per protocol; and patients willing to accept the study-related restrictions 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 a period of 30 days before enrolling in this study; female patients of child bearing 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.

As indicated in Figure 1, after the screening procedure, the eligible patients were randomized in 1:1 ratio via a list of randomly generated numbers on a computer system using the block randomization technique to receive either enteric coated tablet containing a FDC—treatment A or marketed enteric coated tablet—trypsin-chymotrypsin—treatment B for 10 days. Dosing pattern was same in both the groups, and each patient received one tablet thrice daily before meals through oral route. Patients were asked to visit study sites for follow up on day 5±2 and day 10±2. Patients were contacted telephonically to inquire about AE and SAE for follow up.

2.2 Safety Assessment

All the patients who took even a single dose of the therapy were utilized for safety analysis. Safety was assessed throughout the study period by evaluating the incidence of adverse events (AEs) and/or serious adverse events (SAEs). Additionally, lab investigations and tolerability assessments by patients and investigators were also evaluated to determine the safety of the treatment method. 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.

2.3 Efficacy Assessment

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 proportion of patients with complete wound regeneration and improvement in validated BWAT score on day 5±2 and day 10±2. BWAT score included 13 parameters for evaluation which were size, depth, edges, undermining, necrotic tissue type, necrotic tissue amount, exudates type, exudates amount, color of the skin surrounding the wound, peripheral tissue edema and indurations, granulation tissue, and epithelialization. The BWAT score of more than 9 and less than 13 was considered as wound regeneration. A BWAT score of 9 or less was considered as complete healing of wound.¹⁵ Efficacy of study drugs was rated as excellent (wound completely regenerated), good (wound partial regenerated) or poor (wound degeneration) on day 10±2. Efficacy was also assessed by global impression by patients and investigators.

2.4 Sample Size Calculation

Sample size calculation was done by using incidence of adverse events

Statistical analysis software: SAS

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

2.5 Statistical Analysis

Statistical analysis was performed using SAS version 9.4. All the analysis was performed using 2-sided 5% level of significance. Statistical data were on intent to treat population (ITT) for safety and per protocol (PP) for efficacy. The values of $p < 0.05$ were considered as statistically significant. Analysis was done using various statistical methods including chi-square test and Fisher's exact test.

2.6 Study Adherence and Confidentiality

The study monitor verified adherence to the protocol and completeness, consistency, and accuracy of the data by comparing patient source documents with entries in the case report form. Patient confidentiality was maintained at all times.

3. RESULTS

3.1 Study Population

The details of the study population are indicated in Figure 2.

3.2 Baseline Characteristics

Overall 70.16% of patients in the study were males and the mean age of the overall population in treatment A and treatment B was 40.12 and 40.00 years, respectively. The mean weight and height of patients were comparable in both the treatment groups. There was no significant difference in demographic and other baseline characteristics between the treatment groups. Vital signs were found to be normal for all the patients at baseline and are enlisted in Table 1.

