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Research Article

SAFETY AND EFFICACY OF TRYPSIN, BROMELAIN, RUTOSIDE, AND DICLOFENAC COMPARED TO DICLOFENAC IN WOUND HEALING: A RANDOMIZED PARALLEL STUDY

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A B S T R A C T

Objective: To evaluate and compare the safety and efficacy of enteric tablet containing fixed-dose combination (FDC) of trypsin 48 mg, bromelain 90 mg, rutoside trihydrate 100 mg, and diclofenac sodium 50 mg versus similar formulation containing diclofenac sodium 50 mg for healing wounds.
Methods: In a multicenter, open label, randomized, comparative clinical study, patients with surgical wounds were randomized to receive either FDC–ENZOMAC PLUS or diclofenac 50 mg gastro-resistant tablets. Safety was evaluated by assessing incidence of adverse events, while efficacy was evaluated using Bates-Jensen wound assessment tool.
Results: A total of 18 AEs were observed throughout the study period. There was no significant difference in AEs occurrence between both the treatment groups. Majority of AEs were mild in nature with two AEs of moderate intensity. On day 10±2, FDC showed wound regeneration in 94.15% patients while diclofenac showed wound regeneration in 88.30% patients.

INTRODUCTION

Surgical incisions are inseparable procedures in most of the surgeries and cause disruption of the normal structure and function of the skin and its architecture to form a wound (Norman *et al*, 2016). It takes few to several weeks for complete healing of wound in most of the cases as it passes through highly programmed phases, including hemostasis, inflammation, proliferation and remodeling (Guo *et al,* 2010). However, few wounds either heal slowly or fail to heal due to infection by pathogenic organisms leading to impaired wound healing (Guo *et al,* 2010; Bowler, 2002). Moreover, some endogenous factors like dead tissue, poor perfusion, and local inflammatory reactions also play a vital role in the process of wound healing (Bowler, 2002). If proper care is not taken, wound may get infected with pathogens causing pain, redness, swelling, and secretion of pus (Norman *et al*, 2016). Infection prevents healing and affects the patient as it is traumatic and debilitating; it may also be life-threatening in certain cases (Bowler, 2002).

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The first approach to speed up wound healing process is to clean wound regularly. Simultaneous use of antibiotics is also preferred in order to prevent the infection; however, it is unclear if they are advantageous over other wound healing medicines. If wound does not heal for long duration, debridement (removal of dead and inflamed tissue) is done. Proteolytic enzymes are also extensively used for debridement. The majorly used proteolytic enzymes are bromelain, serratiopeptidase, and trypsin for wound management. These enzymes also possess antiedematous, anti-inflammatory, antithrombotic, anti-allergy, immune-modulating activity and fibrinolytic activities helping in wound healing process (InformedHealth.org, 2018; Loo *et al,* 2018; Shah *et al,* 2018; Kaur *et al,* 2014).

In a review article, Kaur R, *et al*. indicated that prolonged use of synthetic analgesics may have side effects. However, enzymes and drugs from natural origin such as trypsin, bromelain and rutoside are potent anti-inflammatory agents with wound healing properties and safe to use (Kaur *et al,* 2014). In a study published in 2004, the combination of trypsin, bromelain and rutoside was found equipotent in hip and knee osteoarthritis patients when compared to diclofenac treatment (Akhtar *et al,* 2004). Another study reported that combination of trypsin, bromelain and rutoside versus serratiopeptidase alone versus combination of trypsin-chymotrypsin were effective and safe in wound healing. In

addition, trypsin and chymotrypsin combination was also more potent than serratiopeptidase monotherapy (Chandanwale *et al,* 2017).

There are very limited clinical evidences available for safety and efficacy of the trypsin, bromelain, rutoside, and diclofenac combination for treatment of surgical wounds. Therefore, this study was conducted to evaluate the safety and efficacy of this FDC for the treatment of patients with wound after minor surgery.

