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Research Article

Assessment of Blood Response to Serratiopeptidase and Chymotrypsin Therapy on Facial Skin Model of Hyaluronic Acid Injection in Rabbits

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Abstract

Aims: The present study aimed to investigate the impact of Serratio peptidase and Chymotrypsin therapy on blood responses following the injection of Hyaluronic Acid in a facial skin model of rabbits. This research is motivated by the growing utilization of Hyaluronic Acid in facial aesthetics and the need to evaluate potential interventions to mitigate any adverse effects on blood parameters.

Materials and Methods: A total of 35 rabbits were used in the experiment. The negative control group (no treatment), positive control (Hyaluronic Acid injection), Serratiopeptidase, and Chymotrypsin groups (serratiopeptidase and chymotrypsin therapy). Blood samples were collected at predetermined intervals for analysis. The blood serum was analyzed 24 hours and 72 hours after the injection. The primary blood parameters analyzed included Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Platelet Count, and C-Reactive Protein (CRP) levels.

Results indicated that both treatments led to anti-inflammatory responses following the HA-induced inflammation.

Conclusion: Chymotrypsin elicited a more pronounced body response to induced inflammation compared to serratiopeptidase, and appears to be better suited for cosmetic applications. However, further research evaluating such responses after more than 72 hours is needed to obtain conclusive results.

Keywords: Chymotrypsin, Dermal Filler, Hyaluronic Acid, Rabbits, Serratiopeptidase

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INTRODUCTION

Hyaluronic acid (HA) is an essential glycosaminoglycan that resides in bodily fluids and the extracellular matrix of tissues. It maintains tissue hydration and regulates key cellular processes, including cell proliferation, differentiation, and the modulation of inflammatory responses (1).

Over the past two decades, there has been an increased surge in interest surrounding HA, mainly due to the demographic shift toward an aging population, an escalating demand for anti-aging solutions, and the rise of age-related diseases. Additionally, the current preference for non-invasive medical techniques, the ever-increasing adoption of aesthetic procedures, and the relentless pursuit of rapid, natural-looking results all contribute to the expanding research and market for HA in the foreseeable future (2).

Once the HA filler is injected into the dermal skin, smaller HA polymers signify distress and serve as potent inducers of inflammation. HA activates inflammatory cells, initiates innate responses to injuries, and regulates the behavior of epithelial cells and fibroblasts (3). Research has attempted to address this inflammation through chemical-based anti-inflammatory drugs. However, recently, research has started focusing on biological molecules, particularly enzymes, as promising alternatives. These enzyme-based therapeutics are able to precisely target specific substrates and efficiently convert them (4).

For instance, alpha-chymotrypsin is one such enzyme that is sourced from bovine pancreases. It activates plasminogen and collagenase, illustrating its potential to mitigate inflammation, as it is utilized to remove necrotic tissue in skin and corneal wounds, thereby preventing the formation of inflammatory edema and hematoma (5). In the context of HA-based procedures, chymotrypsin therapy could reduce the likelihood of adverse reactions and optimize tissue remodeling in response to HA, making it an intriguing area of research.

Serratiopeptidase is another therapeutic protein. It is found naturally in Serratia marcescens, and is a proteolytic enzyme with anti-inflammatory, anti-edema, and analgesic properties. These characteristics allow for utilizing serratiopeptidase for the treatment of various human diseases, such as arthritis and bronchitis (6).

This study aims to investigate the anti-inflammatory potential of Chymotrypsin and Serratiopeptidase therapy, following the injection of HA filler. Through an in-depth examination of these enzymatic interventions within a facial skin model in rabbits, we aim to unlock insights into their benefits, mechanisms, and implications to enhance the outcomes of HA-based cosmetic procedures.

MATERIALS AND METHODS

Ethical Approval

This research was conducted in compliance with ethical standards and received approval from the Research Ethics Committee and Scientific Committee of the Department of Dental Basic Science, College of Dentistry, University of Mosul, under the reference number **UOM. Dent/A.L.23/15**, dated 05.02.2023.

