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Study on the Effect of N-Acetyl Cysteine (NAC) Versus Another Enzyme on Healing of Skin Grafting in Albino Male Rabbits

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***Abstract*— kin grafting is a closure procedure employed in dermatology primarily to seal wounds resulting from the excision of damaged skin. Despite being less preferred than flap closures, grafting can yield an aesthetically pleasing outcome.**

Aim of study: The aim of the present study was to investigate the grafting of the skin of albino male rabbits by N-acetyl cysteine (NAC) and serrati peptidase, then compare between their effect on the skin grafting of rabbits.

Methodology: 9 rabbits were divided into three groups each group have 3 animals as following Group 1 (G1) as a control (skin grafting without any drug), skin was taken from shoulder to femoral area. Group 2 (G2) the skin graft was taken from the same area in the G1 with using serrati peptidase, while the group 3 (G3) the skin graft was taken from the same area in G1 with using NAC.

Results: The results have been shown that both of the NAC and Serrati peptidase good enhancement effects on the skin graft, and showed better enhancement effects on the hematology, histology and AST parameters.

Conclusion: results were show both of NAC and Serrato peptidase can be used to enhance the skin graft will cause increase in the hematology, histology and AST levels.

***Keywords* — N-Acetyl Cysteine; rabbits; serrati peptidase.**

I. INTRODUCTION

AS The skin, the largest organ of the human body, is essential for maintaining homeostasis and safeguarding internal organs from the external environment (1). Cutaneous injuries, particularly chronic wounds, burns, and skin infections, necessitate prolonged treatment, imposing a significant financial strain on global healthcare systems (2). An aging demographic, along with rising incidences of diabetes and obesity, persistently elevates the prevalence of chronic wounds. It is estimated that 1–2% of the population in developed nations will encounter a chronic wound during their lifetime (3). N-acetyl cysteine (NAC) is a chemical sanctioned by the U.S. Food and Drug Administration for therapeutic application as a mucolytic, possessing thiol and carboxyl groups (4,5). NAC may possess extensive therapeutic potential, especially in the context of antioxidants. Serratiopeptidase is a proteolytic enzyme utilized across multiple specialties, including surgery, orthopedics, otorhinolaryngology, gynecology, and dentistry, for its anti-inflammatory, anti-edemic, and analgesic properties (6). Anecdotal evidence indicates it may have anti-atherosclerotic effects, attributed to its fibrinolytic and caseinolytic properties. Serrati peptidase is a proteolytic enzyme derived from the nonpathogenic enterobacterium Serratia E15. It is synthesized in the intestines of silkworms to decompose cocoon walls. This enzyme has served as an alternative to analgesics and nonsteroidal anti-inflammatory drugs, as well as a treatment for chronic sinusitis and postoperative inflammation. (7).

II. MATERIALS AND METHODS

A. Animals

Nine albino male rabbits weighing 750g –1 Kg were used in this study. The rabbits were fed standard rabbits chow and allowed ad libitum access to water. This study was approved by the laboratories in College of Veterinary Medicine, University of Karbala.

Nine rabbits were separated into three groups, each containing three animals as follows. Group 1 (G1) served as a control, using skin transplantation without any pharmacological intervention, with skin harvested from the shoulder to the femoral region. In Group 2 (G2), the skin graft was harvested from the same region as in Group 1 (G1) utilizing serratiopeptidase, however in Group 3 (G3), the skin graft was obtained from the same area in G1 employing N-acetylcysteine (NAC).

Preparation of N-acetyl cysteine

Following the measurement of each ingredient for the formulation, a 3% NAC cream was synthesized, after which the requisite quantity of NAC was pulverized into fine particles and incorporated into water, resulting in a paste. The specified

quantity of this combination was incorporated into cold cream and blended until a homogeneous consistency was attained.

Preparation of serrati peptidase ointment

Serrati peptidase ointments were prepared as 1%. The ointment was kept in plastic containers and stored in refrigerator at 40C until used. Healthy mature male rabbits of body weight of 1.25 ± 0.25kg were incorporated in the study

B. Surgical operation

Preoperative preparation

The subjects of this study underwent surgical preparation by fasting for 12 hours without food and 6 hours without water before to the procedure. The medial tibial region was cut, shaved, and prepped aseptically, as illustrated in Figure 1. The skin was cleansed with 2.5% bovidine iodine. The rabbits were positioned in lateral recumbency and draped with surgical drapes secured to the skin using towel clips. Figure 2

Anesthesia

All procedures were conducted under general anesthesia via intramuscular injection of a Xylazine and Ketamine combination at a dosage of 3:35 mg per kg body weight, with an extra Xylazine dose to enhance depth and duration of unconsciousness.