MATERIALS AND METHODS

A prospective, open-label, multicenter (8 sites in India), randomized, parallel, active-controlled, phase IV clinical study was performed in India from 22nd January, 2018, to 29th April, 2019. The study was conducted per the ethical principles by the Declaration of Helsinki; Schedule Y and other regulatory provisions under the Drugs and Cosmetics Rules; International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use–Good Clinical Practice Guidelines (ICH–GCP); GCP Guidelines issued by Central Drugs Standard Control Organisation; "Ethical Guidelines for Biomedical Research on Human Patients" published by Indian Council of Medical Research and as per the requirements laid down by New Drugs and Clinical Trials Rules, 2019. This trial was approved by 9 ethics committee, including ethics committee of KRM hospital, Sudbhawana hospital, Popular hospital, Vinaya hospital, Anu hospital, Dr. DY Patil vidyapeeth, Bhatia hospital, Sir Sayajirao general hospital, RIMS government general hospital; however, no patients were enrolled at Bhatia hospital, and the study was conducted at only 8 sites. We got this trial registered under the Clinical Trial Registry of India (CTRI no. CTRI/2017/11/010384, Registered on: Nov 06, 2017). We also insured the participants for financial compensation and medical management as per New Drug and Clinical Rules, 2019.

Selection, screening and randomization

The principal investigator enrolled 386 patients of either sexes aged 18 to 65 years with minor surgical wounds. All the patients who were ready to follow the directions of the study; be available for the follow-up visits per the protocol; and those who were able to understand and provide a written informed consent were included in this clinical study.

Patients with clinical conditions, such as uncontrolled diabetes mellitus or any other disorder associated with metabolism, allergy to any of the drugs included in the study, liver or kidney disorder, bleeding disorders, menorrhagia, hematuria, hematemasis, and any other condition that did not justify the participation in the opinion of the investigator, were excluded from the study. Patients on drugs such as tetracycline group, amoxicillin, anticoagulants and aspirin were also excluded from the study. Pregnant women and lactating mothers, women of childbearing age and potential with an active sex life without any contraception, and all the men and women who were enrolled in any other clinical study or all those who had been enrolled in any surgical wound-related clinical trial within 30 days prior to enrolling in this study were not allowed to participate in this study.

After checking for the eligibility of the enrolled participants, the eligible participants were included in the study and randomized in 1:1 ratio by the investigator to receive either enteric-coated tablet containing a FDC–trypsin BP 48 mg, bromelain BP 90 mg, rutoside trihydrate BP 100 mg and diclofenac sodium IP 50 mg tablets–ENZOMAC PLUS (of Macleods Pharmaceuticals Ltd., India)–treatment A or a marketed enteric-coated tablet–diclofenac sodium IP 50 mg–treatment B. The FDC was decided based on a previously published literature by Chandanwale A, *et al* (Chandanwale *et al,* 2017). The statistician used a block randomization technique in a statistic program to generate a list of random numbers on a computer system which were used for randomization procedure. Dose was same in both the arms–one oral tablet was administered twice daily before meals for 10 days. Patients were asked to visit study sites 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.

Safety and tolerability analyses

Safety was evaluated based on the adverse events (AEs) reported and their plausible causal relationship with the study drug. All patients who were administered at least one dose of treatment were included for safety evaluation. The incidences of all AEs were determined. Safety was assessed on day 5±2 and day 10±2.

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.

Efficacy analyses

Efficacy was assessed by determining the number of patients with complete wound regeneration on day 5±2 and day 10±2. Bates-Jensen wound assessment tool (BWAT) score was utilized to assess efficacy. Total score was counted and recorded in case report form for each patient at baseline, day 5±2 and day 10±2. The parameters for BWAT scoring included area, depth, edges, under-mining of wound; necrotic tissue type and amount; type and amount of exudate; skin color surrounding the wound; peripheral tissue edema and indurations; granulation tissue; and epithelialization. BWAT score of >9 and <13 was noted as wound regeneration, while a BWAT score of ≤9 was noted as complete healing of the wound. Efficacy of study drugs was rated as excellent (wound completely regenerated), good (wound partial regenerated) or poor (wound degeneration) on day 10±2 (Harris *et al,* 2010; Bates-Jensen, 2001).

