RESEARCH ARTICLE

Evaluation of Anti-inflammatory Effect of Topical Serratiopeptidase in Mice

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ABSTRACT

Proteolysis enzyme therapy plays a key role in treating a wide range of nutritional and genetic diseases. Serratiopeptidase is a proteolytic enzyme with pain-relieving and anti-inflammatory effects. It is applied topically to guarantee that the enzyme reaches the therapeutic activity.

A total of fifteen healthy adult male white mice were used in this study. They were divided into three groups. All mice have injected a formalin to induce edema in the mouse paw. The first group was left untreated control; the second and third groups were applied 1 and 2 percent serratiopeptidase ointment, respectively, topically to the mouse's right-hand paw.

Results: By analyzing Stage 1 licking time (pain reaction time) at 1 and 2%, the proportion of edema production and inhibition in the serratiopeptidase groups significantly differed from the appearance of the control group. The ointment made a substantial difference in serratiopeptidase concentrations (22.66 ± 1.45), (20.3 ± 2.6) having been applied at various quantities in when compared to just the ointment (61.66 9.93) seconds. While the difference between different Serratiopeptidase ointment concentrations, in the first stage, the percentage of inhibition at the time of licking was (63.25%) and (67.07%) in Serratopeptidase ointment (1.2%), respectively. In contrast, in stage 2, the percentage of inhibition at the time of licking was (37.24 %)(72.04%) when using a 2 % concentration.

Conclusion: Topical use of serratiopeptidase ointment was found to be effective. It contains anti-inflammatory properties as well as modest analgesic properties.

Keywords: Enzyme therapy, Mice, Proteolysis, Serratiopeptidase.

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INTRODUCTION

Inflammation is a pathological process in which the body interacts with infected tissues. Pain, swelling, redness, heat, and loss of function are clinical manifestations of inflammation.[1] Inflammation occurs when the immune system activates to fight pathogens or toxins in the body.[2] Damaged tissues heal by enhancing cellular events and biological processes to restore tissues to normal.[3] Serratiopeptidase (SRP) is a proteolytic enzyme generated from a bacterium called "enterobacilli" that lives in the silkworm's gut wall.[4] SRP is the best enzyme for tissue debridement since it possesses anti-inflammatory and fibrinolytic properties and operates in a wide range of tissues.[5] It is extensively used in numerous medical disciplines as an anti-inflammatory, analgesic, and as health supplement to prevent heart and blood vessel damage.[6] SRP is taken as a tablet. Enzyme delivery methods using stomach acid "topical preparation" were utilized to increase local effects and lessen systemic adverse effects when used frequently in order to increase efficacy and reduce negative effects.[7]

Topical administration of SRP, which has been demonstrated to have superior anti-inflammatory effects than topical NSAIDs, may effectively decrease inflammatory indicate.[8] SRP is beneficial in lowering postoperative stridor in a few studies.

It has stronger anti-inflammatory effects than NSAIDs in the third molar and can be used instead of corticosteroids to reduce inflammation in cases where corticosteroid therapy is inappropriate.[9] Anti-inflammatories in SRP effects as a topical preparation in decreasing edema in mice was the goal of this investigation.

MATERIALS AND METHODS

Animals

Use fifteen healthy white male mice weighing 25–30 grams each. Animals were held in a plastic cage at 23. 2°C in the

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laboratory of the College of Dentistry, the University of Mosul in Iraq, following a 12 h light/12 h dark cycle with a balanced diet.

Preparation of Serratiopeptidase Ointment

Serratiopeptidase (SRP) (pure powder, base, and additives) are the raw materials used. The research was obtained from commercial sources with a particular analytical grade. Prepare a 1% concentrate, a 2% concentrate by adding 1g, and a total active component of 2g. To ensure homogeneity and contact uniformity, 100 g of the preparation were mixed separately. They were using fusion methods by mixing the desired amount of active ingredients in two steps of soil preparation and the liquid phase, with base and additives (PEG 4000, 6000, Tween 80, propylene glycol, and distilled water). Refrigerate until ready to use at 4°C.[10]

