Comparison of the effects of Crystalin® spray and Froximun® powder on the healing of open wounds in cats and dogs

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Abstract

Many topical products are recommended to accelerate wound healing, particularly for patients with chronic or non-healing wounds or when factors delaying healing are present. The potential benefits of these products must outweigh their cytotoxic effects. Hypochlorous acid and clinoptilolite are commonly used for this purpose. This study aimed to comparatively evaluate the effects of Crystalin® Spray and Froximun® Powder, containing these active ingredients, on wound healing. The study involved 12 owned animals (cats and dogs) of various ages, breeds, sexes, and weights. The animals were divided into two groups and treated with either Crystalin® Spray or Froximun® Powder. Wound healing in the Crystalin® Spray group was analyzed using Friedman's test, revealing significant daily wound area reduction ($p=0.001$). Statistically significant healing was observed as early as the 19th day ($p<0.05$). Similarly, the Froximun® Powder group showed significant wound area reduction ($p=0.001$). However, a comparison between the two groups revealed no statistically significant difference in wound healing rates ($p>0.05$). While both products demonstrated effectiveness, Hypochlorous acid preparations offer a cost-effective option for prolonged treatment and are easier for both veterinarians and pet owners to apply. Conversely, clinoptilolite in powder form requires bandaging for effective application, which may pose challenges for pet owners to maintain treatment continuity outside clinical settings.

Keywords: *Cat, clinoptilolite, dog, hypochlorous acid, wound.*

INTRODUCTION

Topical treatments, including antiseptics, silver-based dressings, hyperosmotic dressings, and other dressings that support autolytic debridement, are widely used to effectively reduce microbial load and promote wound healing. In the early stages of wound management, topical agents are often preferred over systemic antibiotics to minimize contamination and infection risk. However, the therapeutic benefits of topical treatments must outweigh their potential cytotoxic effects. A variety of topical antimicrobial agents are available to address these challenges (Davidson, 2015).

Hypochlorous acid (HOCl) is a broad-spectrum microbicidal agent that is highly effective against bacterial, viral, and fungal pathogens. Compared to other antiseptics, HOCl reduces the bacterial load without impairing wound healing and helps prevent biofilm formation. Moreover, HOCl is non-irritating, non-staining, painless, and easy to apply, making it highly advantageous for clinical use (Selkon et al., 2006). Its anti-inflammatory properties help minimize scar formation and alleviate wound-related pain (Gold et al., 2017; Sampson & Muir, 2002).

Clinoptilolite, a natural zeolite mineral derived from volcanic rocks, possesses antiviral, antibacterial, antifungal, and absorbent properties.Froximun®, which contains clinoptilolite silicate and an aluminum tetrahedron in its powder structure, is a non-toxic preparation suitable for wound care applications (Grce & Pavelić, 2005; Ivkovic et al., 2004; Maeda & Nosé, 1999).

This study aimed to comparatively evaluate the effects of Crystalin® Spray, containing hypochlorous acid, and Froximun® Powder, containing clinoptilolite, on wound healing. Given the widespread use and financial implications of these preparations in veterinary medicine, this research investigates their potential to accelerate wound healing and improve treatment affordability. The findings are expected to guide the selection of optimal wound management strategies in veterinary practice.

MATERIALS AND METHODS

The study included 12 animals (cats and dogs) of varying ages, breeds, sexes, and weights that were presented with wound-related complaints to the Balıkesir University Faculty of Veterinary Medicine, Surgery Clinic, and Pati Pet Veterinary Clinic. Animals with natural wounds and no systemic infections were included in the study. These animals were randomly assigned using a computer-generated randomization method. Animals diveded in to two groups of six (G1: Crystalin® and G2: Froximun®) for wound treatment using Hypochlorous Acid (HOCl; Crystalin®, NHP, Türkiye) and Clinoptilolite (Froximun® Toxaprevent Skin, MANC, Germany), respectively.

Initial clinical and hematological examinations were conducted to assess the animals' overall health status, with hematological assessments repeated on the fifth day. The hemogram data were recorded for subsequent analysis. Pathological lesions (wounds) were identified through anamnesis, inspection, and palpation. The hair around the identified lesions was clipped or shaved, depending on the severity of the wound (Figure 1). This process ensured standardized wound preparation providing a consistent basis for comparative analysis.

The exposed wounds were irrigated with 0.9% NaCl solution and mechanically debrided using sterile gauze. After debridement, each wound was photographed with a transparent, colored ruler placed adjacent to the wound edges for measurement and subsequent analysis. In the

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first group, wounds were treated with a hypochlorous acid solution applied twice daily. Photographs were taken after each application to document healing progress, and daily records were maintained until complete healing was observed. Similarly, in the second group, wounds were treated with clinoptilolite powder applied twice daily, following the same documentation procedures.