Statistical analyses

Statistical analysis was performed using Statistical Analysis Software 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) population for efficacy. The values of $p<0.05$ were considered as statistically significant.

Primary safety endpoints: Proportional test was used to for analysis of AEs and SAEs between treatment groups. Student 't' test was used between treatment groups and paired 't' test was used within treatment groups for comparison of parameters on day 10±2.

Secondary efficacy endpoints: Proportional test was used to compare the wound regeneration between the groups. Student 't' test was used between treatment groups and paired 't' test was used within treatment groups for comparison of BWAT

score between baseline and day 10±2. Chi-square test was used for global efficacy impression for patients and investigator.

Determination of sample size

Sample Size Calculation: It was done by using incidence of AEs by SAS®.

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

There were no changes in the conduct of the study or statistical analysis plan during study.

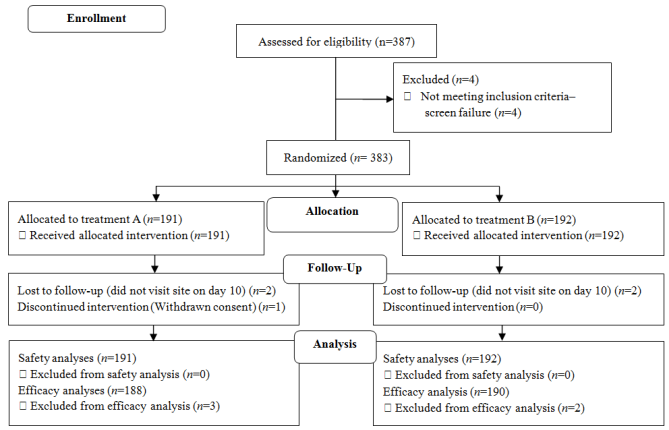
RESULTS

A total of 387 patients were screened in the study, out of which 383 patients (4 patients were screen failures) were randomized. A total 378 patients completed the study. Five patients were discontinued from the study (three patients lost to follow-up from Treatment A and two patients lost to follow-up from Treatment B). The baseline characteristics of patients are summarized in Table 1 and the disposition of patients is summarized in Figure 1.

Table 1 Baseline characteristics

Characteristics (mean [*SD*])	Treatment A	Treatment B
Age (years)	37.89 (12.72)	36.78 (11.46)
Height (*cm*)	159.82 (7.65)	159.01 (7.15)
Weight (*kg*)	62.02 (10.48)	61.85 (8.65)
Sex (male;female) (*n*[%])	127;64 (66.49;33.51)	117;75 (60.94;39.06)
Body temperature (°*F*)	98.04 (0.88)	98.09 (0.80)
Pulse rate (pulse/minute)	79.91 (7.39)	79.86 (8.26)
Respiratory rate (breaths/minute)	17.62 (2.37)	17.35 (2.32)
Blood pressure (*mmHg*)		
Systolic blood pressure	122.29 (7.55)	122.49 (7.71)
Diastolic blood pressure	78.68 (6.78)	78.70 (6.69)

Treatment A: Trypsin 48mg + Bromelain 90mg + Rutoside Trihydrate 100mg + Diclofenac Sodium 50mg enteric coated tablets; Treatment B: Diclofenac Sodium 50mg enteric coated tablets.



