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Efficacy of serratiopeptidase in enhancing wound healing in canines: A randomized controlled trial

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Abstract

The safety and efficacy of serratiopeptidase in canine wound healing were evaluated in a randomized controlled trial involving 12-dogs with surgical wounds. Animals were allocated into a treatment group receiving serratiopeptidase at 5 mg/10 kg BW, twice daily for six days, and a control group receiving no serratiopeptidase. All dogs were concurrently administered Amoxicillin-Cloxacillin (10 mg/kg) and Chlorpheniramine maleate (0.5 mg/kg) via injection. Wound healing was assessed at regular intervals by serial measurements of wound size reduction, wound area contraction, epithelialization, and granulation tissue formation. Early indicators of healing such as scab formation and wound contraction were seen on days 3-4 in the treatment group, compared with days 7-9 in the control group. By day-14, the treatment group demonstrated significantly faster and more complete healing. Infection rates were lower in the serratiopeptidase-treated dogs (1-case) than in controls (2-cases). Hematological and biochemical parameters confirmed good tolerability of serratiopeptidase, with no adverse effects detected. Additionally, elevated ascorbic acid levels were seen in the treatment group. These findings indicate that serratiopeptidase is a safe and potentially effective adjunct to promote wound healing in canines. Further clinical studies with larger numbers are required to confirm and warrant the findings in the study and also elucidate the associated mechanisms.

Keywords: Serratiopeptidase, wound healing, tissue epithelialization, tissue granulation, ascorbic acid

Introduction

Wound healing is a dynamic, sequential biological process that begins immediately after tissue disruption caused by injury or surgery. The initial insult impairs local microcirculation, alters the tissue environment, and activates a cascade of cellular and molecular responses that drive the healing process^[1]. Healing begins with haemostasis, characterized by vasoconstriction and clot formation mediated through platelet activation and the coagulation cascade. Vasoactive mediators such as bradykinin, fibrinopeptides, serotonin, and thromboxane A₂ further promote vascular constriction and stabilize the wound. Following haemostasis, inflammation takes place clearing the necrotic debris and microbes from the wound. This paves the way for proliferation stage, as fibroblasts, smooth muscle cells, and endothelial cells migrate to the wound bed promoting matrix deposition, angiogenesis, initiating epithelialization. Together, they restore tissue integrity through matrix deposition and angiogenesis to restore tissue integrity^[2]. However, dysregulated inflammation may lead to various pathological conditions, delayed healing associated with chronic pain^[3].

In current veterinary practice, post-operative care frequently includes steroids, non-steroidal anti-inflammatory drugs (NSAIDs), analgesics, and antipyretics to control pain and inflammation. While these medications are effective in reducing acute discomfort, their inappropriate or excessive use can impair normal tissue repair. Moderate to high doses of corticosteroids administered within the first five-Day post-injury are known to significantly delay wound healing. Similarly, certain NSAIDs like aspirin and indomethacin, when used in high doses during the acute inflammatory phase, may delay recovery by suppressing the biosynthesis of key mediators such as prostaglandins^[1]. Additionally, prolonged administration of NSAIDs can negatively impact the tensile strength of scar tissue^[4].

These limitations highlight the need for safer, adjunctive therapeutic strategies that support recovery without compromising the physiological healing cascade.

Serratiopeptidase is a proteolytic enzyme widely used across medical specialties such as surgery, orthopaedics, ENT, gynaecology, and dentistry. It is well known for its anti-inflammatory, anti-oedematous, and analgesic properties, making it a useful adjunct in managing post-operative swelling, pain, and tissue inflammation [5, 6, 7]. Serratiopeptidase stands out as a potent caseinolytic and fibrinolytic enzyme, offering therapeutic benefits by promoting pus liquefaction and enhancing antibiotic efficacy [5]. Compared to NSAIDs, serratiopeptidase is generally well tolerated even during prolonged administration [4, 6]. Its broad utility spans trauma, general, and plastic surgery, making it a valuable adjunct in post-operative care.

Proteolytic enzymes bind to circulating inhibitors such as α 1-antitrypsin and α 2-macroglobulin. Following injury or tissue damage, these inhibitor levels rise sharply, leading to temporary fibrinolytic shutdown, persistent inflammatory oedema, and delayed healing. Serratiopeptidase counteracts this effect by supporting plasmin activity, thereby facilitating fibrinolysis, reopening blocked microvasculature, and promoting resolution of inflammatory exudates [8, 9]. It breaks down inflammatory mediators, activates fibrinolysis to clear clots, improve circulation, and reduces immune complexes that prolong inflammation [9]. This action aids in reducing oedema and initiating tissue repair process [8]. Serratiopeptidase has demonstrated notable anti-inflammatory effects, along with strong mucolytic activity helping to break down mucus and relieve congestion, particularly in cases of chronic sinusitis. In veterinary medicine, especially in equine practice serratiopeptidase has been reported to promote pus drainage, reducing infection rates, and enhancing wound recovery [8, 10, 11].