Surgical technique

The procedure executed in this experiment involved the transplantation of a square-shaped skin graft measuring 1cm x 1cm from the forelimb to a defect in the hind limb, which was filled with NAC powder and tetracopeptide powder. The graft borders were sutured to the surrounding skin using a simple interrupted suture technique (figures 2-3) and covered with a gauze pad, which was changed every two days. Postoperatively, a penicillin-streptomycin mixture was administered for five days to avert infection.

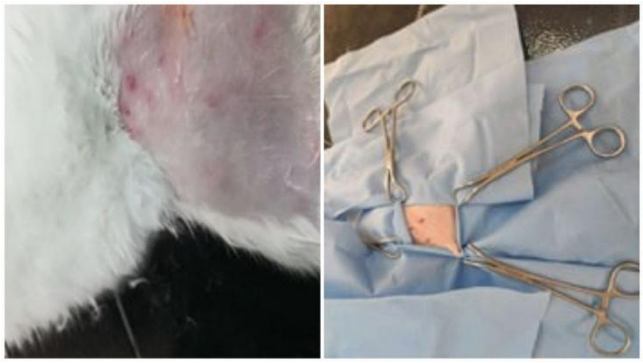

Figure 1: Shaving and cleaning of skin for the surgical operation

blood sample collection

The blood samples were collected directly by syringe through heart puncture (figure 2) then put it in the tubes with and without anticoagulant, these tubes stored cooled in the ice boxes to avoid hemolysis and then send for analysis to make the hematological tests (figure 3).

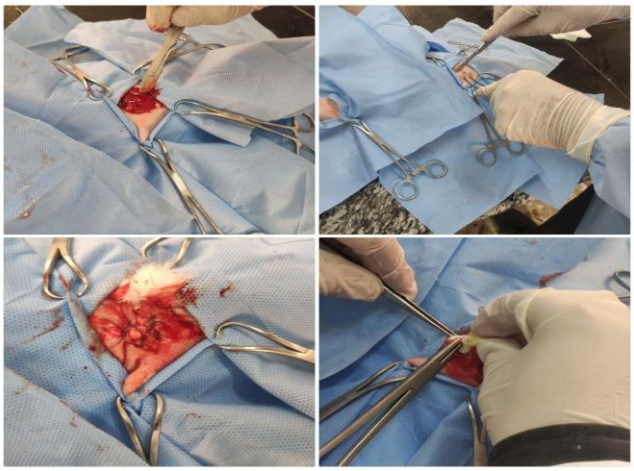

Figure 2: The surgical procedure steps which made during skin grafting

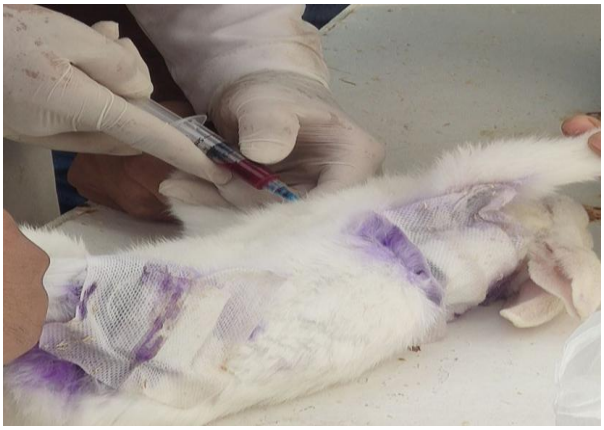

Figure 3: The blood sample collection

III. Results and Discussion

To assess the impact of NAC on the healing of skin grafts in albino male rabbits. This section presents the histological, hematological, and biochemical analyses of skin grafts from albino male rabbits treated with NAC.

A. Histological of G3 treated with NAC

The histological analysis demonstrated a considerable improvement in the healing of skin grafts, as illustrated in Figure 4. Figure 4b illustrates normal healing characterized by the presence of typical keratin, an intact epidermal layer, hair follicles, blood vessels, and muscle layers, in contrast to the control group, which also exhibits a normal epidermis and keratin alongside a standard dermal layer.