Formalin Inflammation and Discomfort Pain

Mice were divided into three groups at random. There are five animals in each group. The formalin assay was carried out using Tjlsen et al. procedure. Edema in both aggregates on the mouse's right paw was induced in the mouse claw. A syringe containing a 30-gauge anesthetic needle and 25 µl of 1 percent formalin was used to inject all animals into the surface of the right posterior paw. Only topical ointment without Serratiopeptidase was applied immediately after formalin injection in the control group, and Serratiopeptidase ointment (1, and 2%) was applied in groups 2 and 3,[11]: group 1 was utilized as a guide. It was placed in a glass cylinder (20 cm wide, 25 cm long) with a 45-degree angle mirror beneath it to allow unobstructed observation of the animals' claws. The entire licking time was 3 seconds, and the injected paw's bite response was measured at intervals of (0–5) minutes (pain phase) and (10–30) minutes. The proportion of pain inhibition and the data on the inflammatory response of mice were used to calculate the inflammatory phase as a function of pain and inflammation.[12,13]

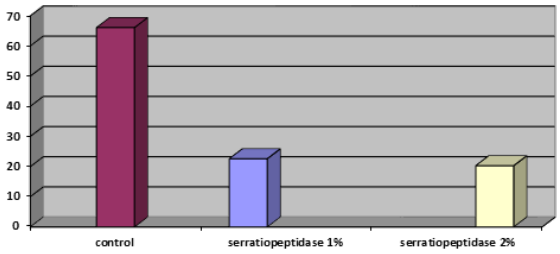

Figure 1: The licking time in stage 1 in mice

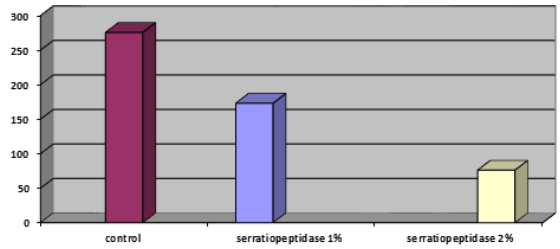

Figure 2: In mice, the licking time in stage 2

Decrease in claw licking time = T0-Tt \ T0 x 100

Tt: refers to the test group's licking and biting time (second)

T0= is the control group's average licking time (second)

The injection of 25 µl of 1% formalin (in 0.9 percent normal saline solution) into the sub-planar tissues of mice causes paw edema. After formalin injection, wait 1,10,30,60 minutes.

The percentage of edema inhibition was then estimated using the formula below:

Percentage of inhibition = ((Vt -V0) / V0) x 100

Vt = volume of the animal's paw after injection

V0 = volume of the animal's paw before injection

V0 is the volume of the animal claws prior to injection.

Analytical Statistics

The results were provided as mean and SD, and the data were analyzed using ANOVA. Duncan's multi-domain test was used to compare the mean of the three sample sets at a probability level less of than 0.05.

RESULTS

The anti-inflammatory activity of serratiopeptidase ointment was tested in mice after formalin was administered into the right-hand paw. In comparison to the ointment-only control group, topical use of 12.2% Serratiopeptidase ointment had good anti-inflammatory benefits.

Assess stage 1 licking time (pain reaction time) at 1% and 2% of the total time. The ointment with serratiopeptidase showed a substantial difference following treatment at various doses, respectively,

There was no significant difference between the ointment alone (61.66 9.93) sec and the ointment plus ointment (61.66 9.93) sec.

Serratiopeptidase ointment comes in a variety of concentrations (Figure 1).

Serratiopeptidase ointment reduced licking duration in the second stage by increasing the dose (1, 2) percent to (173 8.08) (76.04 13.0) seconds respectively, in the second stage (275.66 21.3) sec (Figure 2).

The number of claw licking in the initial stage was shown to be much lower in the current investigation. About the control (35. 8.6) and varied Serratiopeptidase ointment concentrations (Figure 3) shows (22.66 1.45) and (6.34), respectively.

The application of Serratiopeptidase ointment (1, 2) percent was proven effective in the second stage.

As concentration rises, significantly lower the amount of paws licking.

