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RESEARCH ARTICLE

Effect of Topical Serratiopeptidase on Facial Wound Healing in Rabbit

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Abstract

Objective: Serratiopeptidase (SRP), an enzyme with great effect in opposing inflammation. Applications of enzyme topically provide advantage of direct entery to the location of action. This study examined the possibility of using topical SRP preparations in the formula of ointments and to assess the topical effect of SRP in facial wound management in rabbits. Material and Method: healthy male rabbits weight,1.25 ± 0.25kg divided into four groups: group one animals received no treatment, group two animals treated with Vaseline only, group three and four were treated by SRP (0.5% and 1%) respectively. Standard incisions were done on submandibular region of all rabbits. Wound healing assessment was determined by histological method. Results: group one and two showed incomplete closure of the wound with poor re-epithelization, while other groups (three and four) demonstrated complete closure of the wound with good re-epithelization. Conclusion: topical application of SRP has ability to improve facial wound healing in rabbits.

Keywords: *Serratiopeptidase, Facial wound, Rabbit.*

Introduction

Proteolytic enzymes are definite enzymes that break down protein and represent an essential class of proteins and peptides formed by human and additional living organisms. Serratiopeptidase (SRP) is existing in one of the enteric bacilli in the silk worm [1]. It has various actions containing a powerful anti-inflammatory effects, anti-edema in addition to bradykinin-decomposing action as soon as improves the action of antibiotic at the infected site [2, 3].

SRP mostly used as an anti-inflammatory enzyme-based drug alone or in combination with other medicines in treatment of sinusitis, bronchitis, atherosclerosis and arthritis [4]. Some studies concluded that topical SRP was superior substitute to certain nonsteroidal anti-inflammatory drug like diclofenac gel [5, 6].

In addition, in the experimental study SRP was active in eliminating biofilm-forming bacterial infection by improving antibiotic effectiveness in the treatment of staphylococcal infections [7]. Wound healing initiates the homeostasis at the damage tissue, developments to an inflammatory stage charted by production of the epithelial tissues and matrix components and finished with the formation of collagen matrix [8].

Proteolytic enzymes mortify necrotic and damaged cells, throughout mediators and toxic products inactivation, they control the pain, edema and facilitating better wound healing [9]. Certain study showed that healing can enhanced by serratiopeptidase in full thickness wound in rabbit model [10]. The aim of this study was to investigate the effects of topical formulation of SRP as beneficial line in the management of oral wound healing.

Material and Method

The work of the present study was performed in the Dental Basic Science department, Mosul University.

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The study procedure was confirmed by scientific committee of the department.

Preparation of Serratiopeptidase ointment

Serratiopeptidase ointments were prepared in two concentrations (0.5%, 1%). The ointment were kept in plastic containers and stored in refrigerator at 4^0C until used. Healthy mature male rabbits of body weight of 1.25 ± 0.25kg were incorporated in the study. They were divided into four groups. The Group one served as a control and received no treatment while group two treated by vasaline ointment only, whereas group three and four were received SRP ointment.

Topically on the submandibular region of all rabbits. On the first day of study, all rabbits were anesthetized by a mixture of xylazine hydrochloride and ketamine hydrochloride at 0.5, 50 mg/Kg intramuscular respectively, then a standard wound was made on submandibular area of each rabbit (Fig. 1). All animals were sacrificed on day fifteen and tissues of all wounds washed fixed with 10 % neutral buffered formalin for histopathological examination. Specimens were embedded in paraffin, cut into 5 µm sections perpendicular to the surgical line, and stained with hematoxylin-eosin (H&E). These sections were then examined under a light microscope for histopathological changes by blinded pathologists.

Fig. 1: Area of submandibular incision in rabbit model

Results

The histological appearance of skin at wound line of group one, showing poor formation of the granulation tissue at wound line with newly formed blood vessels and poor re - epithelization with incomplete closure of the wound. Figure 2 (A and B).