Treatment A: Trypsin 48mg + Bromelain 90mg + Rutoside Trihydrate 100mg + Diclofenac Sodium 50mg enteric coated tablets; Treatment B: Diclofenac Sodium 50mg enteric coated tablets

Fig 1 Disposition of patients

Safety and tolerability results

Safety was performed in ITT population. A total of 18 (4.70%) AEs were observed throughout the study period including safety follow-up period. There was no significant difference in AEs occurrence between both the treatment groups. Majority of the AEs were mild in nature; only 2 AEs were moderate in nature. 14 AEs were possibly related to respective study treatment. The most frequent AEs observed were nasopharyngitis, cough and pyrexia. No SAE was reported during the study period. Moreover, there were no clinically significant changes observed in laboratory investigations, vital signs, and physical examinations throughout study period. Patients and investigators also rated both the treatments as safe and tolerable throughout the study.

Efficacy results

Efficacy analysis was done in PP population. ENZOMAC PLUS was significantly better ($p<0.05$) in wound regeneration compared to diclofenac on day 10±2 as shown in Figure 2.

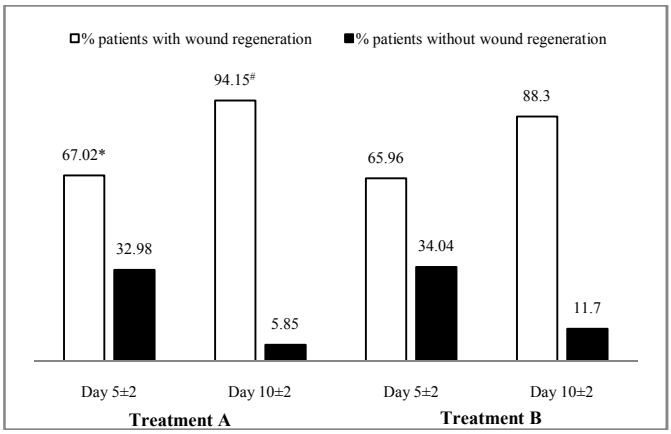


Fig 2 Wound regeneration in patients on day 5±2 and day 10±2

*Between group comparison on day 5±2 ($p>0.05$), #Between group comparison on day 10±2 ($p<0.05$), Treatment A: Trypsin 48mg + Bromelain 90mg + Rutoside Trihydrate 100mg + Diclofenac Sodium 50mg enteric coated tablets; Treatment B: Diclofenac Sodium 50mg enteric coated tablets

The results also revealed that there was a significantly better ($p<0.05$) improvement of total BWAT score in patients who received ENZOMAC PLUS. Out of the 13 BWAT wound characteristics, four characteristics including wound necrotic tissue amount, exudates amount, skin color surrounding wound and epithelialization showed significant improvement ($p<0.05$) at the end of treatment from baseline with both treatments. However, ENZOMAC PLUS was significantly better ($p<0.05$) at end of the treatment in improving these characteristics. Remaining BWAT parameters including wound edge, size, depth, undermining, granulation, necrotic tissue type, exudates type, peripheral tissue edema, and peripheral tissue indurations also improved significantly from baseline in both the treatment groups.

At the end of the treatment, 65.96% patients from treatment A and 44.68% patients from treatment B rated 'excellent' efficacy of study drug and 34.04% patients from treatment A and 50.53% patients from Treatment B rated 'good' efficacy of study drug. 4.79% patients in treatment B were rated for 'poor' efficacy of study drug. No patient had poor rating of efficacy in treatment A.

DISCUSSION

ENZOMAC PLUS, a fixed dose combination of trypsin 48 mg, bromelain 90 mg, rutoside trihydrate 100 mg, and diclofenac sodium 50 mg enteric coated tablet, is safe and tolerable in patients with surgical wound. Safety was comparable between both the groups. Additionally, ENZOMAC PLUS was also found to be significantly more effective in managing wound condition following minor surgery as compared to diclofenac sodium monotherapy.