Animal Housing and Pre-Experiment Procedures

Local, mature rabbits with an average body weight of 1.5 ± 0.5 kg were included in the study. The rabbits were permanently housed indoors, adhering to international, national, and institutional guidelines for animal care and usage. Prior to the initiation of the study, a seven-day adaptation period was observed under standard animal housing conditions, with a constant room temperature maintained at 25 ± 2°C. The rabbits were provided with unrestricted access to a standard diet and water (7).

Drug Administration

Based on the age and body weight of the rabbits, each rabbit in the study received a 0.5cc HA filler injection. The soft filler used in the study was sourced from PROMOITALIA. The rabbits were randomly assigned to one of the four main groups:

1. Group I: This is the control negative group, where a total of 5 rabbits served as the control, providing baseline measurements without any filler injection or treatment, encompassing blood and tissue components.
2. Group II: This is the control positive group, where a total of 10 animals were topically injected with a 0.5 ml HA on the first day of the study period. The rabbits were then divided into two subgroups:

- Subgroup IIa: 5 animals were sacrificed 24 hours post-treatment.
- Subgroup IIb: 5 animals were sacrificed 72 hours post-treatment.

3. Group III: This is the group where 10 animals were treated with Serratiopeptidase after receiving a 0.5 ml HA injection. Serratiopeptidase was administered orally at a dose of 5 mg/kg once daily, mixed with sterile water (3 ml/kg), beginning from the first day of the study period. This group was further divided into two subgroups:

- Subgroup IIIa: 5 animals were sacrificed 24 hours post-treatment.
- Subgroup IIIb: 5 animals were sacrificed 72 hours post-treatment.

4. Group IV: This is the group where 10 animals were treated with chymotrypsin after receiving a 0.5 ml HA injection. Chymotrypsin was injected intramuscularly at a dose of 8.1 units/kg, two hours after the HA injection, on the first day of the study period. This group was also subdivided into two subgroups:

- Subgroup IVa: 5 animals were sacrificed 24 hours post-treatment.
- Subgroup IVb: 5 animals were sacrificed 72 hours post-treatment.

Biochemical Assessments

Blood Sample Collection

For biochemical analysis, two tubes of fresh blood were collected from the jugular vein of each rabbit at the time of sacrifice. One tube was used for a complete blood picture test, while the other was centrifuged at 3000 rpm for 15 minutes to obtain serum. The separated serum was collected using a micropipette and transferred to Eppendorf tubes, which were then stored at -20°C. The primary blood parameters analyzed in this study included Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Platelet Count, and C-Reactive Protein (CRP) levels.

Statistical Analysis

Data were presented as mean values with standard deviations (SD). A significance level of less than 0.05 was considered statistically significant in all conducted tests using the SPSS program. Statistical methods employed included an independent two-sample t-test to compare treatment and control groups, a two-sample t-test to compare within each group, post hoc Waller Duncan's test, and analysis of variances (ANOVA) to detect differences in various parameters among the study groups. A correlation test was also used to examine relationships between parameters within each study group.

RESULTS

Results after the first 24 hours

The ANOVA test showed a highly significant difference among all study groups in serum WBC, RBC, platelets, and CRP, as shown in Table (1). The changes in the levels of these components during the first 24 hours are shown in Figures 1, 2, 3, and4.