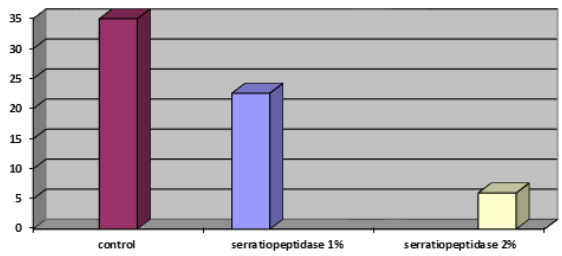

Figure 3: In mice, the number of licking paws in stage 1

In comparison to the control group who did not use the Serratiopeptidase ointment, (29.0 0.0 (16.66 1.7) (Figure 4) Alone (81 10.2)

In the first stage, the percentage of licking time inhibition was measured (63.25 percent). In stage 1, Serratiopeptidase percent 1 and 2 percents were 67.07% and 2%, respectively, whereas in stage 2 they were 67.07% and 2%, respectively. The percentage inhibition of licking time (37.24%) and (72.04) percent, respectively, was discovered.Concentration 1% and 2% (Table 1).

In the current investigation, we discovered that using Serratiopeptidase ointment lowers inflammation.

The impact of formalin in inducing edema in the paw according to ointment dose 1 and 2% vs. The control group received simply ointment treatment. After that, there was a noticeable drop in claw thickness.

In comparison to the control group (3.80 0.25), one hour after ointment application in 1, 2% (2.24 0.21), (2.19 0.20). (Table 2).

The proportion of inhibition in the paw edema was 21.8, 56.25, and 59.37%, respectively.

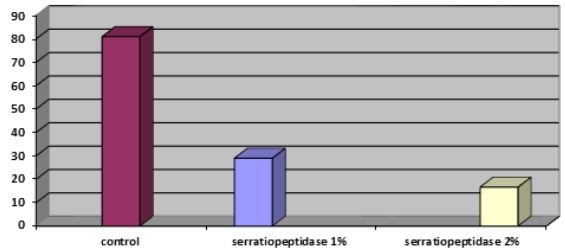

Figure 4: Shows the number of licking paws in mice during phase 2.

After (1,10,30,60) minutes, Serratiopeptidase ointment was reduced by 62.5 percent by 1%, whereas the percentage of inhibition in foot edema was found to be 2% in Serratiopeptidase ointment.

After (1,10,30,60) minutes in a row, 34.42, 31.1, 32.7, 59% (Table 3).

DISCUSSION

Direct use of drugs at the skin has a number of advantages, including easy access to the site of action in the skin.

The site of action is topical, ensuring long-term treatment with adequate and high drug penetration at the application site.[14] The current investigation found that using Serratiopeptidase ointment (1, 2%) topically increases the rate of inhibition of foot edema.

SRP has been demonstrated to lower swelling and inflammation, and these findings back up that claim.

SRP is a proteolytic enzyme that has been shown in other studies to be a good choice for fighting inflammation of infections.[15,16] The primary "COX-I and COX-II" enzymes have a stronger effect when serine protease is used. It's one of the substances linked to inflammatory mediators like interleukins-1, prostaglandins, and cytokines. Thromboxane; serratiopeptidase's molecular mechanism of action is unknown.

It's quite particular, but it's been shown to liquefy dead and injured tissue without harming it. Living tissue is damaged. Formalin can induce inflammation and pain.

The activation of inflammatory mediators causes nerve terminals to release a vasodilating substance and increases the permeability of capillaries (histamine, bradykinin, and serotonin.[18] According to certain research, Serratiopeptidase lowers the permeability of capillaries. In addition to making

Table 1: After using Serratiopeptidase ointment, the proportion of inhibition in stage 1 and stage 2 licking time was 1, and 2%.

	Stage 1		*Stage 2*	
Group treatment	*Licking time (second)*	*Inhibition%*	*Licking time (second)*	*Inhibition%*
control	61.66	-	275.00	-
Serratiopeptidase ointment 1%	23.00	63.00	173.0	37.20
Serratiopeptidase ointment 2%	20.0	68.00	76.00	72.00

Table 2: Paw volume (mm) following application of Serratiopeptidase ointment (1,2%).

Treatment group	*0 min*	*1 min*	*10 min*	*30min*	*60 min*
Control	1.58 ± 0.00	3.19 ± 0.00 AE	3.3 ± 0.00 AE	3.44 ± 0.00 AE	3.80 ± 0.25 ABCD
Serratiopeptidase ointment 1%	1.64 ± 0.00	2.89 ± 0.00 ACDE	2.33 ± 0.07*c AB	2.3 ± 0.06*c AB	2.24 ± 0.21 *AB
Serratiopeptidase ointment 2%	1.57 ± 0.00	2.87 ± 0.10 AE	2.61 ± 0.08*a AE	2.59 ± 0.05*a A	2.19 ± 0.20 *ABC

- Different capital letters indicate that the variation in the same raw is significant enough to warrant a 0.05 significance level.
- Different lowercase letters indicate a significant difference in the same column (p 0.05).

