

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— Background Skin grafting is a closure technique used in dermatology most commonly to close wounds created by the removal of skin damaged. Although currently less favored than flap closures, grafting can produce a good cosmetic result.

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 largest organ of the human body, skin plays a pivotal role in maintaining homeostasis as well as protecting the internal organs from the external environment (Dovi JV et al.,2003) . Cutaneous injuries, especially chronic wounds, burns, and skin wound infection, require painstakingly long-term treatment with an immense financial burden to healthcare systems worldwide (Chen W et al.,2003). An aging population coupled with escalating rates of diabetes and obesity continue to increase the prevalence of chronic wounds. It has been estimated that 1–2% of the population in developed countries will experience a chronic wound in their lifetime (Steed DL,2006). N-acetyl cysteine (NAC) is a compound approved by the U.S. Food and Drug Administration for clinical use as a mucolytic which has thiol and carboxyl groups (Minkov VS and Boldyreva EV,2013; Feng T, et al.,2015). NAC may have a broader therapeutic potential, particularly in the setting of antioxidants. serrati peptidase is a proteolytic enzyme prescribed in various specialties like surgery, orthopedics, otorhinolaryngology,

gynecology and dentistry for its anti-inflammatory, anti-endemic and analgesic effects (Uchi H et al.,2009). Some anecdotal reports suggest it to possess anti atherosclerotic effects also, due to its fibrinolytic and caseinolytic properties. serrati peptidase is a proteolytic enzyme derived from the nonpathogenic enterobacteria Serratia E15 (Gluud, S,2015). It is produced in the intestines of silkworms to break down cocoon walls. This enzyme has been used as an alternative to analgesics and nonsteroidal anti-inflammatory agents and also has been used to treat chronic sinusitis and postoperative inflammation (Hesketh M et al.,2017).

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.

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.

Preparation of N-acetyl cysteine (NAC)

After weighing each component for the mixture, a 3% NAC cream was prepared, and then the necessary number of NAC was crushed into tiny pieces and added to water, producing a paste. The targeted amount of this mixture was added into cold cream and mixed until a thorough mixture was achieved.

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 animals of this study were prepared to surgery by fasting

for 12 hours withheld of food and 6 hours of water before the operation, the area of medial tibial region was clipped and shaved and prepared aseptically figure 1. The skin was scrubbed by bovidine – iodine 2.5%. The rabbits were put at a lateral recumbency and covered with surgical drapes which fixed to the skin with towel clips figure 2

Anesthesia

All the operations were performed under general anesthesia by intramuscular administration the combination of Xylazine and Ketamine at the dose (3:35 mg per kg B.W.) with additional dose of Xylazine to increase depth and prolong anesthesia.

Surgical technique

The technique which performed in this experiment was the transplantation of a square – shape of skin with 1cm×1cm in diameter from forelimb transplanted to a gap made in hind limb filled with NAC powder and tetracopeptide powder then the borders of graft sutured with original skin by simple interrupted suturing figure (2-3) covered by gauze pad changed every two days with 5 postoperative days of treatment with penicillin-streptomycin mixture to prevent any type of 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).

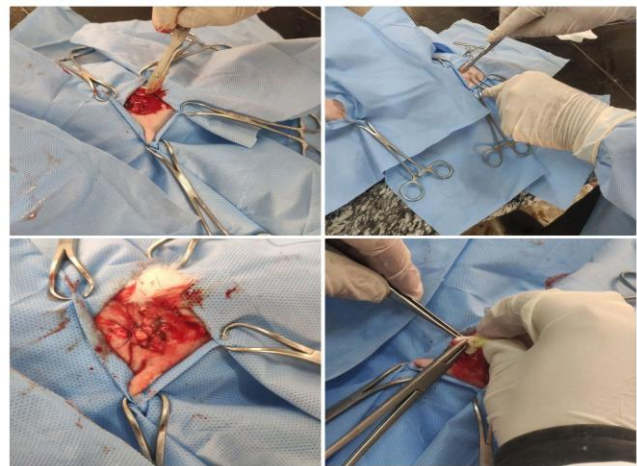


Figure 2: The surgical procedure steps which made during skin grafting



Figure 3: The blood sample collection

III. RESULTS AND DISCUSSION

To determine the effect of N-acetyl cysteine (NAC) on healing of skin grafting in albino male rabbits. In this section the histological, hematological and biochemical analysis for the skin graft of the albino male rabbits after treated with N-acetyl cysteine (NAC).