Table (1): Comparison of serum of White Blood Cell (WBC), Red Blood Cell (RBC), Platelet, and C-Reactive Protein (CRP) among study groups at the end of 24 hours

ANOVA						
Blood Sa	**S.O.V.**	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
WBC	Between Groups	12.688	3	4.229	5.493	0.009
	Within Groups	12.320	16	0.770		
	Total	30.618	19			
RBC	Between Groups	13.907	3	4.636	3.062	0.058
	Within Groups	24.220	16	1.514		
	Total	38.128	19			
Platelet	Between Groups	371684.950	3	123894.983	232.536	0.000
	Within Groups	8524.800	16	532.800		
	Total	380209.750	19			
CRP	Between Groups	401.068	3	133.689	546.787	0.00
	Within Groups	3.912	16	0.244		
	Total	404.980	19			

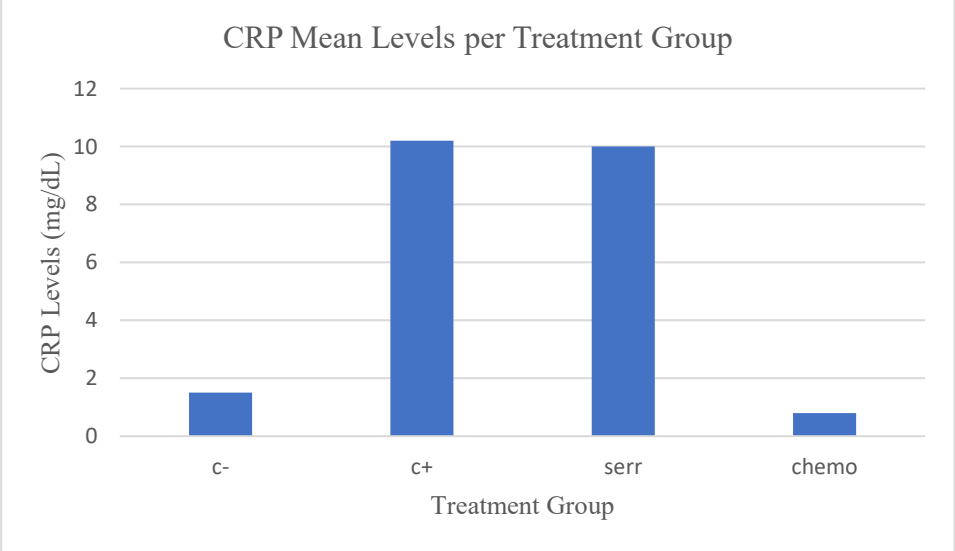

Figure (1): Comparison between CRP mean during the first 24h in all groups

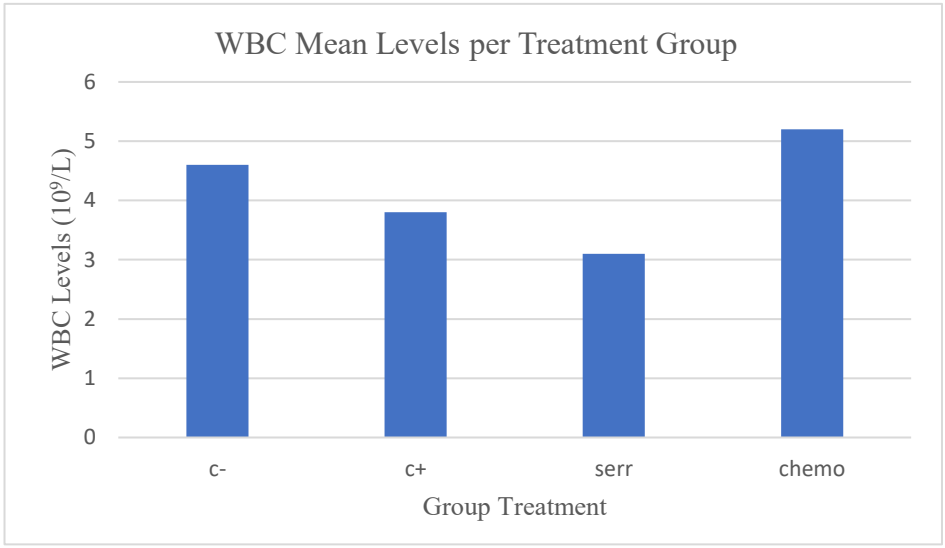

Figure (2): Comparison between WBC during the first 24h in all groups.