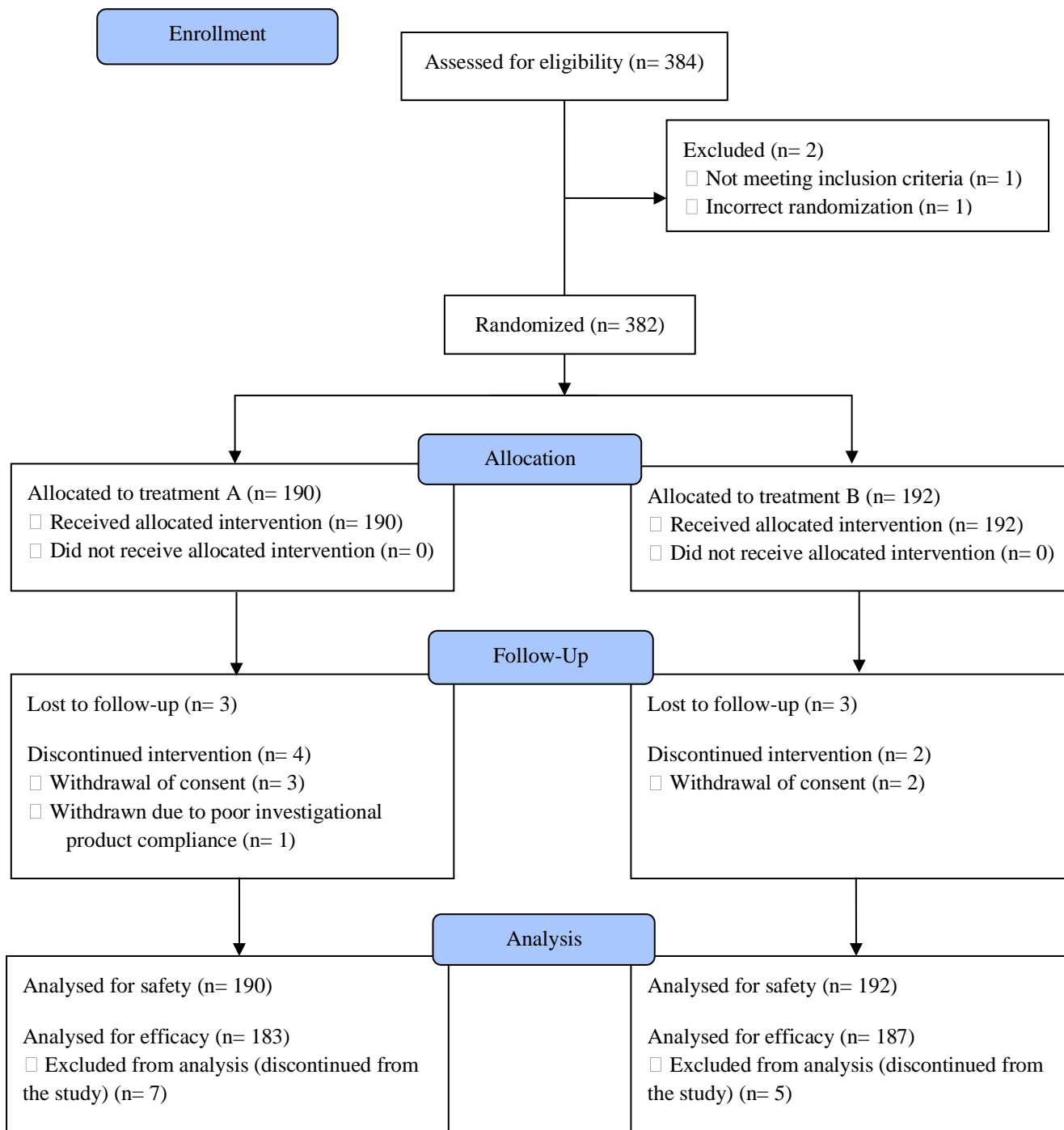


Fig. 2: Disposition of patients

Treatment A: trypsin 96 mg + bromelain 180 mg + rutoside trihydrate 200 mg enteric coated tablet; Treatment B: trypsin-chymotrypsin (100000 AU)

Table 1: Baseline characteristics

Vital signs (Mean [SD])	Treatment A (n=189)	Treatment B (n=192)
Body temperature °F	98.03 (0.73)	98.06 (0.60)
Pulse rate (Pulse/min)	78.75 (7.73)	78.54 (8.30)
Respiratory rate (Breaths/min)	18.12 (2.76)	18.04 (2.67)
Blood pressure (mm/Hg)		
Mean systolic pressure	123.96 (8.55)	123.38 (8.53)
Mean diastolic pressure	77.53 (6.09)	78.11 (6.19)

SD: Standard deviation; Treatment A: trypsin 96 mg + bromelain 180 mg + rutoside trihydrate 200 mg enteric coated tablet; Treatment B: trypsin-chymotrypsin (100000 AU)