A. Histological of G3 treated with NAC

The histological dissection showed significant enhancement of skin grafting healing as shown in the figure 4. Based on the figure 4 b) normal healing with normal keratin and epidermis layer as well as hair follicle, blood vessels and muscular layer in comparison with control group, which show normal epidermis and keratin with normal dermis layer.

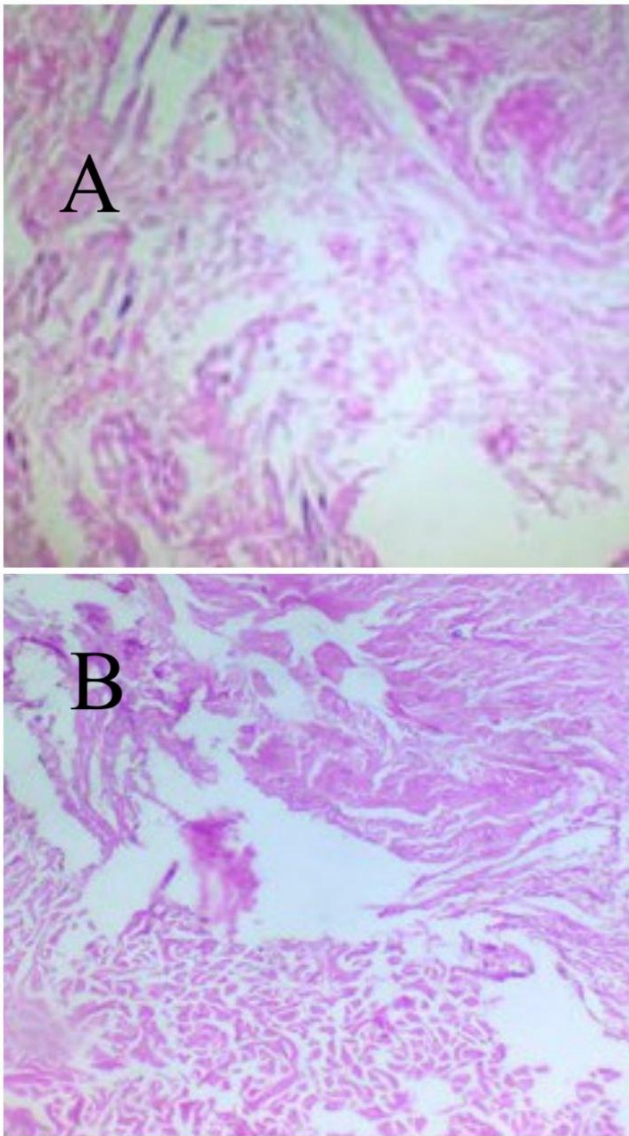


Figure 4: Histological section for skin graft of rabbit with a) without treated and b) treated with NAC

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 (Mecikoglu et al,2006; Pietruski et al.,2021). 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 White Blood Cell (WBC), Red Blood Cell (RBC) and platelets while (PLT) of the albino male rabbits in G1 and G3. As shown in the table 3.1 the WBC, RBC and PLT of the treated rabbits with the NAC were $10.3 \times 10^3 \mu\text{l}$, $3.15 \times 10^6 \mu\text{l}$ and $387 \times 10^3 \mu\text{l}$, respectively, compare with the control animals groups were $6.5 \times 10^3 \mu\text{l}$, $5.21 \times 10^6 \mu\text{l}$ and $315 \times 10^3 \mu\text{l}$. The hematological results showed increase in the WBC and

platelets while the RBC and other hematological parameters were normal in the rabbit which treated by NAC topically, the increase in the WBC and platelets occur due to inflammatory reaction of skin graft.