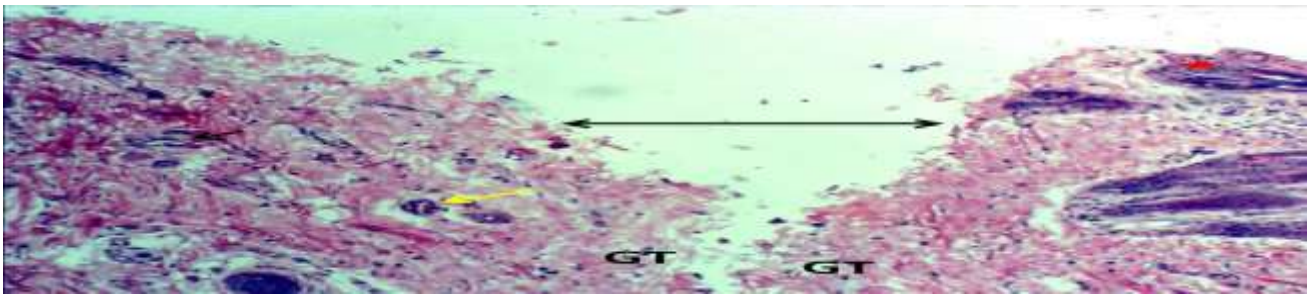

Fig. 2 (A) histological section of skin at wound line of group one. There is poor granulation tissue formation (GT) at wound line, with newly formed blood vessels (yellow arrows) and poor re epithelialization (red star). Note the incomplete closure of the wound (double head arrow) H&E. 100x

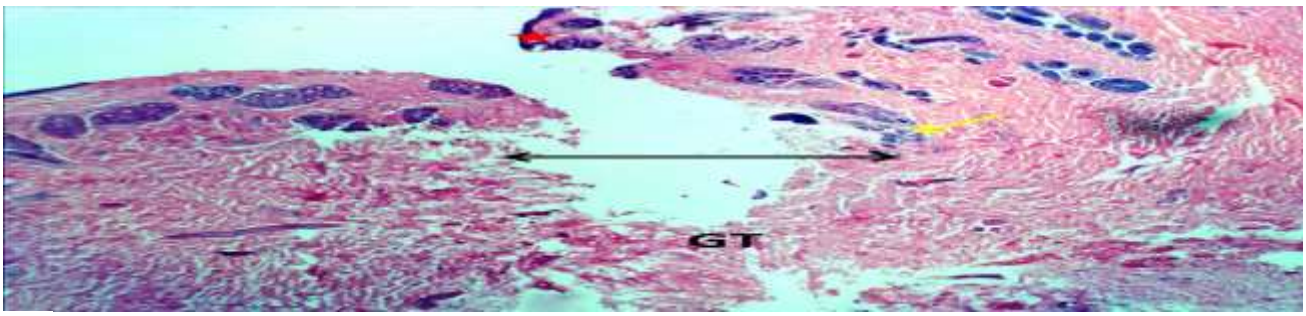

Fig. 2: (B) histological section of skin at wound line of group one. There is poor granulation tissue formation (GT) at wound line, with newly formed blood vessels (yellow arrows) and poor re-epithelialization (red star). Note the incomplete closure of the wound (double head arrow) H&E. 40x

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The histological appearance of skin at wound line of group two treated with vasaline ointment showing the formation of a granulation tissue that occupy most of wound region, with newly formed blood vessels, incomplete closure of the wound and poor re-epithelialization. Fig. 3 (A and B).