Figure 1. *A wound exposed by shaving and visualized using a transparent ruler.*

To prevent systemic sepsis, all animals received amoxicillin-clavulanic acid (17.5 mg/kg, SC) (Synulox®, Zoetis, Germany) once daily for five days. For animals exhibiting leukocytosis based on hemogram results on the fifth day, antibiotic therapy was extended for an additional two days. Throughout the 5–7 day antibiotic treatment period, wound care and general health status were monitored and recorded daily. Subsequently, wound care was continued by the animal owners using the respective preparations assigned to each group. Daily wound photographs were taken by the owners to document the healing process.

The hemograms and wound photographs obtained during the monitoring period were evaluated statistically. Measurements of the daily wound photographs were performed by the same veterinarian using Image J software (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, https://imagej.nih.gov/ij/), generating numerical data (Suarez-Arnedo et al., 2020) (Figure 2). The numerical data were statistically analyzed using the Mann-Whitney U test to compare groups, with a significance level set at $p<0.05$. Data analysis was conducted using a software SPSS Statistics, version 25 (IBM, USA).

RESULTS

This study evaluated a total of 12 owned animals (5 dogs and 7 cats). The demographic details, including age, species, breed, sex, and weight of the animals, are summarized in Table 1. Of the total animals, 5 (41.66%) were dogs and 7 (58.34%) were cats.

The breed distribution among the dogs was 4 (80%) Golden Retrievers and 1 (20%) Maltese Terrier. Their sex distribution included 2 (40%) spayed females, 1 (20%) intact female, and 2 (40%) intact males. The ages of the dogs ranged from 68 to 130 months (average: 106.2 months, approximately 8–9 years). All dogs were presented by their owners with wounds located in various parts of their bodies.

Among the cats, 6 (85.71%) were mixed-breed, and 1 (14.29%) was a Van Cat. Their sex distribution included 1 (14.29%) spayed female, 4 (57.14%) intact females, and 2 (28.58%) intact males. The ages of the cats ranged from 7 to 120 months (average: 50.3 months, approximately 4.2 years). Similar to the dogs, all cats were presented by their owners with wounds located in various parts of their bodies.

Hematological examinations conducted on the animals included in the study revealed significant changes in several parameters between the 1st and 5th days. The mean WBC count decreased from 21.95 ± 19.11 on the 1st day to 13.65 ± 9.23 on the 5th day. Similarly, the RBC count showed a slight increase from 7.45 ± 1.51 to 7.70 ± 1.00. Hemoglobin (HGB) levels also increased, with a mean of 14.34 ± 3.88 on the 1st day and 14.80 ± 2.73 on the 5th day. Hematocrit (HCT) values exhibited a rise from 34.90 ± 10.07 on the 1st day to 37.62 ± 9.03 on the 5th day. Platelet (PLT) counts demonstrated the most notable increase, with a mean of 319.83 ± 225.97 on the 1st day and 391.91 ± 126.51 on the 5th day. These findings indicate a trend toward normalization and recovery in the hematological parameters over the course of the study, as summarized in Table 2.

The comparison of hematological parameters between Days 1 and 5 revealed that the median WBC value on Day 1 was 15.76, while it decreased to 10.99 on Day 5 (Wilc=-2.746, p=0.006). This difference was statisti-

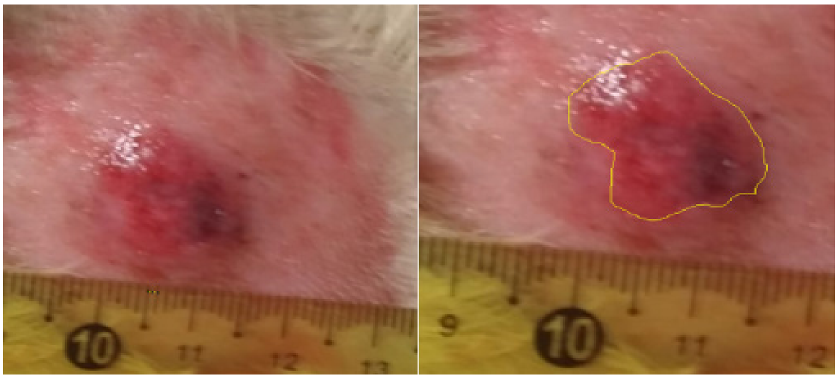

Figure 2. Wound measurement.