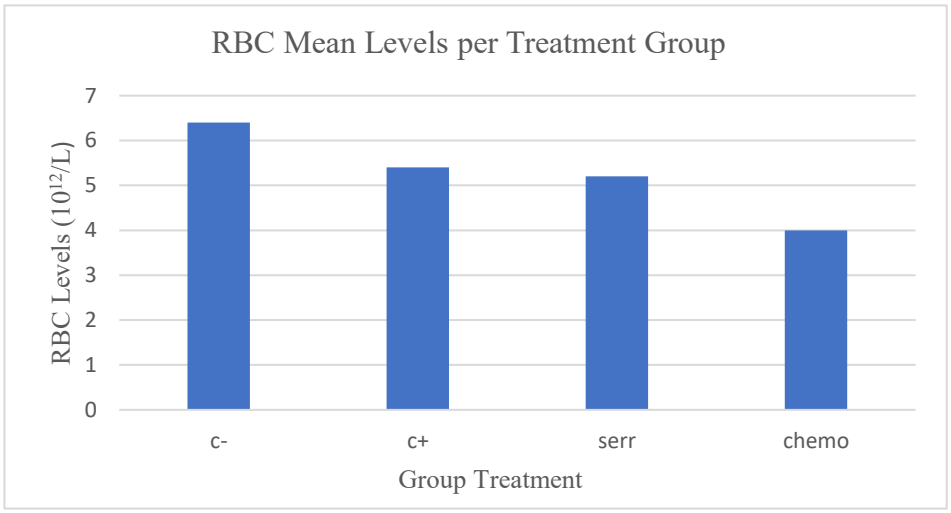

Figure (3): Comparison between RBC during first 24h in all groups.

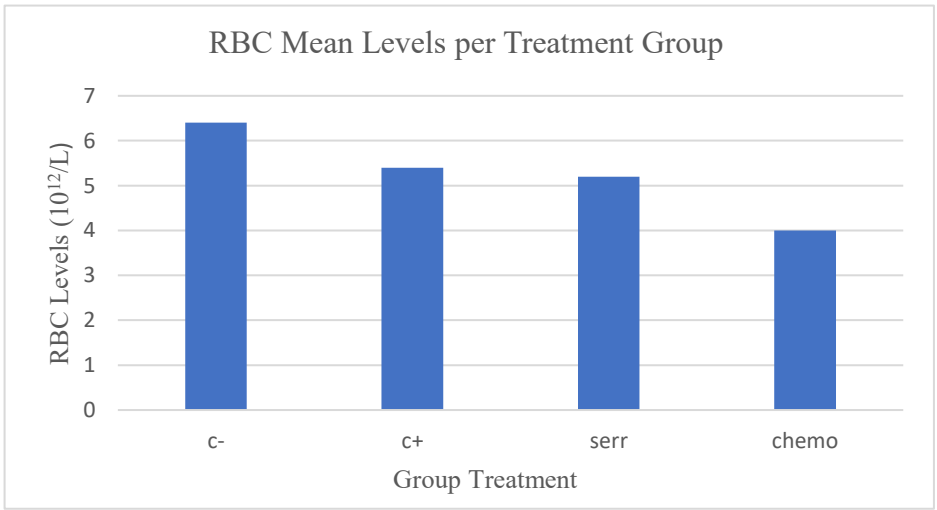

Figure (4): Comparison between Platelet levels during the first 24h in all groups

Results after the first 72 hours

As shown in Table 2, the ANOVA test showed a significant difference among the groups for WBC, RBC, and CRP levels. On the other hand, the changes in platelet levels were not significant. The mean levels for all groups are shown in Figures 5,6,7, and 8.