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

Parameters	WBC x $10^3 \mu\text{l}$	RBC x $10^6 \mu\text{l}$	PLT $10^3 \mu\text{l}$
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 (Tsai et al.,2014), 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 analysis for the G3 have been done by using aspartate aminotransferase (AST). (Table 2) shows the AST analysis of G1 and G3, which were 0.4 mg/ dl and 0.6 mg/ dl, respectively. According to the results that were obtained from this analysis showed mild increase in this enzyme in comparison with control group, this increase in the level in the enzyme maybe occur due to inflammatory reaction of the skin grafting in the NAC treated rabbit, the result of chemical analysis in this group showed the better enhancement than the serratopeptide treated rabbit in comparison with control group, the enhancement effects of NAC due to antioxidant properties of it (Nejatifar et al.,2022).

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 serratopeptidase can be clearly seen in this section due to the

antibacterial effects (Mecikoglu, et al.,2006).as well as antioxidant properties (El-Abd, et al.,2020).

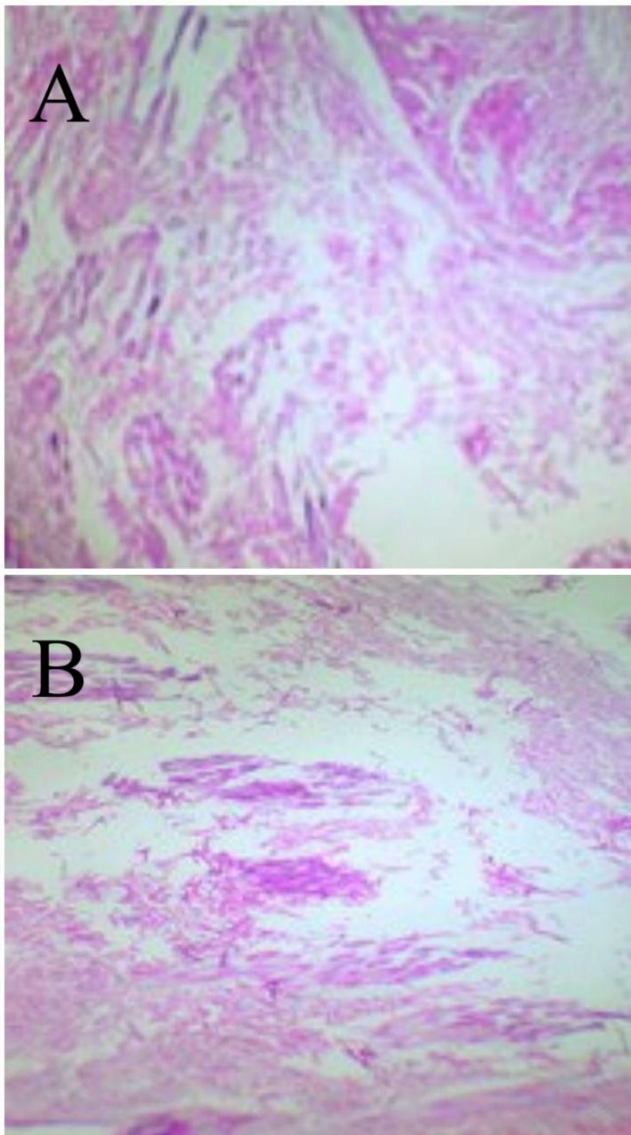


Figure 5: Histological section for skin graft of rabbit a) control group and b) treated group with using serattopeptidase

Previous studies were reported to study the histological of the skin of the animals after treated with the serattopeptidase for the grafting purpose. (Rath et al., 2011), reported to enhancement of the wound healing by using serattopeptidase their results the wounds had partial epithelization, inflammatory cell predominance with few fibroblasts, 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