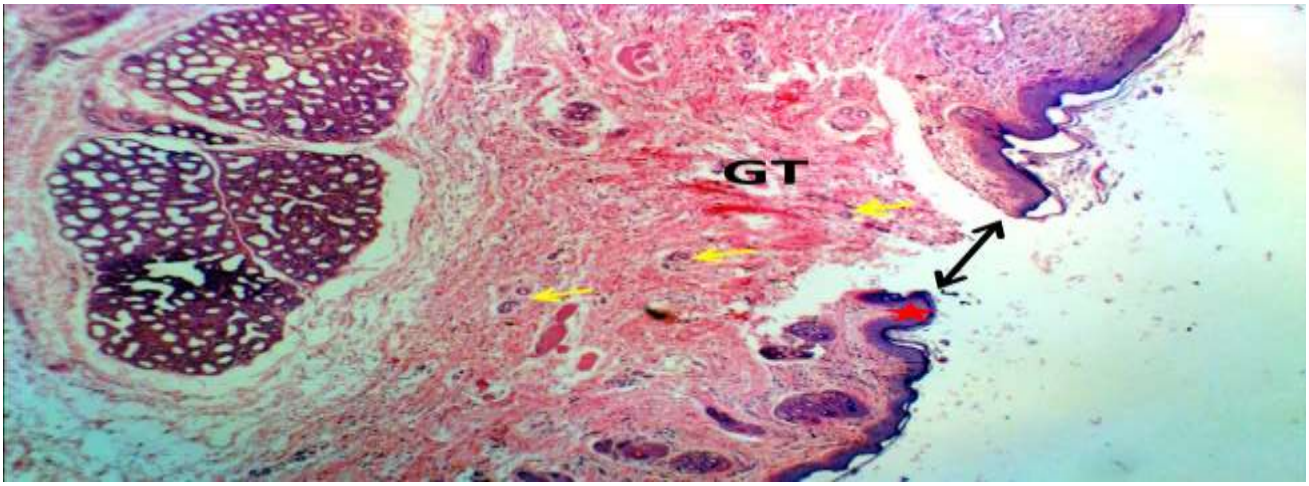

Fig. 3 (A) histological section of skin at wound line of group two. There is granulation tissue formation occupy most of wound region (GT), with newly formed blood vessels (yellow arrows). Note the incomplete closure of the wound (double head arrow) and re- epithelialization (red star) H&E. 40x

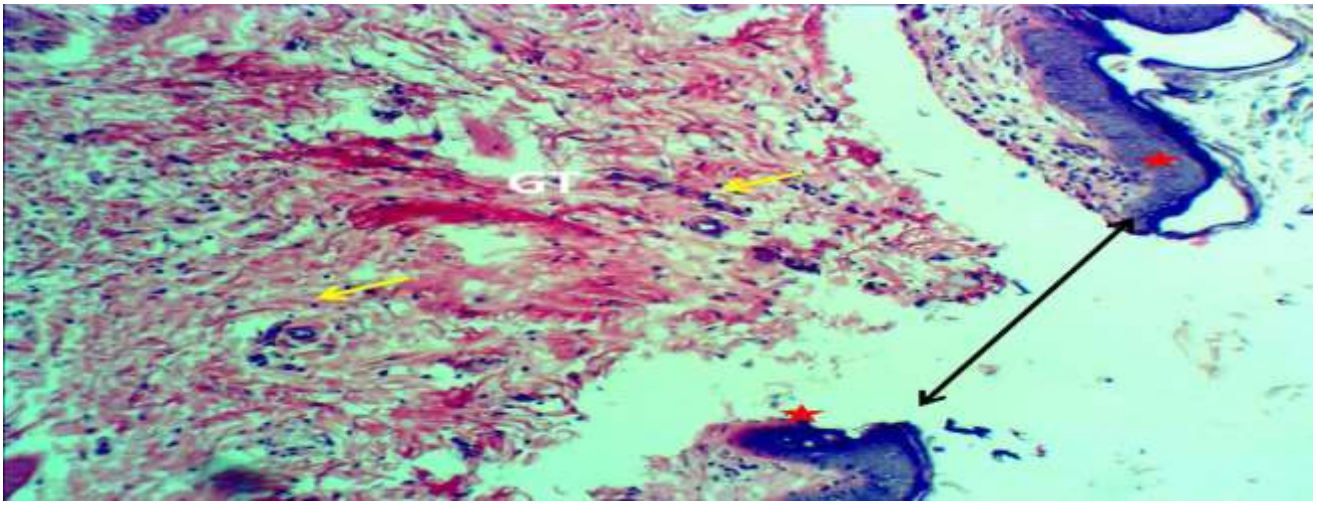

Fig. 3 (B) histological section of skin at wound line of group two. There is granulation tissue formation occupy most of wound region (GT), with newly formed blood vessels (yellow arrows). Note the incomplete closure of the wound (double head arrow) and re- epithelialization (red star) H&E. 100x

The newly formed blood vessels near the epidermis at the incision line and re-epithelialization showing in Fig. 3 (C).