Table (2): Comparison of serum WBC, RBC, platelets, and CRP among study groups at the end of the first 72 hours

ANOVA						
Blood Sample	**S.O.V.**	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
WBC	Between Groups	12.354	3	4.118	6.690	0.004
	Within Groups	9.848	16	0.615		
	Total	22.202	19			
RBC	Between Groups	3.013	3	1.004	8.159	0.002
	Within Groups	1.970	16	0.123		
	Total	4.983	19			
Platelet	Between Groups	143039.350	3	47679.783	1.013	0.413
	Within Groups	752971.200	16	47060.700		
	Total	896010.550	19			
CRP	Between Groups	12.688	3	4.229	5.493	0.009
	Within Groups	12.320	16	0.770		
	Total	25.008	19			

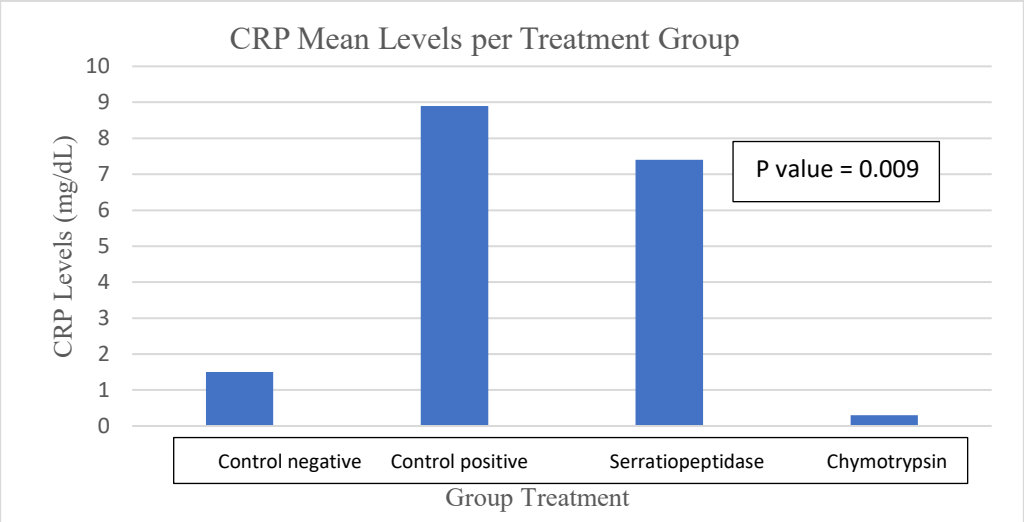

Figure (5): Comparison between CRP mean during the first 72h in all groups.