The present study evaluated the safety and efficacy of oral serratiopeptidase in promoting the healing surgical wounds (both lacerated and incised) in canines. 12-Dogs were randomly assigned to either a control group or a treatment group receiving serratiopeptidase at 5 mg/10 kg BW. All dogs received standard post-operative therapy consisting of Amoxicillin-Cloxacillin (10 mg/kg) and Chlorpheniramine maleate (0.5 mg/kg). Wound healing was monitored through parameters including wound area reduction, contraction, epithelialization, infection, and oedema. Infected wounds were subjected to antibiogram testing to determine microbial sensitivity. Safety was evaluated through haematological profiling, while biochemical tests assessed the serum antioxidant levels and total proteins. Histomorphological analysis of wound tissue provided additional insights into the healing process.

Materials and Methods

Materials

Amoxicillin-Cloxacillin injection (Inj. Intamox) was procured from Intas Pharmaceuticals Ltd., Ahmedabad. Chlorpheniramine Maleate injection (Zeet) was sourced from Umedica (Alembic) Laboratories Pvt. Ltd.

Study design and participants

12-dogs with lacerated or surgically induced wounds present at the Veterinary Polyclinic, Veterinary College, Parbhani, were randomly allocated into two groups: a control group and a Treatment group. Allocation was done irrespective of age, sex, breed, weight, or wound type.

On Day 0 (baseline/SOT), all dogs received intramuscular Amoxicillin-Cloxacillin (10 mg/kg) and Chlorpheniramine maleate (0.5 mg/kg). Surgical intervention was performed either to excise pedunculated masses or to even out the uneven wound margins. All incised wounds were cleansed daily with normal saline and covered with adhesive tape to protect against environmental contamination. Preventive measures were followed to avoid maggot infestation.

Sutures were intentionally avoided for the ease of observation of natural wound healing processes, including granulation and epithelialization. Infected wounds were irrigated with 1:1000 potassium permanganate solution and cauterized using 2% copper sulfate. Postoperatively, each dog received its designated therapeutic regimen. Wounds were cleaned with sterile cotton, flushed with normal saline, and dressed with cotton gauze and adhesive tape. Wound characteristics including size, epithelialization, infection, and scar development were recorded from day 0 (start of treatment, SOT) till day 14 (end of treatment, EOT).

Surgical procedure

Prior to surgical intervention, the operative site was shaved, cleaned and washed with soap, and disinfected with spirit. Anaesthesia was administered based on wound status: Dogs with lacerated wounds and those undergoing excision of pedunculated growths were given intramuscular Xylazine hydrochloride (1 mg/kg of BW), followed by Ketamine hydrochloride (10 mg/kg BW). Dogs with infected wounds were given intravenous sedation with Xylazine hydrochloride (0.5 mg/kg BW). No sedatives were administered to calm and cooperative dogs.

Treatment

Postoperatively, all dogs received intramuscular Amoxicillin-Cloxacillin (10 mg/kg BW) and Chlorpheniramine maleate (0.5 mg/kg BW) for seven consecutive Days. Only the Treatment group received oral Serratiopeptidase at a dose of 5 mg (10,000 U) per 10 kg BW, administered twice daily (BID) for six days.

Wound healing assessments

Vital parameters - including body temperature, heart rate, and respiration rate were monitored on days 0, 4, 7, and 14 to evaluate post-operative recovery. To assess the effect of serratiopeptidase on wound healing, two primary indicators were evaluated: Wound contraction and Epithelialization. Wound size was traced from the margins onto a transparent gelatin sheets during each evaluation. These tracings were transferred onto graph paper to calculate wound area. Wound contraction was determined by comparing the change in area from day 0 to subsequent observations. The percentage of wound contraction was calculated using the formula.

$$\text{Wound contraction (\%)} = \frac{\text{Wound Area on '0' day} - \text{Wound area on 'n' day}}{\text{Wound area on '0' day}} \times 100$$

Hematological and antioxidant assessments

Blood samples were collected on days 0, 4, 7, and 14 for hematological and biochemical evaluation. The hematological parameters included hemoglobin concentration, packed cell volume (PCV), total leukocyte count (TLC) differential leucocyte count (DLC), and total erythrocyte count (TEC). Serum ascorbic acid levels were also quantified to assess antioxidant status and evaluate the potential influence of Serratiopeptidase on systemic oxidative balance.

Histopathological assessment

Histomorphological evaluation was performed on days 0, 7, 14, and 21 to monitor tissue regeneration and characterize the progression of wound repair. Biopsy samples were collected from the wound edges of dogs from both the groups control and treatment on each time points. After collection, the samples were rinsed with normal saline to remove blood and debris and preserved in 10% neutral buffered formalin, dehydrated through graded alcohol series, cleared in xylene, and embedded in paraffin wax. Thin sections (4-5 microns) were obtained and stained with Hematoxylin and Eosin, as well as Van Gieson's stain, to observe cellular changes. These assessments provided detailed insights into the comparative wound-healing dynamics between the two groups^[12].

Statistical analysis

Statistical analysis was performed using the 'F' test (ANOVA) with Web based Agricultural Statistical Procedure (WASP 1.0) available at www.icargoa.res.in. Differences were considered statistically significant based on p-values obtained from the model.