Clinical characteristics of the wound at baseline: A total of 22 patients (5.76%) from total 382 patients had wound at four different sites including sacrum and coccyx (0.26%), lateral ankle (2.62%), medial ankle (2.09%), heel (0.79%). Remaining 360 (94.24%) patients had wound at other sites on the body. A total of 89 (23.36%) patients from total 381 patients had irregular wound, 155 (40.68%) patients had linear or elongated wound, 109 (28.61%) patients had round or oval wound, 4 (1.05%) patients had bowl or boat wound and 24 (6.30%) patients had other shapes. However, wound shape data was not available for one patient.

3.3 Patient Compliance And Concomitant Medications

Concomitant medications: A total of 368 patients were receiving concomitant medication(s) during the study period.

Patient compliance: Out of 382 randomized patients, a total of 370 patients (97.33% in treatment A and 98.95% in treatment B) had $\geq 80\%$ of treatment compliance at the EOS. Five patients (2.67%) from treatment A, and two patients (1.05%) of treatment B had compliance $<80\%$. Data were not available for three patients from treatment A and two patients from treatment B.

3.4 Primary Endpoint

Safety and tolerability evaluation: During the study period, 4 patients (1.05%) experienced 1 AE each and 1 patient (0.26%) experienced 3 AEs. AEs were experienced by 5 patients throughout the study period. No significant difference ($p>0.05$) in AE occurrence was observed between both the treatment groups throughout study period and safety follow up. Table 2. lists the AEs that affected the patients.

Table 2: Adverse events related and non-related to the study treatment

AE	Mild n (%)		Moderate n (%)		Severe n (%)		Total n (%)
	R	NR	R	NR	R	NR	
Treatment A							
Dyspepsia	-	1 (0.26)	-	-	-	-	1 (0.26)
Pyrexia	1 (0.26)	1 (0.26)	-	-	-	-	2 (0.52%)
Vomiting	-	-	-	1 (0.26)	-	-	1 (0.26)
Diarrhea	-	-	-	1 (0.26)	-	-	1 (0.26)
Abdominal pain	-	-	-	-	-	1 (0.26)	1 (0.26)
Treatment B							
Gastritis	-	1 (0.26)	-	-	-	-	1 (0.26)
Total AEs							7 (1.83)

AE: Adverse event, R: Related, NR: Non-related; Treatment A: trypsin 96 mg + bromelain 180 mg + rutoside trihydrate 200 mg enteric coated tablet; Treatment B: trypsin-chymotrypsin (100000 AU)

No clinically significant AEs or changes were observed in hematology and biochemistry parameters in any of the patients throughout study period. No abnormality other than study indication was observed in any patients during the study and follow up period. No clinically significant abnormality was observed in vital signs of all study patients throughout the study period except three patients who suffered from fever. Both the treatments were found to be safe when assessed through clinical laboratory evaluation. Global impression of tolerability by investigator and patients are described in Table 3.

Table 3: Global impression of tolerability at the end of the treatment

Global impression: Tolerability	By investigator n (%)		By patients n (%)	
	Treatment A	Treatment B	Treatment A	Treatment B
Excellent	181 (98.91)	186 (99.47)	180 (98.36)	186 (99.47)
Good	2 (1.09)	1 (0.53)	3 (1.64)	1 (0.53)
Poor	0	0	0	0

Treatment A: trypsin 96 mg + bromelain 180 mg + rutoside trihydrate 200 mg enteric coated tablet; Treatment B: trypsin-chymotrypsin (100000 AU)

3.5 Secondary Endpoint

Efficacy evaluation: Patients who showed $\geq 80\%$ treatment compliance were included for the efficacy analysis. Out of 382 patients enrolled, data of 370 patients were included in the efficacy analysis. Compliance to the treatment was $<80\%$ for 7 patients and no data was available for 5 patients, and hence not included in the efficacy analysis. As indicated in Fig. 3, the regeneration of wound on day 5 ± 2 was non-significant between both the groups. However, on day 10 ± 2 that effect of treatment A was significantly better ($p<0.05$) in wound regeneration compared to treatment B. The significance in the efficacy analysis was determined by chi-square test.