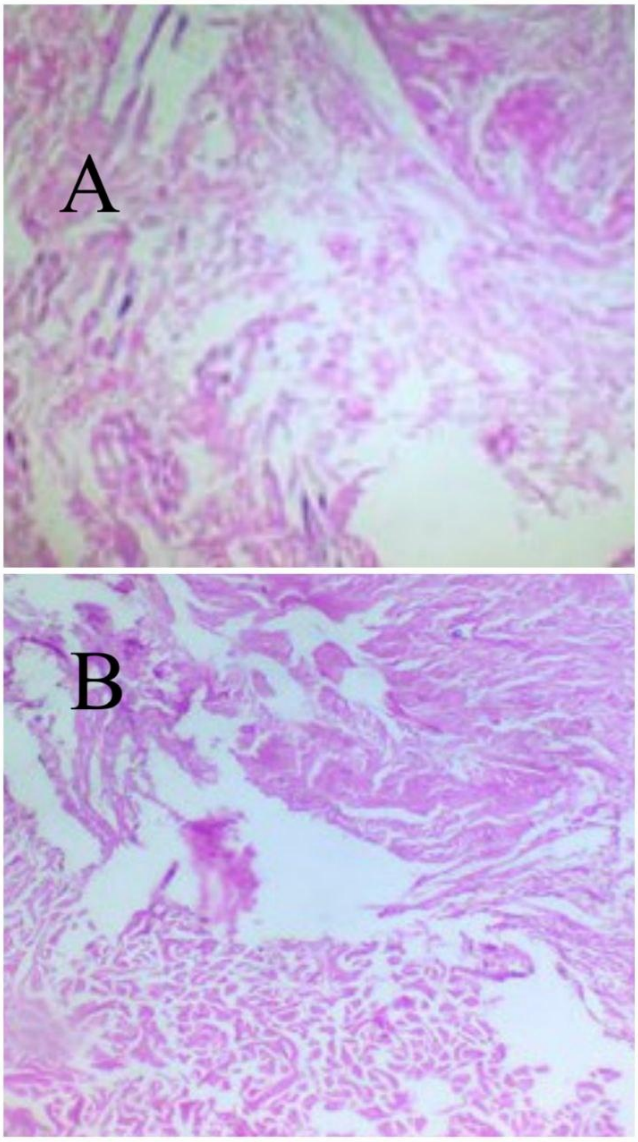

Figure 4: Histological section for skin graft of rabbit with a) without treated and b) treated with NAC

The results of the present study were showed that the enhancement effects of NAC on the skin grafting and healing have been observed clearly. According to previous studied the effect of NAC to enhance the grafting of the skin due to antioxidant effect of the NAC (8,9). The obtained results in the present study in line with the results obtained from the studies that were reported previously. The present study was applied on albino male rabbits which considered as a new study about those types of the rabbits. The hematological analyzed include to check the Red Blood Cell (RBC), White Blood Cell (WBC) and platelets while (PLT) of the albino male rabbits in G1 and G3. As shown in the table 1 the WBC, RBC and PLT of the treated rabbits with the NAC were 10.3 x 10x^3 µl, 3.15 x 10x^6 µl and 387 x 10x^3 µl, respectively, compare with the control animals groups were 6.5 x 10x^3 µl , 5.21 x 10x^6 µl and 315 x10x^3 µl. The hematological data indicated an elevation in white blood cells and platelets, while red blood cells and other hematological parameters remained normal in the rabbit treated topically with NAC. The rise in WBC and platelets is attributed to the inflammatory response associated with the skin graft.

Table 1: The comparison of blood parameters between NAC group and control group

Parameters	WBC x $10x^3$ µl	RBC x $10x^6$ µl	PLT $10x^3$ µl
Control	6.5	5.21	315
NAC group	10.3	3.15	387

Based on the results that have been obtained from the hematological study the leave of the WBC and PLT of the G3 were increased compare with the G1, according to the previous studies enhance the WBC maybe due to the effect of the body by injury or get inflammatory, however, our study includes to injured the animals of the G3 before treated with the NAC that gave the reason to the WBC and PLT increased.

Biochemical analysis of G3 treated with NAC

The biochemical investigation for G3 was conducted utilizing aspartate aminotransferase (AST). Table 2 presents the AST analysis for G1 and G3, recorded at 0.4 mg/dl and 0.6 mg/dl, respectively. The analysis results indicated a mild increase in this enzyme compared to the control group. This elevation in enzyme levels may be attributed to the inflammatory response associated with skin grafting in the NAC-treated rabbits. Chemical analysis revealed superior enhancement in this group compared to the serattopeptide-treated rabbits relative to the control group, with the beneficial effects of NAC stemming from its antioxidant properties. (10).