Results

Twelve clinical cases presenting with various types of lacerated wounds at the Veterinary Polyclinic, College of Veterinary and Animal Sciences, Parbhani were enrolled and evenly allocated to either the Control or Treatment group. Of the 12 dogs, six were male and six were female. The cohort comprised six non-descript dogs, three Golden Retrievers, one Pomeranian, one Pashmi, and one Doberman.

Post-surgical evaluation revealed that 11 dogs had lacerated wounds, one had an incised wound. Three dogs presented with infected wounds at baseline (Table 1). The incised wound exhibited a bright red appearance with sharply demarcated edges after 24-hour post-surgery, along with reduced gaping, mild exudation, and hyperaemia when compared to the lacerated or infected wounds. In cases of lacerated wounds, surgical trimming of uneven edges and removal of dead tissue and debris resulted in enhanced epithelialization for bridging the gap between two wound edges. The infected wounds were thoroughly cleansed and irrigated with 1:1000 potassium permanganate to wash out remove

necrotic debris and purulent exudate, followed by cleansing with 1% povidone iodine solution. Postoperative administration of intramuscular Amoxicillin-Cloxacillin and Chlorpheniramine maleate effectively controlled infection and mitigated histamine mediated inflammation in most cases. However, two infected wounds one from each group required alternative antibiotics based on antibiogram results.

Quality of wound healing

Infected wounds were observed in three cases, two from control and one from treatment group. Samples from infected sites were collected using sterile swabs and underwent culture and antibiotic sensitivity testing and targeted therapy was initiated accordingly. From day 4 onward, all three animals demonstrated a noticeable reduction in pus discharge and exudation indicating a favourable response to therapy.

Animal wise details of epithelialization and scar formation are presented in Table 1. In the Treatment group, wound moistness with mild exudation persisted till day 2, followed by early scab formation as early as day 4. Conversely, Control group wounds retained a fresh, hyperaemic appearance through day 2, with delayed scab formation observed only by day 5, the wound remained reddish with moderate retraction from the edges.

Wound contraction commenced with subtle peripheral shrinkage. Treatment group wounds developed a healthy pinkish-red coloration as early as day 3, reflecting active tissue repair. By day 5, a marked reduction in wound area was evident, accompanied by well-formed granulation tissue. In contrast, the Control group showed only moderate peripheral scarring and contraction by day 9. Collectively, the treatment group demonstrated a significantly early granular tissue formation compared to the control group resulting in faster healing process (Table 1).

The clinical observation of healing was recorded on day 4, 7, and 14 post surgery and the details of wound area and contraction are reported in Tables 2 and 3. The treatment group exhibited healthy granulation tissue and epithelialization on day 5, accompanied by a statistically significant reduction in wound size up to day 7. In contrast, the control group showed initial signs of granulation only after day 7.

Table 1: Clinical observations of wound healing (Type of wound, Wound infection, Epithelialization, Scar formation and Oedema)

Group	Breed	Types of wound	Wound infection				Epithelialization				Scar formation				Oedema
			0	4	7	14	7	14	21	28	7	14	21	28	
Control	Non-descript	Lacerated	-	+	-	-	+	-	-	-	-	-	+	+	
	Doberman	Lacerated	-	-	-	-	+	-	-	-	-	-	+	-	
	Pomeranian	Lacerated	-	-	-	-	+	-	-	-	-	-	+	-	
	Non-descript	Lacerated	+	-	-	-	+	-	-	-	-	-	+	+	
	Non-descript	Lacerated	-	-	-	-	+	-	-	-	-	-	+	-	
	Pashmi	Lacerated	-	-	-	-	+	-	-	-	-	-	+	-	
Treatment	Golden retriever	Lacerated	+	-	-	-	+	-	-	-	-	-	+	-	
	Golden retriever	Lacerated	-	-	-	+	-	-	-	-	-	-	+	-	
	Non-descript	Lacerated	-	-	-	+	-	-	-	+	-	-	-	-	
	Golden retriever	Lacerated	-	-	-	+	-	-	-	+	-	-	-	-	
	Non-descript	Lacerated	-	-	-	+	-	-	-	-	-	-	+	-	
	Non-descript	Incised	-	-	-	+	-	-	-	-	-	-	+	-	

+ = Present; - = absent

Table 2: Mean value of wound area of control and treatment groups

Interval in Days	Average wound area (cm ²)		T value
	Control	Treatment	
0 Day	3.45 ± 0.19	3.57 ± 0.20	0.451 ^{NS}
4 th Day	2.50 ± 0.14	1.93 ± 0.11	3.189**
7 th Day	1.75 ± 0.10	1.18 ± 0.07	4.748**
14 th Day	0.97 ± 0.05	0.48 ± 0.05	7.335**

Values are expressed as mean ± SE (n = 6 animals / group); ** Values are significant at 1% (3.169) level of significance

Table 3: Mean value of wound contraction of control and treatment groups

Interval in Days	Percent wound area (%)		T value
	Control	Treatment	
4 th Day	27.41 ± 0.36	46.05 ± 0.75	22.491**
7 th Day	49.32 ± 0.74	67.08 ± 0.31	22.219**
14 th Day	71.65 ± 0.74	86.75 ± 0.76	14.236**

Values are expressed as mean ± SE ($n = 6$ animals / group); ** Values are significant at 1% (3.169) level of significance.