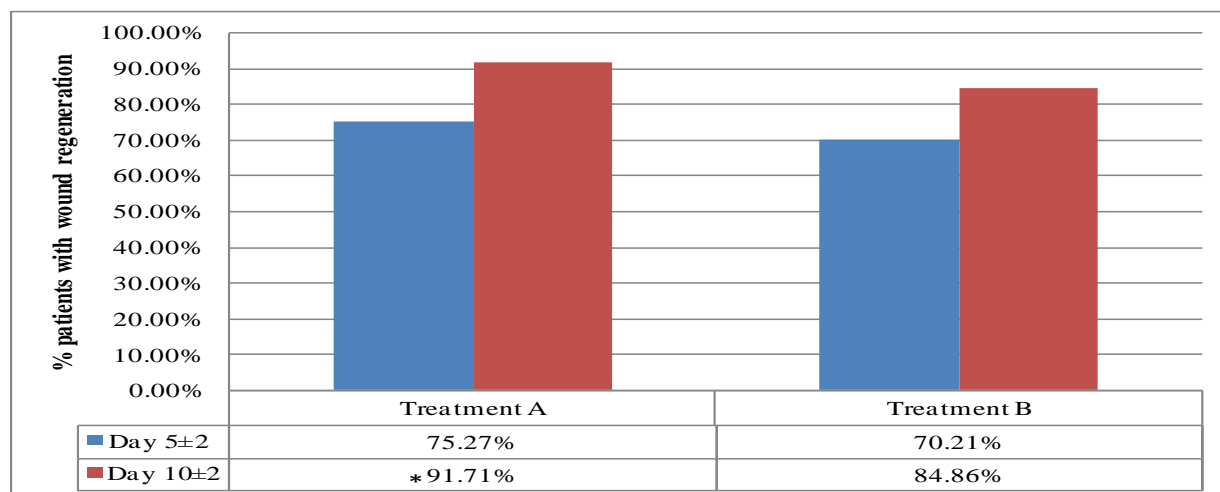


Fig. 3: Wound regeneration status

*Significant ($p < 0.05$) difference from treatment B on day 10; Treatment A: trypsin 96 mg + bromelain 180 mg + rutoside trihydrate 200 mg enteric coated tablet; Treatment B: trypsin-chymotrypsin (100000 AU)

Global impression of efficacy by investigator and patients are described in Table 4. Analysis by chi-square test followed Fisher's exact test revealed that the patient's global impression of efficacy for study drugs was rated non-significantly different for Treatment A as compared to Treatment B.

Table 4: Global impression of efficacy at the end of the treatment

Global impression: Efficacy	By investigator n (%)		By patients n (%)	
	Treatment A	Treatment B	Treatment A	Treatment B
Excellent	134 (74.03)	138 (74.59)	134 (74.03)	143 (77.30)
Good	47 (25.97)	47 (25.41)	47 (25.97)	42 (22.70)
Poor	0	0	0	0

Treatment A: trypsin 96 mg + bromelain 180 mg + rutoside trihydrate 200 mg enteric coated tablet; Treatment B: trypsin-chymotrypsin (100000 AU)

4. DISCUSSION

The investigators found that the FDC in this study was safe and well-tolerated by the patients. It was more effective than the marketed product containing trypsin-chymotrypsin. Safety results indicated that there were no SAEs reported during the study or follow up. No significant difference in the AEs reported in this study was observed between the treatment groups. From all the experienced AEs, four AEs were mild, two AEs were moderate and one AE was severe in nature. Physical and vital signs, and lab investigations indicated no significant difference between the treatment groups.