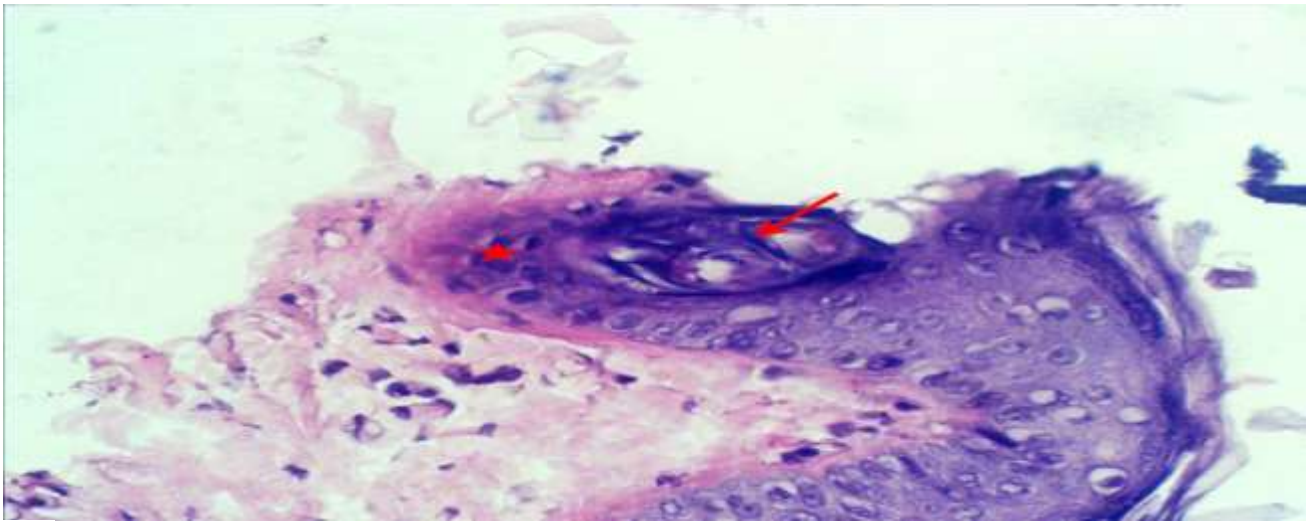

Fig. 4: (A) histological section of skin at wound line of group two. There is a newly formed blood vessel near the epidermis at the incision line (red arrow) and re- epithelialization (red star) H&E. 400x

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The histological appearance of skin at wound line of group three treated with (0.5% SRP) showing granulation tissue formation occupy most of wound region and moderate re-epithelialization with complete closure of the wound and newly formed hair follicles Fig. 4 (A).

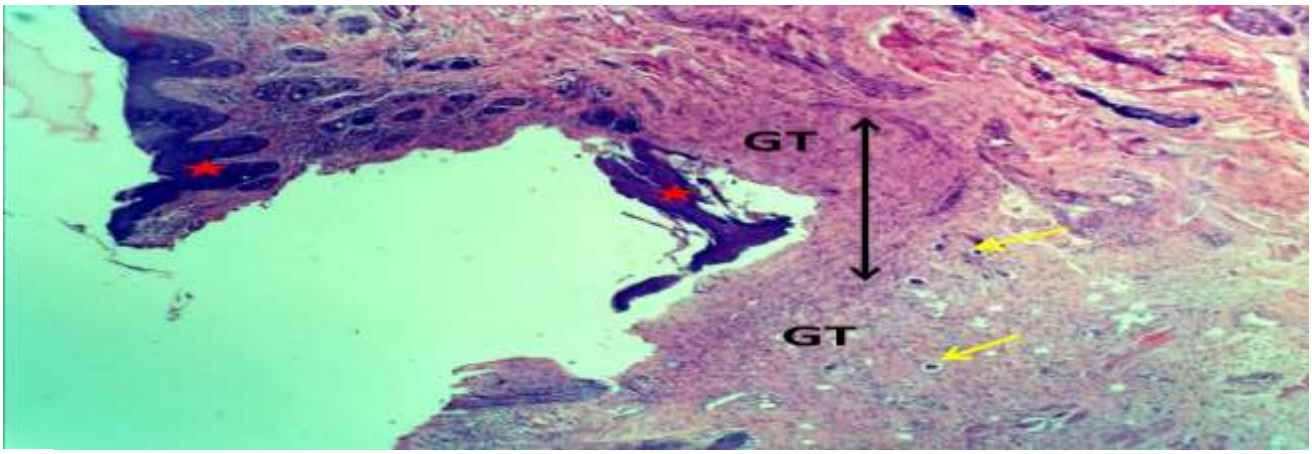

Fig. 4: (A) histological section of skin at wound line of 0.5% SRP group three. There is granulation tissue formation occupy most of wound region (GT), and moderate re-epithelialization (red star), Note the complete closure of the wound (double head arrow) newly formed hair follicles (yellow arrow). H&E. 40x

The proliferation of fibroblast showing in Fig. 4 (B).