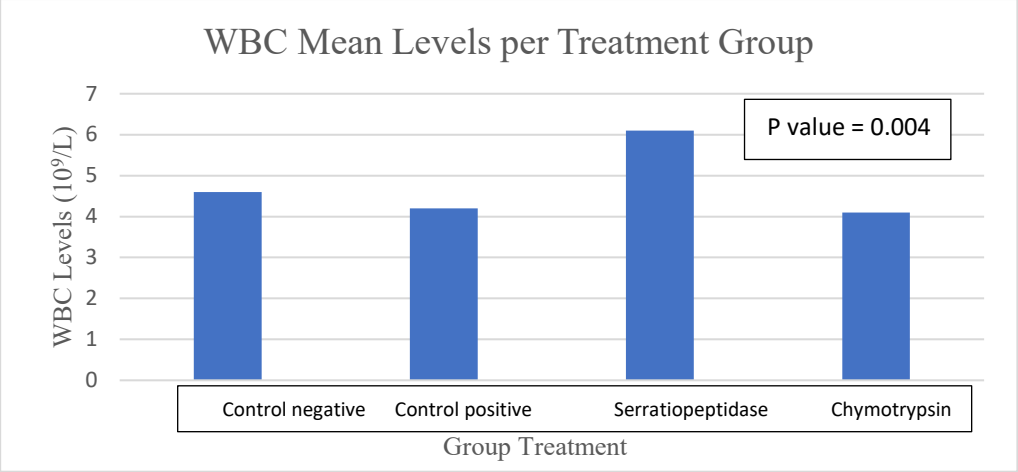

Figure (6): Comparison between WBC during the first 72h in all groups

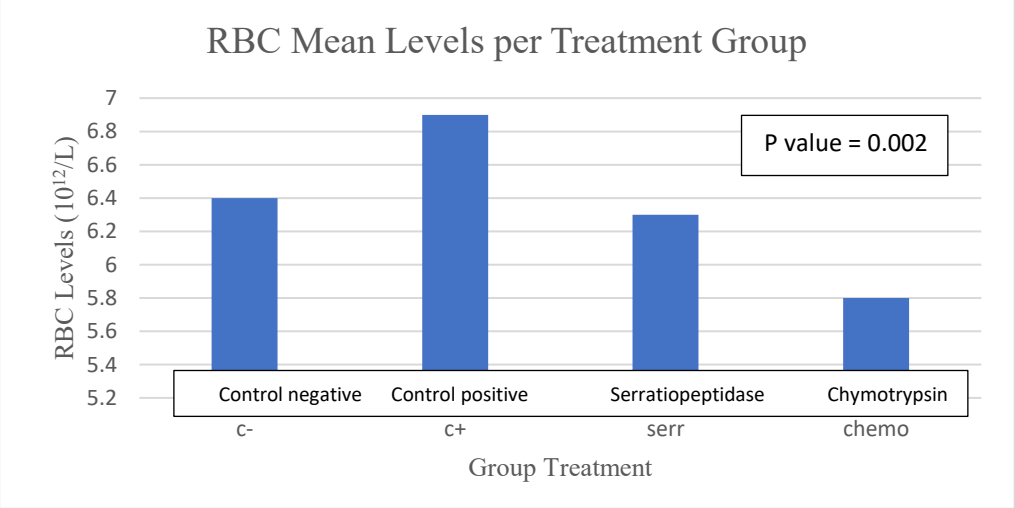

Figure (7): Comparison between RBC during the first 72h in all groups

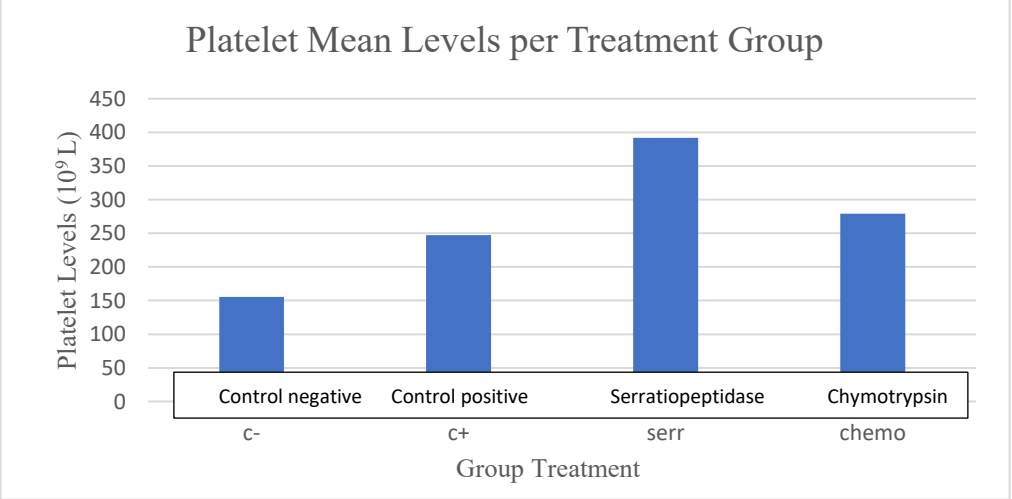

Figure (8): Comparison between Platelet levels during the first 72h in all groups

DISCUSSION

After the injection of HA filler in the control positive, a significant increase in the CRP levels was observed after 24 and 72 hours. CRP levels increase with the level of inflammation (8,9). Therefore, this suggests that HA had induced inflammation in the rabbit. The levels of CRP decreased significantly after 72 hours of treatment with Serratiopeptidase. Similarly, the chymotrypsin treatment significantly decreased the levels of CRP after 24 and 72 hours, as compared to the control positive group. This decrease was significantly lower than that following Serratiopeptidase treatment.