As per the efficacy results, proportion of patients with wound regeneration was significantly more ($p < 0.05$) in treatment A compared to treatment B group. From the 13 parameters of BWAT score which significantly improved at the end of study from baseline in both study treatment groups; 11 parameters, including depth, edges, undermining, necrotic tissue type, necrotic tissue amount, exudates type, exudates amount, skin color surrounding wound, peripheral tissue edema, peripheral tissue indurations and epithelialization, significantly improved ($p < 0.05$) with treatment A as compared to treatment B; no significant difference in change was observed in wound size and granulation tissue between both the treatment groups.

The results obtained in this study are comparable to the results of other clinical trials published online. A published literature indicated a clinical trial evaluating and comparing the efficacy, safety and tolerability of trypsin-chymotrypsin with serratiopeptidase and oral FDC (trypsin:48 mg, bromelain:90 mg, rutoside trihydrate:100 mg). It included 75 patients who were randomized 1:1:1 in each group. A significant reduction in erythema score, edema score and pain VAS score was observed in each treatment group. Although improvement in the condition was noted in all the treatment groups, the maximum change was observed in trypsin-chymotrypsin group. However, the dose of FDC of trypsin, bromelain, and rutoside trihydrate used in this study is half the dose as compared to our clinical study.¹⁶ Therefore, with the available data, it can be stated that the increase in dose of trypsin, bromelain and rutoside trihydrate increases the efficacy without affecting the safety and tolerability.

Another double blind, randomized, placebo-controlled clinical study, proved the effectiveness of bromelain 500 mg capsule in wound healing following free gingival grafting. This capsule was given once a day for 10 days starting from the day of surgery. Placebo group received capsule containing 90% microcrystalline cellulose, 5% corn starch, 2% magnesium stearate, and 3% FD and C yellow dye. The patients in both the groups were assessed 7 days after the surgery to evaluate the effectiveness of bromelain oral capsule in the management of pain, epithelialization and bleeding at the donor site. The final assessment was conducted on day 10. Overall, bleeding was observed in 18% of the patients, and the mean (SD) VAS score of pain was 3.84 (2.29). Complete epithelialization on day 7 after the surgery was noted in 53.8%, which increased to 80.8% on day 10 post-operatively.¹⁷ The FDC used in the current study has bromelain as one of the active constituents indicating its role in the effectiveness of the formulation in the management of wound healing.

In addition, several review articles support the efficacy and safety of the three drugs used in formulating the study treatment formulation in the management of wound.^{4,7,16,17} Therefore, the results obtained from the current clinical study conducted are similar to those published in the literature indicating the safety and efficacy of a fixed dose combination of trypsin, bromelain and rutoside trihydrate in the management of wound due to minor surgical procedures.

5. CLINICAL TRIAL LIMITATIONS

All the participants were of Indian origin; thus, the effects on different races and participants with varying food habits could not be found. Individuals under 18 years and over 65 years of age, and pregnant women were not included in this trial; thus, the safety and efficacy data for this population could not be identified.

6. CONCLUSION

Owing to the multiple pharmacological effects, trypsin (96 mg), bromelain (180 mg), and rutoside trihydrate (200 mg) combination hastens the wound healing process after minor surgical procedure. Although trypsin-chymotrypsin combination is also effective for the same and is one of the currently used methods for the management of wound healing, the trypsin, bromelain, and rutoside trihydrate FDC used in our study has proven to be more effective in the same period of time indicating the efficacy of this FDC. It is also safe and tolerable with no SAEs. Therefore, this combination can be used to reduce the recovery time after a minor surgical procedure and has emerged as a promising method in the management of wound.

7. CONFLICT OF INTEREST

All authors are full-time employees of Macleods Pharmaceuticals Ltd.