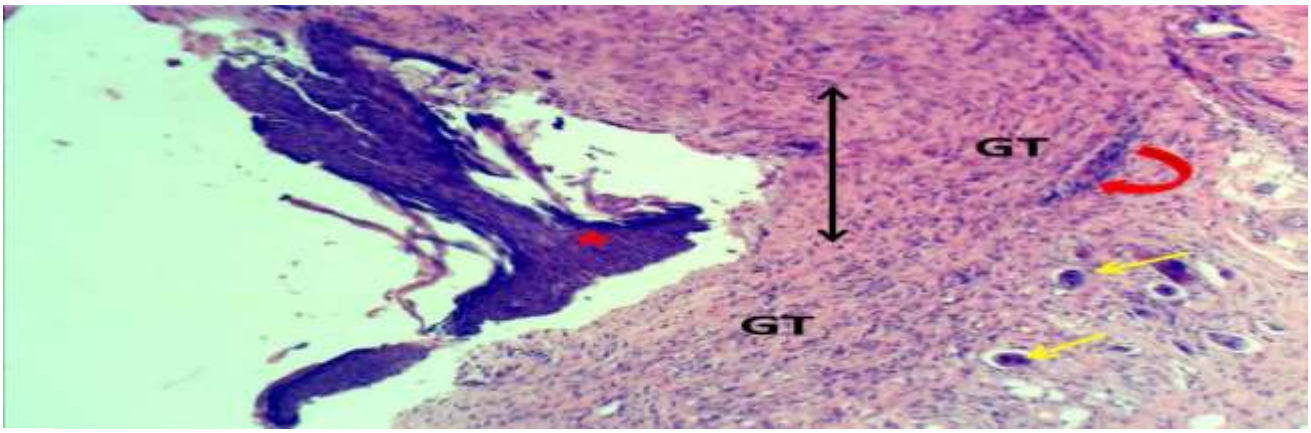

Fig. 4: (B) histological section of skin at wound line of 0.5% SRP group three . There is granulation tissue formation occupy most of wound region(GT), and moderate re-epithelialization(red star), Note the complete closure of the wound(double head arrow) newly formed hair follicles(yellow arrow) and proliferation of fibroblast(curved arrow). H&E. 100x

The histological appearance of skin at wound line of group four treated with (1% SRP) showing a good wound healing represented by granulation tissue formation occupy most of wound region and good re epethelization, the complete closure of the wound and newly formed hair follicles . Fig. 5 (A and B).The proliferation of fibroblast and good re - epithelialization showing in Fig. 5 (C).

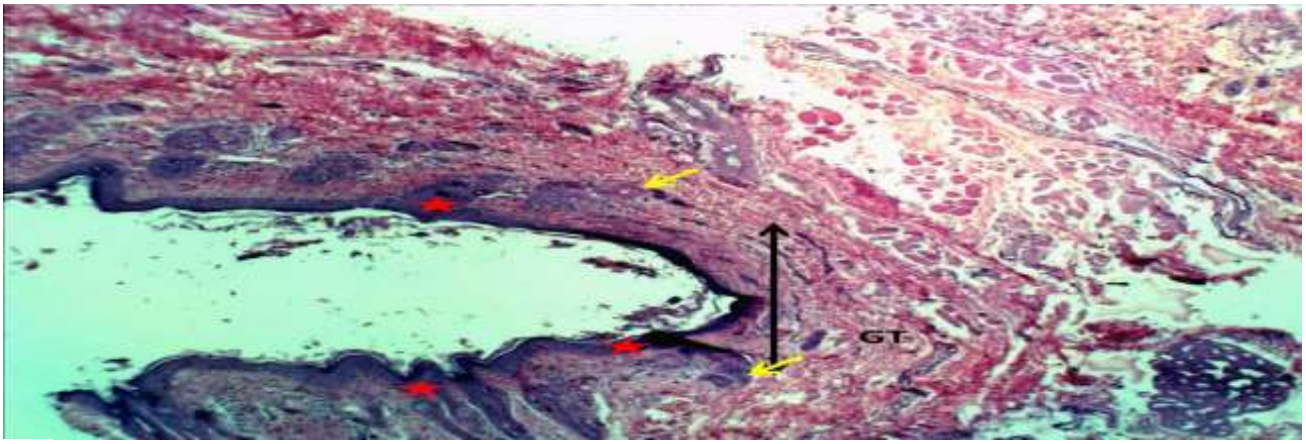

Fig. 5: (A) histological section of skin at wound line of 1% SRP group four. There is good wound healing represented by granulation tissue formation occupy most of wound region (GT), and re-epithelialization (red star). Note the complete closure of the wound (double head arrow), newly formed hair follicles (yellow arrow) H&E. 40x