The wound contraction could possibly be attributed to epithelialization and partially to the reduction of inflammation at the wound site. At the end of the treatment (day 14), the wound area in the treatment group had reduced by $86.75 \pm 0.76\%$ of its original area (from $3.57 \pm 0.20 \text{ cm}^2$ to $0.48 \pm 0.05 \text{ cm}^2$), indicating substantial healing. In comparison, the control group showed a comparatively slower contraction of $71.65 \pm 0.74\%$ of the initial wound area (from $3.45 \pm 0.19 \text{ cm}^2$ to $0.97 \pm 0.05 \text{ cm}^2$), suggesting a slower healing response. A more rapid wound contraction was observed in the treatment group, may be attributed to the anti-inflammatory and anti-oedematous properties of

serratiopeptidase^[13].

Physiological parameters

The body temperature, heart rate (beats per min), and respiration rate (Breaths per min) of all the subjects were monitored from baseline to the end of treatment and the statistical comparison is presented in Table 4. Analysis of rectal temperature showed no significant differences between the groups at any time point; however, the control group consistently showed marginally higher mean temperatures compared to the treatment group. Similarly, no statistically significant intergroup differences were observed in the mean heart rate. Both the groups exhibited a mild increase in heart rate on days 4 and 7, followed by a mild drop in heart rate on day 14. Further, the intergroup comparison of mean respiration rate also revealed no statistically significant differences and respiratory values remained stable throughout the study period. Importantly, all recorded physiological parameters - temperature, pulse, and respiration remained within the normal physiological range throughout the study, indicating that the treatment was well tolerated and physiologically safe.

Table 4: Mean value of changes in physiological parameters of control and treatment groups during intervention

Parameter	Interval (Days)	Control	Treatment	T value
Temperature (°F)	0 Day	101.13 ± 0.22	100.92 ± 0.20	0.994 ^{NS}
	4 th Day	101.22 ± 0.14	100.85 ± 0.20	1.539 ^{NS}
	7 th Day	101.22 ± 0.14	100.87 ± 0.22	1.342 ^{NS}
	14 th Day	101.00 ± 0.26	100.97 ± 0.22	0.096 ^{NS}
Heart rate (Beats / min)	0 Day	84.50 ± 1.34	83.67 ± 0.88	0.531 ^{NS}
	4 th Day	86.83 ± 1.49	84.00 ± 0.73	1.705 ^{NS}
	7 th Day	86.67 ± 1.63	85.00 ± 0.73	0.935 ^{NS}
	14 th Day	84.17 ± 1.08	84.83 ± 0.95	0.465 ^{NS}
Respiration (Breaths / min)	0 Day	22.67 ± 0.67	22.83 ± 0.48	4.000 ^{NS}
	4 th Day	23.50 ± 0.62	23.33 ± 0.33	0.237 ^{NS}
	7 th Day	23.33 ± 0.67	23.17 ± 0.60	1.512 ^{NS}
	14 th Day	23.17 ± 0.87	22.67 ± 0.71	0.443 ^{NS}

The values are expressed as mean ± SE ($n = 6$ animals / group)

Antibiogram analysis

Three subjects, two from control group, and one from treatment group were identified as infected. Pus samples from each infected site were collected in sterile test tubes and submitted for bacterial culture and sensitivity test. Both the cases from the control group revealed the presence of Gram positive cocci specifically *Staphylococci* and *Streptococci* with high sensitivity to ciprofloxacin. One of the cases exhibited moderate sensitivity to ceftriaxone while the other case was moderately sensitive to ceftriaxone and gentamicin. Both isolates were resistant to enrofloxacin, chloramphenicol, amoxicillin, ampicillin, and cloxacillin.

The infected case from the treatment group involved a large lacerated wound. The culture analysis identified the mixed bacterial growth comprising Gram positive cocci and Gram negative *Escherichia coli*. These organisms exhibited high sensitivity to ciprofloxacin, moderate sensitivity to gentamicin, and were resistant to ceftriaxone, streptomycin, and oxytetracycline. Detailed antibiotic sensitivity assessment of all the infected cases is detailed in Table 5. Following a change in antibiotic therapy based on sensitivity analysis, a marked reduction in pus formation was observed, leading to a considerable improvement in wound healing across all affected subjects.