Table 2: The comparison of AST between NAC and control group

Parameters	**AST mg/ dl**
Control	0.4
NAC group	0.6

Determine the effect of serrati peptidase on healing of skin grafting in albino male rabbits

In this section the Group 2 of the animals (albino male rabbits) was treated with serrati peptidase, then the histological, hematological and biochemical analysis have been done.

Histological of G2 treated with serrati peptidase

The histological study of this part was measured for the G2 of the animals after treated with serrati peptidase. The histological dissection showed moderate enhancement of skin grafting healing as shown in the figure 5, which show healing with increase in formation of the collagen fiber and epidermis layer while dermis layer is normal, compare with non-treatment group of the rabbits which show normal epidermis and keratin with normal dermis layer. the enhancement effects of serattopeptidase can be clearly seen in this section due to the antibacterial effects (8).as well as antioxidant properties (11).

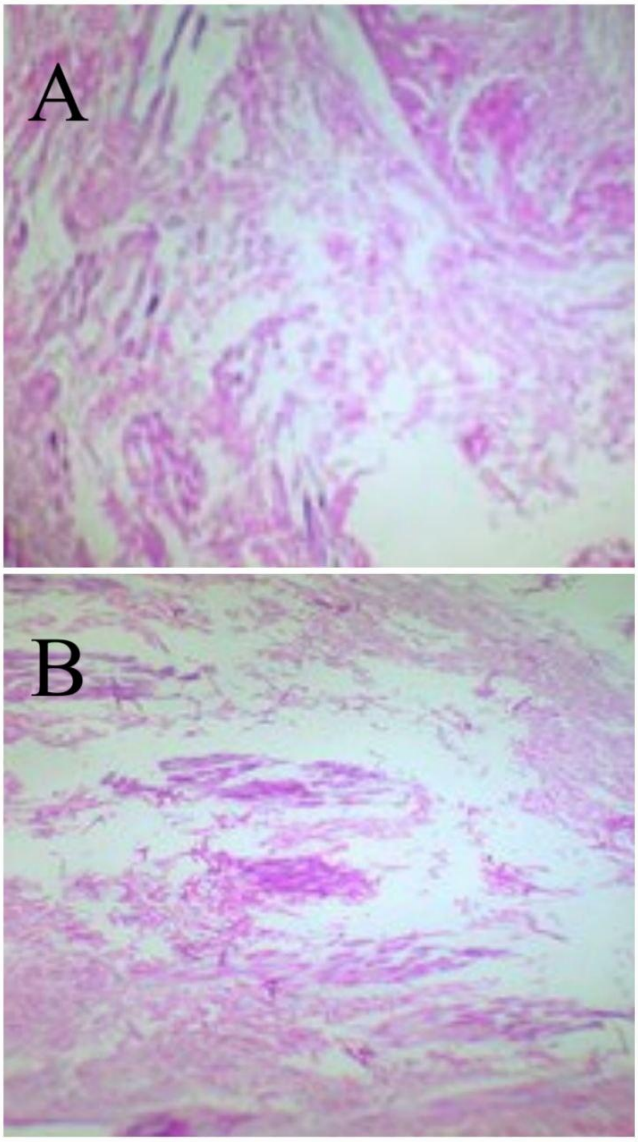

Figure 5: Histological section for skin graft of rabbit **a)** control group and **b)** treated group with using serattopeptidease

Previous studies were reported to study the histological of the skin of the animals after treated with the serattopeptidease for the grafting purpose (12), reported to enhancement of the wound healing by using serattopeptidease their results the wounds had partial epithelization, inflammatory cell predominance with few fi broblasts, and sparse collagen deposition were noticeable, especially at the wound centers. In the present study similar results obtained.