After the first 24 hours of chymotrypsin treatment, there was a significant increase in WBC levels. This initial increase might indicate that chymotrypsin treatment triggered an immune response, as an increase in WBC levels can be a sign of the body responding to some form of injury, infection, or inflammation (10). However, after 72 hours, the study found that WBC levels significantly decreased. This decrease suggests that the initial immune response triggered by chymotrypsin may have been short-lived, and the body's immune system returned to a state of equilibrium. Alternatively, chymotrypsin might have a suppressive effect on the immune system over time, leading to decreased WBC levels. On the other hand, Serratiopeptidase is an enzyme with known anti-inflammatory and anti-edematous (swelling-reducing) properties [6]. After the first 24 hours of Serratiopeptidase treatment, there was a significant decrease in WBC levels. This initial decrease might indicate that Serratiopeptidase treatment initially had a suppressive effect on the immune system. It could have been reducing inflammation or immune activity, resulting in lower WBC levels. However, after 72 hours, the study found that WBC levels significantly increased. This suggests that the initial suppressive effect of Serratiopeptidase on the immune system might have been temporary, and the immune system rebounded, leading to increased WBC levels. It is possible that the enzyme's anti-inflammatory properties, initially reducing WBCs, gave way to a subsequent immune response.

Red blood cells (RBC) are responsible for transporting oxygen from the lungs to various tissues in the body and returning carbon dioxide to the lungs for exhalation.

RBC levels are usually stable in healthy individuals and are closely regulated by the body (11). However, when an injury or inflammatory stimulus occurs (such as the introduction of hyaluronic acid filler), the body responds by releasing pro-inflammatory cytokines and chemokines (12). Some of these pro-inflammatory molecules can affect erythropoiesis, the process by which RBCs are produced in the bone marrow. Thus, inflammation can disrupt the balance between RBC production and RBC clearance or destruction by reducing the responsiveness of the bone marrow to erythropoietin, a hormone that stimulates RBC production (13). This can lead to a temporary decrease in RBC levels as new RBCs are not being produced as rapidly (11,13).

This could explain the initial decrease in RBC levels after the first 24 hours. However, after the initial inflammatory stimulus or injury is controlled, the body enters the resolution phase, characterized by anti-inflammatory responses (14). This explains the increase after the first 72 hours. Since Serratiopeptidase and chymotrypsin are anti-inflammatory, they both lead to an increase after 72 hours, as compared to the first 24 hours.

Platelets are small blood cells that play a crucial role in the blood clotting process (15). When they encounter an injury, they become activated. This activation causes them to release various bioactive molecules, including growth factors like platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-beta). The growth factors released by activated platelets contribute to wound healing and tissue repair. They stimulate cell proliferation, collagen production, and other processes necessary for tissue regeneration and recovery (15,16). After the first 24 hours, platelet levels increased in the positive control group compared to the control group. As the changes are temporary, these levels decreased after the first 72 hours. Serratiopeptidase treatment resulted in a significant decrease in platelet levels after the first 24 hours. This decrease might be attributed to its anti-inflammatory properties, which could temporarily suppress the platelet response to injury or inflammation. However, the platelet levels then increased after the first 72 hours. This could be due

to a delayed inflammatory response or a rebound effect following the initial decrease. In contrast, chymotrypsin treatment first increased platelet levels during the first 24 hours, which then significantly decreased during the first 72 hours. This immediate increase may be related to the action of the enzyme or an acute response to the treatment. The following decrease in platelet levels could potentially be due to the resolution of the initial injury or inflammation.