8. FUNDING

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10. REFERENCES

- [1] American Academy of Family Physicians. (2020). Caring for your incision after surgery available at <https://familydoctor.org/caring-for-your-incision-after-surgery/> referred on 06/03/2020.
- [2] Guo S, DiPietro LA. (2010). Factors affecting wound healing. *J Dent Res* 89(3): 219-229.
- [3] World Health Organization. (2009). Wound management available at <https://www.who.int/surgery/publications/WoundManagement.pdf> referred on 06/03/2020.
- [4] Kaur R, Abmwani S, Mehta B. (2014). Trypsin, rutoside and bromelain alone and fixed dose combination: a natural, safer and effective anti-inflammatory agent. *J drug deliv* 4(1): 108-110.
- [5] Izaka KI, Yamada M, Kawano T, Suyama T. (1972). Gastrointestinal absorption and antiinflammatory effect of bromelain. *Jpn J Pharmacol* 22(4): 519-534.
- [6] Taussig SJ, Batkin S, Bromelain. (1988). The enzyme complex of pineapple (*Ananas comosus*) and its clinical application. An update. *J Ethnopharmacol* 22(2): 191-203.
- [7] Muhammad ZA, Ahmad T. (2017). Therapeutic uses of pineapple-extracted bromelain in surgical care - a review. *J Pak Med Assoc* 67(1): 121-125.
- [8] Kameníček V, Holán P, Franěk P. (2001). [Systemic enzyme therapy in the treatment and prevention of posttraumatic and postoperative swelling]. *Acta Chir Orthop Traumatol Cech* 68(1): 45-49.
- [9] Golezar S. (2016). *Ananas comosus* effect on perineal pain and wound healing after episiotomy: a randomized double-blind placebo-controlled clinical trial. *Iran Red Crescent Med J* 18(3): e21019.
- [10] Howat RC, Lewis GD. (1972). The effect of bromelain therapy on episiotomy wounds--a double blind controlled clinical trial. *J Obstet Gynaecol Br Commonw* 79(10): 951-953.
- [11] Ordesi P, Pisoni L, Nannei P, Macchi M, Borloni R, Siervo S. (2014). Therapeutic efficacy of bromelain in impacted third molar surgery: a randomized controlled clinical study. *Quintessence Int* 45(8): 679-684.
- [12] Majid OW, Al-Mashhadani BA. (2014). Perioperative bromelain reduces pain and swelling and improves quality of life measures after mandibular third molar surgery: a randomized, double-blind, placebo-controlled clinical trial. *J Oral Maxillofac Surg* 72(6): 1043-1048.
- [13] de la Barrera-Núñez MC, Yáñez-Vico RM, Batista-Cruzado A, Heurtebise-Saavedra JM, Castillo-de Oyagüe R, Torres-Lagares D. (2014). Prospective double-blind clinical trial evaluating the effectiveness of Bromelain in the third molar extraction postoperative period. *Med Oral Patol Oral Cir Bucal* 19(2): e157-e162.
- [14] Shah D, Mital K. (2018). The role of trypsin:chymotrypsin in tissue repair. *Adv Ther* 35(1): 31-42.
- [15] Barbara Bates-Jensen. (2001). Bates-Jensen wound assessment tool. 2001 available at <https://www.woundcare.ca/Uploads/ContentDocuments/BWAT.pdf> referred on 14/05/2020.
- [16] Chandanwale A, Langade D, Sonawane D, Gavai P. (2017). A randomized, clinical trial to evaluate efficacy and tolerability of trypsin:chymotrypsin as compared to serratiopeptidase and trypsin:bromelain:rutoside in wound management. *Adv Ther* 34(1): 180-198.
- [17] Soheilifar S, Bidgoli M, Hooshyarfard A, Shahbazi A, Vahdatinia F, Khoshkhouie F. (2018). Effect of oral bromelain on wound healing, pain, and bleeding at donor site following free gingival grafting: a clinical trial. *J Dent (Tehran)* 15(5): 309-316.