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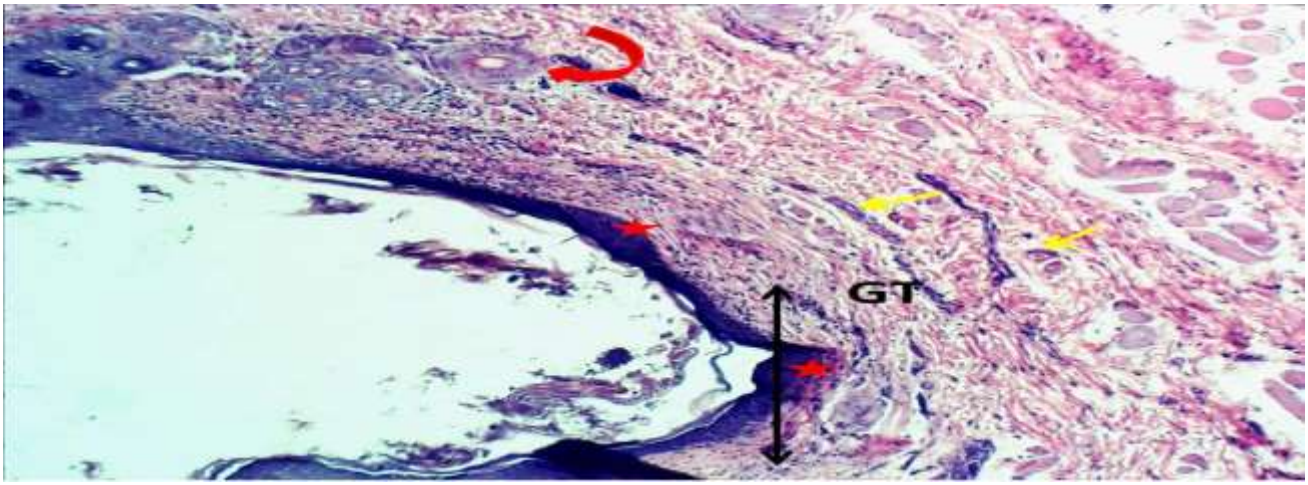

Fig. 5: (B) histological section of skin at wound line of 1% SRP group four. There is granulation tissue formation occupy most of wound region(GT), with newly formed blood vessels (yellow arrows) and good re-epithelialization(red star) and newly formed hair follicles (curved arrow). Note the complete closure of the wound (double head arrow). H&E. 100x