Table 5: Cultural and sensitivity pattern of wound swab of animals from groups A and B

Control group	Organisms identified	Sensitivity pattern	Resistant
Non-descript	Gram positive cocci (Staphylococci and streptococci)	Ciprofloxacin ++++ Ceftriaxone +++	Enrofloxacin Chloramphenicol Ampicillin-cloxacillin Amoxicillin
Non-descript	Gram positive cocci (Streptococci and staphylococci)	Ciprofloxacin ++++ Ceftriaxone +++ Gentamicin ++	Enrofloxacin Chloramphenicol Ampicillin cloxacillin
Treatment group	Organisms identified	Sensitivity pattern	
Pomerinian	Mixed infection (Gram positive staphylococci and Gram negative <i>E. coli</i>)	Ciprofloxacin ++++ Gentamicin +++	Ceftriaxone Streptomycin Oxytetracycline

++++ Highly sensitive; +++ Moderately sensitive; ++ Mild sensitive

Haematological assessments

Haematological assessments were performed from baseline (SOT) to the end of the treatment period, and the mean values of each parameter were compared between the control and treatment groups. Haemoglobin concentration and PCV remained within normal range across both the control and treatment groups. The concentration remained relatively stable throughout the study period and hence no statistically significant difference was observed across both the groups. The mean TLC showed a mild increase from baseline to EOT in both the groups, although this change was not statistically significant. TEC values remained within the

reference range for all subjects, with no significant differences detected between groups from SOT to EOT. Neutrophil percentages remained relatively stable throughout the study, with no statistically significant intergroup variation (Table 6). A noticeable but statistically non-significant decline in the lymphocyte percentage was recorded in both the groups from baseline to Day 7. Subsequently, lymphocyte levels increased again and remained steady until the EOT. At no point during the study were statistically significant differences observed between the groups for either parameter. Basophils were absent in all samples collected from both the groups throughout the study period.

Table 6: Mean values of haematological assessments

Parameter	Interval in Days	Control	Treatment	T Value
Haemoglobin (gm/dL)	0 Day	14.22 ± 0.18	14.27 ± 0.08	0.512 ^{NS}
	4 th Day	14.17 ± 0.12	14.27 ± 0.22	0.403 ^{NS}
	7 th Day	14.33 ± 0.15	14.63 ± 0.23	1.077 ^{NS}
	14 th Day	14.70 ± 0.09	14.50 ± 0.22	0.855 ^{NS}
Packed Cell Volume (%)	0 Day	43.17 ± 0.87	44.50 ± 0.78	1.150 ^{NS}
	4 th Day	42.83 ± 1.33	44.00 ± 1.29	0.630 ^{NS}
	7 th Day	43.17 ± 1.35	44.00 ± 1.57	0.402 ^{NS}
	14 th Day	44.50 ± 1.52	43.33 ± 1.63	0.524 ^{NS}
TLC (thousand per cu mm.)	0 Day	12.25 ± 0.09	12.37 ± 0.12	0.426 ^{NS}
	4 th Day	14.28 ± 0.23	13.65 ± 0.26	1.826 ^{NS}
	7 th Day	12.57 ± 0.15	12.48 ± 0.22	0.110 ^{NS}
	14 th Day	12.20 ± 0.09	12.37 ± 0.07	1.7 ^{NS}
TEC (thousand per Cu mm.)	0 Day	6.46 ± 0.15	6.55 ± 0.18	0.398 ^{NS}
	4 th Day	6.49 ± 0.12	6.48 ± 0.08	0.081 ^{NS}
	7 th Day	6.51 ± 0.11	6.53 ± 0.06	0.124 ^{NS}
	14 th Day	6.45 ± 0.04	6.61 ± 0.10	1.365 ^{NS}
Neutrophil (%)	0 Day	65.00 ± 0.58	65.67 ± 0.42	0.933 ^{NS}
	4 th Day	73.33 ± 0.71	74.00 ± 0.68	0.674 ^{NS}
	7 th Day	67.17 ± 0.48	67.33 ± 0.71	0.194 ^{NS}
	14 th Day	63.50 ± 0.62	64.67 ± 0.33	0.987 ^{NS}
Lymphocyte (%)	0 Day	29.50 ± 0.50	28.33 ± 0.42	1.784 ^{NS}
	4 th Day	20.83 ± 1.01	20.67 ± 0.49	0.148 ^{NS}
	7 th Day	26.67 ± 0.67	26.67 ± 0.88	0
	14 th Day	29.83 ± 0.54	29.33 ± 0.33	0.685 ^{NS}
Eosinophil (%)	0 Day	3.17 ± 0.31	3.67 ± 0.33	1.103 ^{NS}
	4 th Day	3.50 ± 0.22	3.67 ± 0.21	0.542 ^{NS}
	7 th Day	3.17 ± 0.40	3.83 ± 0.31	1.319 ^{NS}
	14 th Day	3.50 ± 0.22	3.50 ± 0.22	0
Monocyte (%)	0 Day	2.50 ± 0.43	2.33 ± 0.21	0.349 ^{NS}
	4 th Day	2.33 ± 0.56	2.50 ± 0.22	0.277 ^{NS}
	7 th Day	2.67 ± 0.33	2.33 ± 0.42	0.620 ^{NS}
	14 th Day	3.33 ± 0.56	2.67 ± 0.33	1.026 ^{NS}

The values are expressed as mean ± SE (n = 6 animals / group)

Biochemical assessments

Serum ascorbic acid levels (mg/dL) were measured at baseline and at the EOT. Both groups exhibited a progressive increase in serum ascorbic acid concentrations over time, with a statistically significant intergroup difference noted up to day 14. Although the control group showed a moderate rise, the treatment group demonstrated a substantially greater increase, suggesting an enhanced physiological response associated with the administered therapy.