Akhtar NM, *et al*. compared the safety and effectiveness of enteric coated tablet containing a combination of trypsin 48 mg, bromelain 90 mg, rutoside 100 mg and diclofenac 50 mg

with diclofenac enteric coated tablets in the management of osteoarthritis. The results suggested that this FDC was equally safe and more effective than diclofenac monotherapy. Thus, supporting the safety data in our study (Akhtar *et al,* 2004). However, the effects of diclofenac in incisional wound healing is controversial as per Krischak GD *et al*; unimpaired healing, both macroscopically and microscopically, was observed in both placebo and diclofenac group in their study involving Wistar rat (Krischak *et al,* 2007). Another clinical trial by Chandanwale, *et al*. reported the safety and effectiveness of trypsin, bromelain and rutoside combination in wound healing (Chandanwale *et al,* 2017).

In addition, a review article by Sinclair RD, *et al*. stated that proteolytic enzymes are cheaper and simpler alternatives for wound debridement as compared to surgical methods. Also, proteases and collagenases are the most easily available non-surgical debriders (Sinclair *et al,* 1994).

Trial limitations: Our study included participants of Indian origin only due to which the changes in the effects of drug based on the geographical origin of the individuals could not be assessed.

Innovation: Several marketed formulations containing a combination of trypsin, bromelain, rutoside with diclofenac are currently available. Data has been published in the past for the safety and efficacy of this combination in osteoarthritis. However, no published evidence is available for its effectiveness in wound healing.

CONCLUSION

ENZOMAC PLUS is equally safe when compared to diclofenac in the management of wound. The BWAT score indicated that this FDC is more effective than diclofenac monotherapy. In addition, the global impression efficacy evaluation by investigators and patients rated that ENZOMAC PLUS is more efficacious than diclofenac in treating wound condition.

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References

1. Akhtar NM, Naseer R, Farooqi AZ, Aziz W, Nazir M. 2004. Oral enzyme combination versus diclofenac in the treatment of osteoarthritis of the knee--a double-blind prospective randomized study. Clin Rheumatol, 23(5): 410-415.
2. Bates-Jensen B. Bates-Jensen wound assessment tool Instructions for use [online]. 2001 [cited 2020 Dec 22]. Available from: URL: https://wwwoundcare.ca/Uploads/ContentDocuments/BWAT.pdf.
3. Bowler PG. 2002. Wound pathophysiology, infection and therapeutic options. Ann Med, 34(6): 419-427.
4. 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.
5. Guo S, Dipietro LA. 2010. Factors affecting wound healing. J Dent Res, 89(3): 219-229.
6. Harris C, Bates-Jensen B, Parslow N, Raizman R, Singh M, Ketchen R. 2010. Bates-Jensen wound assessment tool: pictorial guide validation project. J Wound Ostomy Continence Nurs, 37(3): 253-259.
7. InformedHealth.org, Cologne, Germany: Institute for Quality and Efficiency in Health Care (IQWiG). What are the treatment options for chronic wounds? [online]. 2018 [cited 2020 Dec 21]. Available from: URL: https://www.ncbi.nlm.nih.gov/books/NBK326436/.
8. 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.
9. Krischak GD, Augat P, Claes L, Kinzl L, Beck A. 2007. The effects of non-steroidal anti-inflammatory drug application on incisional wound healing in rats. J Wound Care, 16(2): 76-78.
10. Loo YL, Goh BKL, Jeffery S. 2018. An overview of the use of bromelain-based enzymatic debridement (Nexobrid®) in deep partial and full thickness burns: appraising the evidence. J Burn Care Res, 39(6): 932-938.
11. Norman G, Dumville Jo C, Mohapatra DV, Owens GL, Crosbie EJ. 2016. Antibiotics and antiseptics for surgical wounds healing by secondary intention. Cochrane Database Syst Rev, 3(3): CD011712.
12. Shah D, Mital K. 2018. The role of trypsin:chymotrypsin in tissue repair. Adv Ther, 35(1): 31-42.
13. Sinclair RD, Ryan TJ. 1994. Proteolytic enzymes in wound healing: the role of enzymatic debridement. Australas J Dermatol, 35(1): 35-41.

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