Hematological of G2 treated with serrati peptidase

The hematological examination was conducted on the G2 group of rabbits following treatment with the Serrato peptidase enzyme. The blood samples collected from the rabbits at various intervals during the experiment will be compared with those from other groups. Table 3 presents the levels of WBC, RBC, and PLT for Groups 1 and 2 of the rabbits. The G2 results indicated 11.5 x 10^3 µl, 5.07 x 10^6 µl, and 405 x 10^3 µl for WBC, RBC, and PLT, respectively, whereas the control group exhibited 6.5 x 10^3 µl, 5.21 x 10^6 µl, and 315 x 10^3 µl for WBC, RBC, and PLT, respectively. The hematological analysis of the serratopeptidase-treated rabbits revealed a mild elevation in the white blood cell count attributable to inflammatory responses from the skin graft, while other hematological parameters remained normal compared to the control group. The observed enhancements in these parameters are likely due to the anti-inflammatory properties of serratopeptidase (13). The results indicated a considerable increase in WBC, RBC, and PLT attributable to the inflammatory response of the skin graft (14).

Table 3: The comparison of blood parameters between the Serrato peptidase and control group

Parameters	WBC x $10x^3$ µl	RBC x $10x^6$ µl	PLT $10x^3$ µl
Control	6.5	5.21	315
Serrato peptidase	11.5	5.07	405

According to the findings from the hematological study, the levels of WBC and PLT in G2 were elevated compared to G1. Previous studies suggest that the increase in WBC may be attributed to the body's response to injury or inflammation (10). However, our study involved injuring the animals in G2 prior to treatment with NAC, which accounts for the observed increase in WBC and PLT.

Biochemical analysis of G2 treated with serrati peptidase

Table 4 presents the biochemical analysis of AST levels in animals treated with serratiopeptidase, revealing a moderate increase in this enzyme relative to the control group. This elevation is attributed to the inflammatory response associated with skin grafting in the treated rabbits. The chemical analysis results indicate that the enhancement in this group is less pronounced than that observed in the NAC-treated rabbits when compared to the control group. The beneficial effects of serratiopeptidase are ascribed to its antioxidant properties (15).

Table 4: The comparison of AST enzyme between Serrato peptidase and control groups

Parameters	AST mg/ dl
Control	0.4
Serrato peptidase	0.9

To compare the effects of NAC and serrati peptidase on the healing of skin grafting in albino male rabbits. This objective included to compare the G2 and G3 of the rabbits after treated by serrati peptidase and NAC, respectively, via a histological, Hematological and Chemical analysis. Histological study of the G2 of the rabbits as shows in figure (b), moderate enhancement of skin grafting healing after treated with serrati peptidase, while figure (b) shows the G3 of the rabbits after treated with NAC. The results that obtained form that figure shows normal healing with normal keratin and epidermis layer as well as hair follicle, blood vessels and muscular layer, The findings of the current study indicate that NAC is superior to serratiopeptidase

for usage in skin grafting. The hematological analysis of G1, G2, and G3 was determined based on three criteria previously mentioned: WBC, RBC, and PLT, as clearly illustrated in Table 3.5. The white blood cell (WBC) count in group G1 was 6.5 x 10^3 µl, which is within the normal range. WBC counts were elevated in groups G2 and G3, measuring 11.5 and 10.3, respectively. The WBC levels in rabbits treated with serratiopeptidase surpassed those in the N-acetylcysteine (NAC) treated group. Given that this increase was attributed to inflammation, we conclude that G3 demonstrated superior grafting progress compared to G2, suggesting that NAC may be more effective than serratiopeptidase in hematological contexts. Furthermore, the level of PLT in G2 exceeded that of G3, indicating a higher degree of inflammation in G2 for the same reasons previously stated.

Table 5: The comparison of blood parameters of Serrato peptidase, NAC and control group

Parameters	WBC x $10x^3$ µl	RBC x $10x^6$ µl	PLT $10x^3$ µl
Control	6.5	5.21	315
Serrato peptidase	11.5	5.07	405
NAC	10.3	3.15	387

This section entails a comparison of the ASTs of G1, G2, and G3. Table 3.6 displays the AST levels of all groups. The AST level rose from 0.4 mg/dl in the untreated group to 0.9 mg/dl in the group treated with serratiopeptidase, while the NAC-treated group exhibited an AST level of 0.6 mg/dl. This elevation in AST levels is attributable to inflammatory responses, with greater inflammation correlating to higher levels. This suggests that NAC is a superior improvement compared to serratiopeptidase.