Total serum proteins (gm/dL) were also analysed from the baseline to the EOT. Both groups showed a slight, statistically insignificant decline on day 4, followed by an increase by day 7. These fluctuations remained within normal physiological limits and did not differ significantly between the groups. The values for serum ascorbic acid levels and total serum protein levels are summarized in Table 7.

Table 7: Mean values of parameters in biochemical assessments

	Interval in Days	Control	Treatment	T value
Serum Ascorbic acid (mg/dL)	0 Day	0.46 ± 0.02	0.49 ± 0.02	0.999 ^{NS}
	4 th Day	0.49 ± 0.03	0.55 ± 0.03	1.440 ^{NS}
	7 th Day	0.52 ± 0.02	0.58 ± 0.04	1.400 ^{NS}
	14 th Day	0.55 ± 0.03	0.66 ± 0.03	2.598*
Total serum protein (gm/dL)	0 Day	5.44 ± 0.19	5.30 ± 0.12	0.621 ^{NS}
	4 th Day	5.34 ± 0.21	5.35 ± 0.19	0.024 ^{NS}
	7 th Day	5.59 ± 0.18	5.68 ± 0.21	0.337 ^{NS}
	14 th Day	5.59 ± 0.29	5.86 ± 0.19	0.800 ^{NS}

Values are expressed as mean ± SE (n = 6 animals / group)

Histopathological assessments

In the control group, biopsy samples obtained from granulation tissue revealed active neovascularization

characterized by a dense vascular network and early granulation tissue formation. However, collagen fibre development was initially absent (Evident figures in supplementary file). With progression of healing, fibroblastic proliferation gradually increased, marked by the appearance of mature fibroblasts and early collagen fibres toward the EOT. These changes were more evident in sections stained with Van Gieson's stain. By day 21, a marked increase in mature fibroblast proliferation was observed accompanied by evident collagen fibre deposition. In contrast, fibroblastic activity was notably more pronounced in the treatment group along with the evident granulation tissue formation. Until day 14, tissue sections from treatment group, consistently demonstrated pronounced significant fibroblast proliferation, prominent neovascularization, and substantial collagen deposition, which were comparatively less evident in the control group.

Discussion

The present study demonstrates the efficacy of serratiopeptidase in accelerating surgical wound healing in canines. Key indicators such as reduction in wound area, contraction of wound margins, granulation, and epithelialization were notably improved in the treatment group receiving serratiopeptidase compared with the control group. Early and progressive signs of healing were evident in the treatment group, where wound contraction and epithelialization were observed as early as days 3 and 4. In contrast, the control group exhibited pronounced exudation and hyperemia during the same period, indicating a slower progression toward repair. The incidence of infection rates was lower in the treatment group, with only one affected case, compared with two cases in the control group. Targeted antibiotic therapy based on culture and sensitivity results was effective in resolving infections in all affected subjects, further supporting optimal healing outcomes.

Serratiopeptidase, a proteolytic enzyme, was administered orally in this study to evaluate its safety and therapeutic potential in canine wound healing. The enzyme is well documented for its mucolytic activities in both in-vitro and in-vivo settings [6], and its role in surgical wound repair and infection control has been supported by previous research [7]. Experimental evidence further indicates that serratiopeptidase facilitates the breakdown of viscous exudates; for instance, sputum liquefaction was observed within 1-3 h of administration in bronchitic rabbit models [14]. A similar mechanism may explain the reduction in pus formation and improved wound cleanliness observed in the present study.

Clinically, the treatment group demonstrated early and progressive healing responses. Wounds remained moist for the initial two days, with scab formation beginning by day 4. The presence of pinkish red tissue, an indication of healing, appeared by day-3, and the contraction of the wound area from the edges was visible by day-5, accompanied by granulation tissue development. By day-5, a significant reduction in wound area was recorded. The control group, however, showed granulation only from day-7 onward, with moderate scarring and contraction noted by day-9. These findings underscore the accelerated healing observed in the treatment group. The observed benefits may be attributed to

the enzyme's anti-inflammatory and fibrinolytic actions [13]. Serratiopeptidase facilitates fibrinolysis, dissolves necrotic tissue, and hydrolyzes inflammatory mediators such as bradykinin, serotonin, and histamine. This activity enhances microcirculation at the wound site, supporting tissue repair and reducing infection rates [2, 15]. Further observations discovered that serratiopeptidase reduced fluid accumulation at inflamed wound sites and improved vascular perfusion, aiding tissue regeneration [2, 7, 15]. By day-14, the average wound size in the treatment group had reduced to 13.25% of their initial area, compared to 28.35% in the control group. This reduction could be attributed to epithelialization and reduced inflammation, facilitated by serratiopeptidase. The reduction in edema improved the circulation and distribution of oxygen and nutrients over the wound area resulting in wound contraction and healing process.