Table 3: After applying Serratiopeptidase ointment at a concentration of 1.2%, the percentage of inhibition in paw edema was calculated.

Treatment group	*1 min*	*10 min*	*30min*	*60 min*
Control	-	-	-	-
Serratiopeptidase ointment 1%	21.9 %	56.20%	59.38%	62.6%
Serratiopeptidase ointment 2%	34.40 %	31.10 %	32.8%	59.1%

product clearance through the blood and lymphatic arteries easier.[4]

The use of an H1. According to our findings, Blocker is similar to what has been found in earlier investigations. Due to the blockade of H1 and receptors, diphenhydramine decreases formalin-induced edema in rats. The action of histamine blockers.[19,20] SRP was more effective at controlling immune cell mobility. All the way from the lymph node to the inflamed and damaged tissues. This one-of-a-kind mechanism refers to.

The enzyme's involvement in keeping tissues in a healthy state (maintaining homeostasis).[15,21] In one research, the anti-inflammatory activity of SRP is attributable to a neutrophilic vector, according to a rat model. Apoptosis, suppression of neutrophil migration at the site of inflammation, decreased vascular permeability, and the removal of inflammatory debris are all effects of this drug.[16,22] in addition to preventing SRP. As a result of the hydrolysis of inflammation develops.

Our study's pain assessment findings revealed that SRP ointment is important. In the first stage, the differences in licking time are smaller than in the control group. Serratiopeptidase improves pain, according to the findings of other investigations. By preventing inflammatory tissues from secreting bradykinin 6 and 17. Furthermore, this outcome.

SRP, according to another study, lowers the amount of fluid in tissues and relieves pain. Decongestion allows fluid to flow, which reduces swelling and speeds tissue recovery.

It relieves pain and dissolves dead tissue in the vicinity of the affected area without harming healthy tissue. In addition, it relieves pain and swelling without inhibiting prostaglandins. Its proteolytic activity promotes blood circulation, eliminating damaged proteins, denatured proteins, and cellular detritus, as well as having negative effects on the digestive system.[16]

As a result of the foregoing, we may conclude that. Some analgesia is caused by the use of Serratiopeptidase ointment at various concentrations. The decrease in the time and quantity of claw lickings in the first stage, this stage, demonstrates this. Stage 1 denotes the pain stage, and stage 2 denotes the inflammatory stage after inflammation. A total of 1% formalin induces this reaction. The neurological stage refers to the body's reaction to pain (phase I or early stage). It is a stage that occurs shortly after formalin injection and depicts the irritating action of formalin on sensory fibers. Stages of inflammation (late-stage) are characterized by inflammatory discomfort.[25] A mixture of inflammatory reactions occurs when formalin is injected for 10 minutes.[26]

It contains the drug's involvement in preventing immune cells from migrating from the lymph node to the site of inflammation tissue that has been injured. An endogenous source of peripheral physiological receptor activation is one option.

Immune cells create beta-endorphins, which are released into wounded or inflammatory tissues. Inflammatory pain is particularly susceptible to these opioid activities in the peripheral nervous system. Sensory neurons have functional receptors at their peripheral ends. The concept that stimulation of peripheral receptors causes a reduction in sensorimotor function is supported.[27] Substantiating our claims with scientific proof Serratiopeptidase alone is insufficient to justify its usage as a general analgesic. In this field, both experimental and clinical work is required. Scientific evidence currently available for the order of serratiopeptidase's use as a pain reliever is absent thus, there is a need for it. This section needs further clinical trials and studies.

CONCLUSION

Serratiopeptidase is a commonly used anti-inflammatory and anti-pain agent. Serratiopeptidase its systemic use has a variety of adverse effects, including an anticoagulant impact. As a result, applying it topically can help reduce systemic side effects while increasing topical effects. According to this research, SRP has an anti-inflammatory and anti-edematous impact in mice with moderate analgesic effectiveness in paw edema.

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