In this part the hematological analysis was done for the G2 of the rabbits after treated with the Serrato peptidase enzyme. The blood samples taken from the rabbits at different times of experiment and compare with other groups. Table 3, shows the levels of WBC, RBC, and PLT for the G1 and G2 of the rabbits. The results of the G2 showed $11.5 \times 10^3 \mu\text{l}$, $5.07 \times 10^6 \mu\text{l}$

and $405 \times 10^3 \mu\text{l}$ for WBC, RBC, and PLT, respectively, while the control group showed 6.5×10^3 , $5.21 \times 10^6 \mu\text{l}$ and $315 \times 10^3 \mu\text{l}$, for WBC, RBC, and PLT, respectively. The hematological analysis for the serratopeptidase treated rabbits showed mild increase in the WBC count due to inflammatory reactions of skin graft while the other hematological parameters were normal in comparison with control group, the enhancement effects of serattopeptide on these parameters due to anti-inflammatory effects of serattopeptide (Tiwari, M., 2017). The results showed significant increase in the WBC, RBC and PLT due to inflammatory reaction of the skin graft (Dixit, S., et al,2017).

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

Parameters	WBC x $10^3 \mu\text{l}$	RBC x $10^6 \mu\text{l}$	PLT $10^3 \mu\text{l}$
Control	6.5	5.21	315
Serrato peptidase	11.5	5.07	405

Based on the results that have been obtained from the hematological study the level of the WBC and PLT of the G2 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 (Nejatifar, F., et al.,2022), however, our study includes to injured the animals of the G2 before treated with the NAC that gave the reason to the WBC and PLT increased.

Biochemical analysis of G2 treated with serrati peptidase

Table 4 shows the biochemical analysis for the aspartate aminotransferase (AST) for the animals which treated by serratopeptidase, which showed moderate increase in this enzyme in comparison with control group, this increase in this enzyme occur due to inflammatory reaction of the skin grafting in the treated rabbit, the result of chemical analysis in this group showed the enhancement lower than the NAC treated rabbit in comparison with control group, the enhancement effects of serattopeptidase due to antioxidant properties of it (Tamimi, Z ,et al. 2017)

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, which gave indicted to use NAC in skin grafting is better than serrati peptidase based on the results that were obtained in the present study. The hematological study of the G1, G2 and G3 were determine according to three parameters as mentioned in previously parts which included WBC, RBC and PLT as it shows clearly in table 3.5. WBC of the G1 was $6.5 \times 10^3 \mu\text{l}$ which normal level, WBC were increased in the G2 and G3 (11.5 and 10.3), the levels of WBC in the rabbits that were treated with serrati peptidase is more than the NAC treated group, and since this increase was due to inflammation, we conclude that the G3 was better than G2 in the progress of the grafting, which gave indicated to that the use of the NAC can be more effective that serrati peptidase in the Hematological. Moreover, the level of PLT in G2 was more that G3 which also gave another indicted to be G2 more inflammation than G3, due to same reason that were mentioned above.

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

Parameters	WBC $\times 10^3 \mu\text{l}$	RBC $\times 10^6 \mu\text{l}$	PLT $10^3 \mu\text{l}$
Control	6.5	5.21	315
Serrato peptidase	11.5	5.07	405
NAC	10.3	3.15	387

This part includes to compare the AST of G1, G2 and G3. Table 3.6 show the AST level of all groups. AST level was increased from 0.4 mg/ dl in the group without treated and reached to 0.9 mg/ dl in the treated group with serrati peptidase, while the treated group with NAC was 0.6 mg/ dl, the increase in the AST level due to inflammatory reaction and the greater the inflammation, the higher the level. That gave indicate to use of NAC the better enhancement than the serrati peptidase

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

The present study has been conducted to determine the effect of NAC, and Serrato peptidase on the skin grafting of the albino male rabbits. The results were divided into three parts according to Histological, hematological and Chemical analysis. The

conclusions from these results show that the NAC showed good enhancement effects on the skin graft, and showed better enhancement effects on the hematology, histology and AST parameters, while the Serrato peptidase show good enhancement effects on the hematology, histology and AST parameters but less than NAC. Our 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.

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