Hematological assessments revealed no significant changes in hemoglobin concentration, PCV, TLC, and TEC in either group. Differential leukocyte counts including neutrophils, lymphocytes, eosinophils, and monocytes, remained within normal physiological limits and showed no notable fluctuations between the control and treatment groups. Basophils were absent in all samples at any point in both the groups. These findings indicate that serratiopeptidase administration did not disrupt hematological homeostasis or immune cell profiles, thereby supporting its hematological safety and suitability as an adjunctive agent in canine wound healing.

Biochemical analyses showed elevated levels of serum ascorbic acid in both groups, with the treatment group exhibiting significantly higher rise by the end of the study. This elevation is likely associated with increased collagen biosynthesis, as ascorbic acid is essential for the hydroxylation of proline to hydroxyproline, a critical step in stable collagen formation. Supporting evidence from previous studies has demonstrated that administration of ascorbic acid to rats undergoing laparotomy accelerated wound healing. The beneficial effects of ascorbic acid in tissue repair are attributed to its role in enhancing hydroxyproline content, promoting neovascularization, facilitating fibroblast maturation, and improving collagen deposition at the wound site [16]. The mobilization of ascorbic acid from body stores to granulation tissue was evident during the healing process and is consistent with the findings reported by Gould [17]. Total serum protein levels remained within physiological limits throughout the experimental period, with no significant differences between groups, further reinforcing the safety of serratiopeptidase supplementation.

Histopathological evaluation provided additional insights into the healing process. Fibroblastic proliferation was more pronounced in the treatment group, accompanied by earlier and more robust granulation tissue formation. By day 14, neovascularization was markedly higher in the treatment group. On day 21, collagen fiber deposition appeared denser when compared to the control group. These findings collectively indicate that serratiopeptidase accelerates wound healing by stimulating fibroblast activity, promoting collagen fiber proliferation and improved vascularization within the healing tissue.

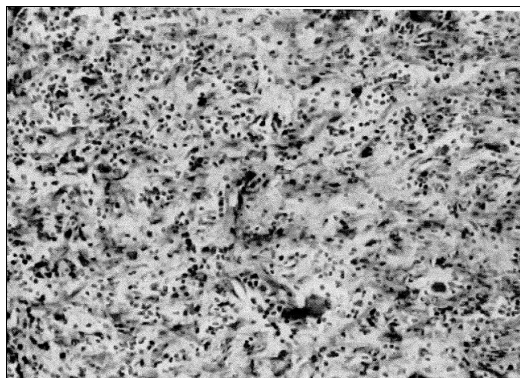
Histopathological analysis

Fig 1: Neovascularization with granulation tissue on Day 7 in Control group (Group A) (H and E X 200)

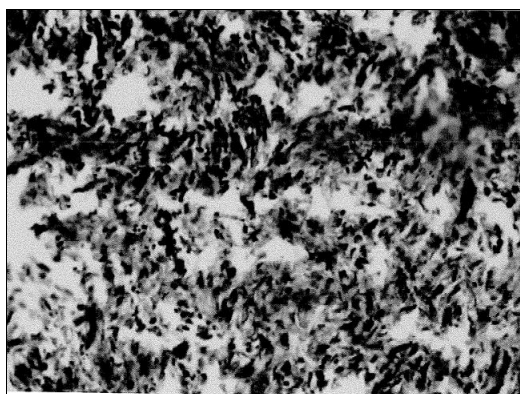


Fig 2: Fibroblastic proliferation with granulation tissue at Day 14 in Control group (Group A) (H and E X 400)

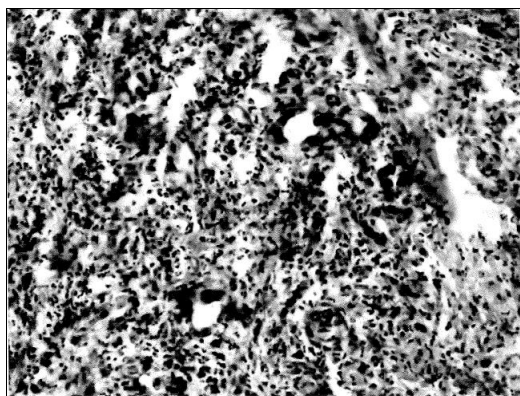


Fig 3: A section showing pronounced fibroblastic proliferation and granulation tissue at Day 14 in Control group (Group A) (Van Gieson's, X 400)

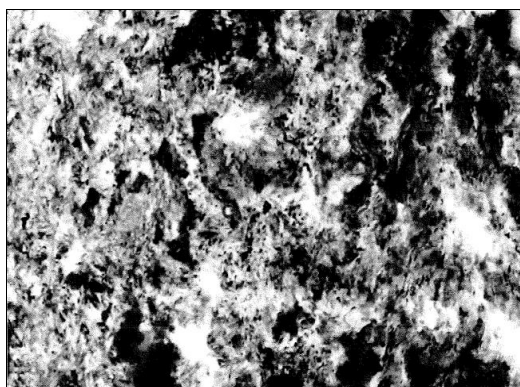


Fig 4: Pronounced Collagen fibre development in a section at Day 21 interval in Control group (Group A) (Van Gieson's, X 400)