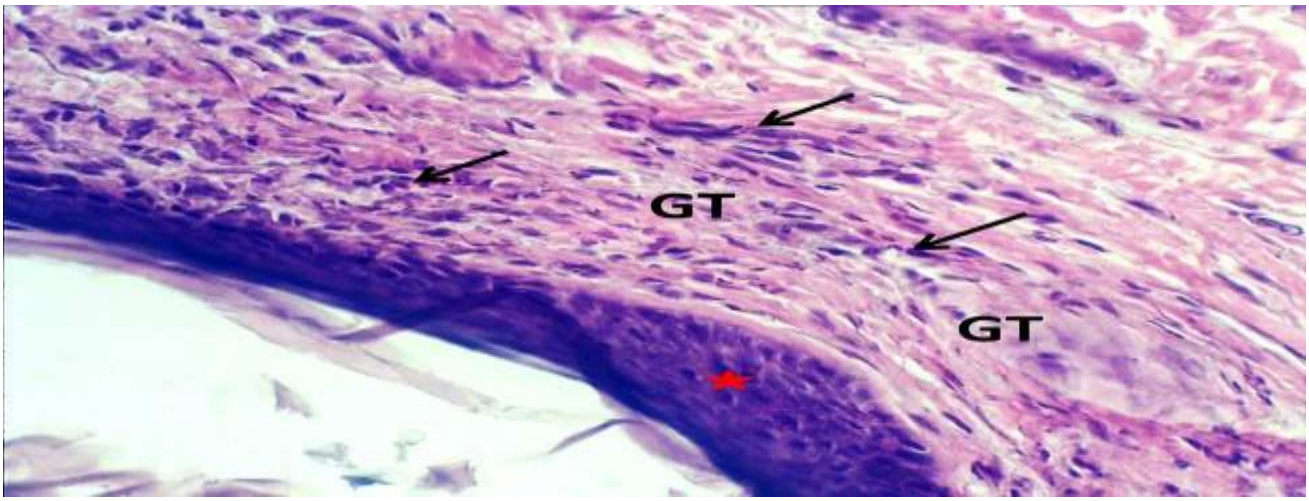

Fig. 5: (C) histological section of skin at wound line of 1% SRP group four There is granulation tissue formation occupy most of wound region (GT), proliferation of fibroblast(arrows) and good re-epithelialization(red star). H&E. 400x

Discussion

Enzymic treatment can take part in maintaining regular inflammatory process inside the body and in this manner it may support and pace up healing [9].Proteolytic enzymes have important pharmacological use as anti-inflammatory agents. Among this category, SRP, a metalloprotease produced by Serratiamarcescens, provides a better management for painful and inflammatory situations with prevalent use [11]. Available dosage forms of SRP are mainly enteric coated tablet.

The oral route bioavailability of these peptide drugs is usually very little, due to the acidity of stomach environment, proteolytic activity of gastrointestinal tract, and difficult permeability through mucosa of intestine. SRP has been reported to show systemic adverse effects which include anticoagulant effect and gastrointestinal disturbances. Consequently application of topical enzyme provide probable advantages of introducing the enzyme directly to the site of action with reduction of systemic side effects and increase in local effects of SRP by topical route [6-12].Wound healing is complicated process .The response of normal tissue to injury is a function of cells and cellular mediators. It starts with homeostasis at the site of wound, followed by inflammatory phase with proliferation of the epithelium and ends with the formation of a highly organized tissue. In chronic wounds, inflammation demonstrates increased levels of proteases and proinflammatory cytokines.

So the control of their expression is necessary for regular healing process of wound. Proteases and their inhibitors donate to the equilibrium between extracellular matrix destruction and deposition, creating a balance that is important for the accurate coordinated healing of skin wounds [13]. Facial wounds in rabbit models are usually

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recovered to baseline by postoperative day10 to11. These process which are controlled by proteases, which is one of the important constituent of tissue repair that have a task in different wound-healing mechanisms [14]. Wound healing is an complicated progression of biological steps relating to contraction and closure of wounds and re-establishment of functional barriers [15].

Results of this study revealed that histological appearance of facial skin at wound area of SRP treated groups showed a good wound healing represented by granulation tissue formation occupy most of wound region and good re-epithelialization with complete closure of the wound and formation of new hair follicles, this could be explained by the fact that hydrolysis by enzymes proteases, like SRP, is the most proficient, selective and least disturbing way of wound debridement which can accelerate granulation tissues formation and re-epithelization which can help in healing process of cutaneous wounds [15].

In agreement with results of this study, Rath Get al suggested that healing of wound is enhanced by means of SRP in full thickness wounds in rabbits [10]. So, enzyme can promote wound repair and restores the cutanuous condition of skin to normal [16]. On other hand, disagreement with results of this study stated that systemic use of SRP (5mg/kg orally) cannot improve healing of buccal mucosal wound in rabbits [14-17].

Which can be explained by the fact that topical SRP act in the vicinity and do not suffer classic systemic metabolic modifications which cannot be achieved when was used orally [6]. In addition to that, topical SRP mainly accelerate wound healing on skin which differ from oral mucosal wounds in relation to healing capacities glucose transporter protein-1 (GLUT-1) which is membrane protein act as carrier for glucose uptake into cell thus its expression can be a parameter of cell proliferation activity and wound healing. In the skin, glucose transporter protein-1 (GLUT-1) observed in the epidermal basal cell layer while the buccal mucosa showed no GLUT-1 protein expression in its basal cell layer, and this can affect the healing rate of wounds in skin and oral buccal mucosa. So, different formulations are required for oral mucosal wound healing [18].

Serratiopeptidase is a principal enzyme which has an extended history in dental and therapeutic fields as an efficient anti-inflammatory agent. Current study emphasizes present circumstances and future outlook of SRP as topical drug that can help in process of wound healing.