Table 6: The comparison of AST enzyme between Serrato peptidase, NAC and control groups

Parameters	AST mg/ dl
Control	0.4
Serrato peptidase	0.9
NAC	0.6

IV. Conclusion

This study aims to assess the impact of NAC and Serrato peptidase on skin grafting in albino male rabbits. The data were categorized into three sections based on histological, hematological, and chemical analyses. The findings indicate that NAC significantly improved skin graft outcomes and exhibited superior enhancement effects on hematological, histological, and AST parameters compared to Serrato peptidase, which also demonstrated beneficial effects on these parameters, albeit to a lesser extent than NAC. Our findings indicate that both NAC and Serratiopeptidase can be utilized to improve skin transplant outcomes, resulting in elevated hematological, histological, and AST levels.

References

1) Dovi, J. V., He, L. K., & DiPietro, L. A. (2003). Accelerated wound closure in neutrophil-depleted mice. *Journal of Leucocyte Biology, 73(4), 448-455.*
2) Chen, W., Palazzo, A., Hennink, W. E., & Kok, R. J. (2017). Effect of particle size on drug loading and release kinetics of gefitinib-loaded PLGA microspheres. *Molecular pharmaceutics, 14(2), 459-467.*
3) Steed, D. L. (2006). Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity ulcers. *Plastic and reconstructive surgery, 117(7S), 143S-149S.*
4) Minkov, V. S., & Boldyreva, E. V. (2013). Weak hydrogen bonds formed by thiol groups in N-Acetyl-L-Cysteine and their response to the crystal structure distortion on increasing pressure. *The Journal of Physical Chemistry B, 117(46), 14247-14260.*
5) Feng, T., Pi, B., Li, B., Jiang, L., Wang, Y. M., Zhu, X. S., & Yang, H. L. (2015). N-Acetyl cysteine (NAC)-mediated reinforcement of alpha-tricalcium phosphate/silk fibroin (α-TCP/SF) cement. *Colloids and Surfaces B: Biointerfaces, 136, 892-899.*
6) Uchi, H., Igarashi, A., Urabe, K., Koga, T., Nakayama, J., Kawamori, R., ... & Furue, M. (2009). Clinical efficacy of basic fibroblast growth factor (bFGF) for diabetic ulcer. *European Journal of Dermatology, 19(5), 461-468.*
7) Hesketh, M., Sahin, K. B., West, Z. E., & Murray, R. Z. (2017). Macrophage phenotypes regulate scar formation and chronic wound healing. *International journal of molecular sciences, 18(7), 1545.*
8) Mecikoglu, M., Saygi, B., Yildirim, Y., Karadag-Saygi, E., Ramadan, S. S., & Esemenli, T. (2006). The effect of proteolytic enzyme serratiopeptidase in the treatment of experimental implant-related infection. *JBJS, 88(6), 1208-1214.*
9) Pietruski, P., Paskal, W., Paluch, Ł., Paskal, A. M., Nitek, Ż., Włodarski, P., ... & Noszczyk, B. (2021). The impact of N-acetylcysteine on autologous fat graft: first-in-human pilot study. *Aesthetic plastic surgery, 45, 2397-2405.*
10) *Nejatifar, F., Abdollahi, M., Attarchi, M., Roushan, Z. A., Deilami, A. E., Joshan, M., & Kojidi, H. M. (2022). Evaluation of hematological indices among insecticides factory workers. Heliyon, 8(3), e09040*
11) El-Abd, M. A., & Ibrahim, E. A. (2020). Production and one-step purification of serratiopeptidase enzyme from Serratia marcescens with potent anti-inflammatory and antioxidant power. *Egyptian Pharmaceutical Journal, 19(3), 238.*
12) Rath, G., Johal, E. S., & Goyal, A. K. (2011). Development of serratiopeptidase and metronidazole-based alginate microspheres for wound healing. *Artificial Cells, Blood Substitutes, and Biotechnology, 39(1), 44-50*

13) Tiwari, M. (2017). The role of serratiopeptidase in the resolution of inflammation. *Asian journal of pharmaceutical sciences, 12(3),* 209-215

14) Dixit, S., Baganizi, D. R., Sahu, R., Dosunmu, E., Chaudhari, A., Vig, K., ... & Dennis, V. A. (2017). Immunological challenges associated with artificial skin grafts: available solutions and stem cells in future design of synthetic skin. *Journal of biological engineering, 11, 1-23.*

15) Tamimi, Z., Al Habashneh, R., Hamad, I., Al-Ghazawi, M., Roqa'a, A. A., & Kharashgeh, H. (2021). Efficacy of serratiopeptidase after impacted third molar surgery: a randomized controlled clinical trial. *BMC oral health, 21(1), 1-9*