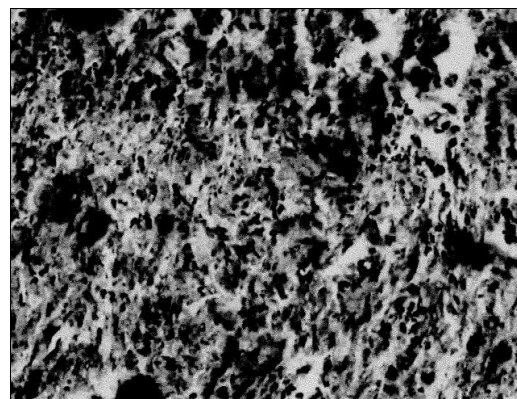


Fig 5: Pronounced Fibroblastic Proliferation with granulation tissue formation at Day 7 in Treatment group (Group B) (H and E X 400)

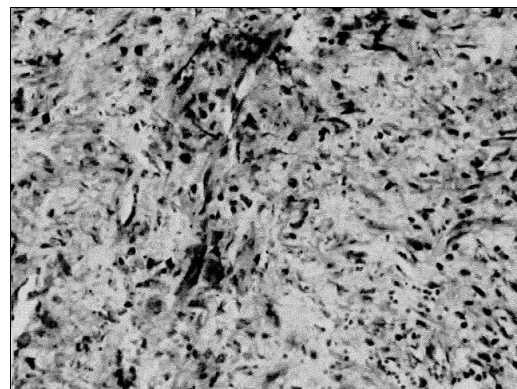


Fig 6: A section of granulation tissue showing appreciable fibroblastic activity with angiogenesis at Day 14 interval in Treatment group (Group B) (H and E X 400)

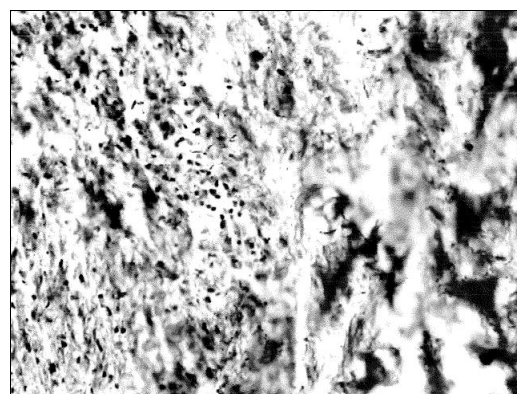


Fig 7: Collagen fibre formation at Day 14 interval in Treatment group (Group B) (Van Gieson's, X 400)

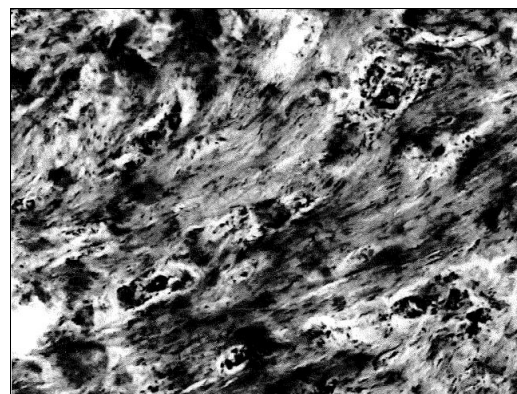


Fig 8: A section showing mature fibrous tissue formation and formed vascularization at Day 21 interval in Treatment group (Group B) (H and E, X 400)

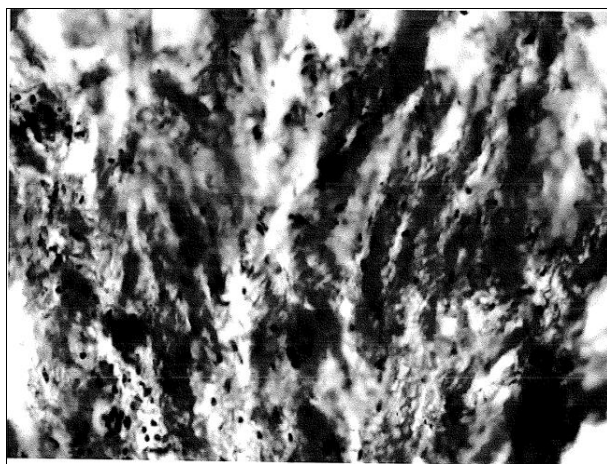


Fig 9: Remarkable collagen fibre formation at Day 21 interval in Treatment group (Group B) (Van Gieson's, X 400)

Conclusion

This study highlights the therapeutic potential of the serratiopeptidase in enhancing wound healing in canines. Dogs in the treatment group exhibited earlier signs of recovery, with noticeable granulation tissue formation, epithelialization, and significant wound contraction beginning as early as day-3. A marked reduction in edema and lower incidences of wound infection was observed in the treatment group compared to the control group. The enzyme's contribution to faster tissue repair was clearly apparent, and the administered dose was well tolerated, with no adverse clinical or hematological effects reported. These findings support the use of serratiopeptidase as a safe adjunctive therapy for canine surgical wounds. Further studies with larger sample sizes and extended follow-up durations are recommended to evaluate its effects on pain modulation, long-term scar quality, and overall functional outcomes during recovery.

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Conflict of interest

None.

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