Conclusion

Therapeutics enzyme became an important part of contemporary dentistry and medicine mainly owing to their efficiency and selectivity. Serratiopeptidase became widely used one, as effective drug in numerous diseases precisely during dental and medical surgical events, but there is a need of more research confirmation and studies. The current work emphasizes the ability of the enzyme to accelerate wound healing withminimal possible side effects and complications since it is applied topically. Serratiopeptidase could be used safely and effectively to improve facial wound healing.

Source of Funding

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References

1. Vandana Gupte, UmeshLuthra (2017) Analytical techniques for serratiopeptidase: A review-. J. Pharm. Anal., 7(4): 203-207.
2. Al-Khateeb TH, Nusair Y (2008) Effect of proteolytic enzyme serrapeptase on swelling, pain and trismus after surgical extraction of mandibular third molars. Int. J. Oral. Maxillofac. Surg., 37: 264-68.
3. Chappi D M, Suresh KV, Patil MR, Desai R, Tauro DP, Bharani K N S S, Parkar MI, Babaji HV (2015) Comparison of clinical efficacy of methylprednisolone and serratiopeptidase for reduction of postoperative 289equel after lower third molar surgery. J. Clin Exp. Dent., 7(2): e197-202.
4. ManjuTiwari (2017) The role of serratiopeptidase in the resolution of

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inflammation .Asian Journal of Pharmaceutical Sciences, 12 (3): 209-215.

5. Ujwala A Shinde, Shivkumar S Kanojiya (2014) Serratiopeptidase Niosomal Gel with Potential in Topical Delivery. J. Pharm. (Cairo) 382-959.
6. Nirale NM1, Menon MD (2010) Topical formulations of serratiopeptidase: development and pharmacodynamic evaluation. Indian J. Pharm. Sci., 72(1): 65-71.
7. Mecikoglu M, Saygi B, Yildirim Y, Karadag-Saygi E, Ramadan SS, Esemenli T (2006) The Effect of Proteolytic Enzyme Serratiopeptidase in the Treatment of Experimental Implant-Related Infection. J. Bone Joint Surg. Am., 88(6):1208-14.
8. Akriti Gupta and Pramod Kumar (2015) Assessment of the histological state of the healing wound. Plast Aesthet. Res., 2: 239-42.
9. Chandanwale A, Langade D, Sonawane D and 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.
10. G Rath, ES Johal, Amit K Goyal (2011) Development of Serratiopeptidase and Metronidazole Based Alginate Microspheres for Wound Healing, Artificial Cells, Blood Substitutes, and Biotechnology, 39:1, 44, 50.
11. Vélez-Gómez JM, Melchor-Moncada JJ, Veloza LA, Sepúlveda-Arias JC (2019) Purification and characterization of a metalloprotease produced by the C8 isolate of Serratiamarcescens using silkworm pupae orcasein as a protein source. Int. J. Biol. Macromol., 15(135): 97-105.
12. Vijayan, K, Jayaraja Kumar, SelvaduraiMuralidharan, S Parasuraman (2015) Formulation and Characterization of Novel Ethanolic Carrier for Improves Penetration of Serratiopeptidase. Research & Reviews: A Journal of Drug Formulation, Development and Production, 2(1): 1-6.
13. McCarty SM, Percival SL (2013) Proteases and Delayed Wound Healing. Advances in wound care, 2 (8438-447): 438- 447.
14. AL-MASHHADANE FA (2019) The activity of serratiopeptidase on oral wound healing in rabbits. International Journal of Pharmaceutical Research, 11 (2): 562-567.
15. Singh D, Singh MR, Saraf S (2011) Development of delivery cargoes for debriding enzymes effective in wound healing. Trends in applied sciences research, 1-14.
16. Tiwari M (2017) The role of serratiopeptidase in the resolution of Inflammation. Asian journal of pharmaceutical sciences, 12: 209-215.
17. Chopra D, Rehan HS, Mehra P, Kakkar AK (2009) A randomized, double-blind, placebo-controlled study comparing the efficacy and safety of paracetamol, serratiopeptidase, ibuprofen and betamethasone using the dental impaction pain model, 38(4):350-5.
18. Al-Mashhadane FAM (2012) The Clinical Effect of Phenytoin on Oral Wound. Al-Rafidain Dent J., 12(1): 165-170.

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