CONCLUSIONS

Within the limitations of the current study, it is possible to conclude that:

Injection of HA-filler induced inflammation in the rabbits. Both treatments of Serratiopeptidase and Chymotrypsin result in anti-inflammatory responses, thereby changing the mean levels of the components of the blood serum: RBCs, WBCs, platelets, and CRPs. Based on the results of this study, we conclude that treatment with chymotrypsin led to a better body response to the induced inflammation, as compared to serratiopeptidase. Further studies should be conducted to evaluate the anti-inflammatory response of the two treatments for longer periods of time, that is, more than 72 hours, to observe the changes in the blood components.

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Authors' Contribution

Conceptualization: Al-Najjar AZ and Al-Mashhadane FA. Formal analysis: Al-Najjar AZ and Al-Mashhadane FA. Funding acquisition: Al-Najjar AZ and Al-Mashhadane FA. Investigation: Al-Najjar AZ and Al-Mashhadane FA. Methodology: Al-Najjar AZ and Al-Mashhadane FA. Project administration: Al-Mashhadane FA. Resources: Al-Najjar AZ and Al-Mashhadane FA. Software: Al-Najjar AZ. Supervision: Al-Mashhadane FA. Validation: Al-Mashhadane FA. Visualization: Al-Najjar AZ and Al-Mashhadane FA. Writing–original draft: Al-Najjar AZ and Al-Mashhadane FA. Writing–review editing: Al-Mashhadane FA. All authors have read and approved the final manuscript.

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Ethical statement: This research was conducted in compliance with ethical standards and received approval from the Research Ethics Committee and Scientific Committee of the Department of Dental Basic Science, College of Dentistry, University of Mosul, under the reference number **UOM. Dent/A.L.23/15**.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declaration of Generative AI and AI-assisted technologies

During the preparation of this work, the authors did not use AI tools. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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تقييم اســـتجابة الدم لعلاج الســـيراتيوبيبتيداز والكيموتريبســـين على نموذج جلد الوجه لحقن حامض الهيالورونيك في الأرانب

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الملخص

الأهداف: تهدف الدراســة الحالية إلى دراســة تأثير العلاج بالسـيراتيوببتيداز والكيموتريبسـين على اسـتجابات الدم بعد حقن حمض الهيالورونيك في نموذج جلد الوجه للأرانب. الدافع وراء هذا البحث هو الاســتخدام المتزايد لحمض الهيالورونيك في جماليات الوجه والحاجة إلى تقييم التدخلات المحتملة للتخفيف من أي آثار ضـــارة على معايير الدم. **المواد وطرق العمل**: تم اســتخدام ما مجموعه 35 أرنبًا في التجربة. مجموعة المراقبة الســلبية (بدون علاج)، الســيطرة الإيجابية (حقن حمض الهيالورونيك)، مجموعات السيراتيوببتيداز، والكيموتريبسين (العلاج بالسيراتيوببتيداز، والكيموتربسين). تم جمع عينات الدم على فترات زمنية محددة لتحليلها. تم تحليل مصـل الدم بعد 24 سـاعة و 72 سـاعة من الحقن. شـملت بارامترات الدم الأولية التي تم تحليلها عدد خلايا الدم الحمراء (RBC)، وعدد خلايا الدم البيضــاء (WBC)، وعدد الصــفائح الدموية، ومسـتويات البروتين التفاعلي (CRP). **النتائج**: أشـارت النتائج إلى أن كلا العلاجين أدى إلى استجابات مضـادة للالتهابات بعد الالتهاب الناجم عن HA. **الاســـتنتاجات**: أثار الكيموتريبســـين اســـتجابةً جســـديةً أكثر وضـــوحًا للالتهاب المُســـتحث مقارنةً بالسـيراتيوببتيداز، ويبدو أنه أنسـب للاسـتخدامات التجميلية. مع ذلك، يلزم إجراء المزيد من الأبحاث لتقييم هذه الاسـتجابات بعد أكثر من 72 ساعة للوصول إلى نتائج قاطعة.

الكلمات المفتاحية: كيموتريبسين، حشو جلدي، حمض الهيالورونيك، أرانب، سيراتيو بيبتيداز