Table 1. *Animals included in the study.*

Case	Name	Species	Breed	Age (months)	Weight (kg)	Sex
G1C1	Joy	Dog	Golden Retriever	68	30	Spayed Female
G1C2	Yağmur	Cat	Mixed Breed	51	4	Spayed Female
G1C3	Fıstık	Dog	Maltese Terrier	117	7	Spayed Female
G1C4	Sarı	Cat	Mixed Breed	38	2.6	Female
G1C5	Susurluk	Cat	Mixed Breed	120	4.4	Female
G1C6	Tarçın	Dog	Golden Retriever	94	26	Female
G2F1	Dakdevir	Cat	Mixed Breed	7	3.9	Female
G2F2	Max	Dog	Golden Retriever	122	27	Male
G2F3	Pars	Dog	Golden Retriever	130	25	Male
G2F4	Kadife	Cat	Van Cat	101	3.8	Female
G2F5	Ka	Cat	Mixed Breed	25	4.8	Male
G2F6	Huzur	Cat	Mixed Breed	13	5.2	Male

Table 2. *Comparison of Hematological Parameters on Days 1 and 5.*

	X±SS	Median	Min-Max	Wilc*	p
WBC (1st Day)	21.95±19.11	15.76	5,87-70.15	-2.746	0.006
(5th Day)	13.65±9.23	10.99	7.10-41.09		
RBC (1st Day)	7.45±1.51	7.32	4.24-10.10	-0.941	0.347
(5th Day)	7.70±1.00	7.75	6.04-9.67		
HGB (1st Day)	14.34±3.88	15.40	5.30-19.10	-0.511	0.609
(5th Day)	14.80±2.73	15.40	9.90-18.80		
HCT (1st Day)	34.90±10.07	38.28	11.95-45.44	-1.098	0.272
(5th Day)	37.62±9.03	37.55	25.69-54.42		
PLT (1st Day)	319.83±225.97	321.50	34.00-704.00	-1.177	0.239
(5th Day)	391.91±126.51	349.00	230.00-618.00		

*Wilc: Wilcoxon Signed-Rank Test

cally significant ($p<0.05$). The median RBC value on Day 1 was 7.32, and on Day 5 it was 7.75 (Wilc=-0.941, p=0.347), showing no statistically significant difference between the two days ($p>0.05$). The median HGB value remained the same on both Day 1 and Day 5 at 15.40 (Wilc=-0.511, p=0.609), indicating no statistically significant difference ($p>0.05$). Similarly, the median HCT value was 38.28 on Day 1 and 37.55 on Day 5 (Wilc=-1.098, p=0.272), showing no significant difference ($p>0.05$). For PLT, the median value was 321.50 on Day 1 and 349.00 on Day 5 (Wilc=-1.177, p=0.239), also indicating no statistically significant difference between the two days ($p>0.05$).

When the wound areas treated with Crystalin spray and Froximun powder were statistically analyzed, no statistically significant difference was observed between the two groups ($p>0.05$). The distribution of wounds in the group treated with Froximun powder (G2) was evaluated using Friedman variance analysis. The analysis revealed that changes in wound size over time showed a statistically significant difference (p=0.001). A statistically significant difference in wound healing with Froximun powder was observed as early as the 19th day ($p<0.05$) (Figure 3, 4, 5, Table 3).

DISCUSSION

Wound treatment aims to create the conditions necessary for healing. These conditions, depending on the state of the wound, include temporary antisepsis and preparation for debridement to facilitate healing or closure. Definitive wound treatment should begin once the patient is stabilized. Initially, it is crucial to ensure antisepsis according to the condition of the wound and the patient, while also preventing further contamination caused by the clinic environment or the animal itself. After achieving antisepsis, the debridement of necrotic tissues and debris from the wound should be performed. These steps contribute to infection control and the formation of a healthy wound bed, facilitating proper closure(Anderson, 1996; Davidson, 2015). For infection control, systemic antibiotic therapy is the first recommendation. Although it may not reach therapeutic levels in severely traumatized wounds, it is suggested for preventing bacterial invasion and as prophylaxis in systemic infections. Broad-spectrum antibiotics with low systemic toxicity and good tissue penetration are indicated (Anderson, 1996). In this study, amoxicillin-clavulanic acid was used as prophylaxis to reduce bacterial invasion. Hematological examinations conducted before and after the treatment revealed positive outcomes, consistent with previous

Figure 3. *Case G1C6 (Tarçın): Appearance of the wound on Day 1 (A), Day 7 (B), and Day 13 (C).*

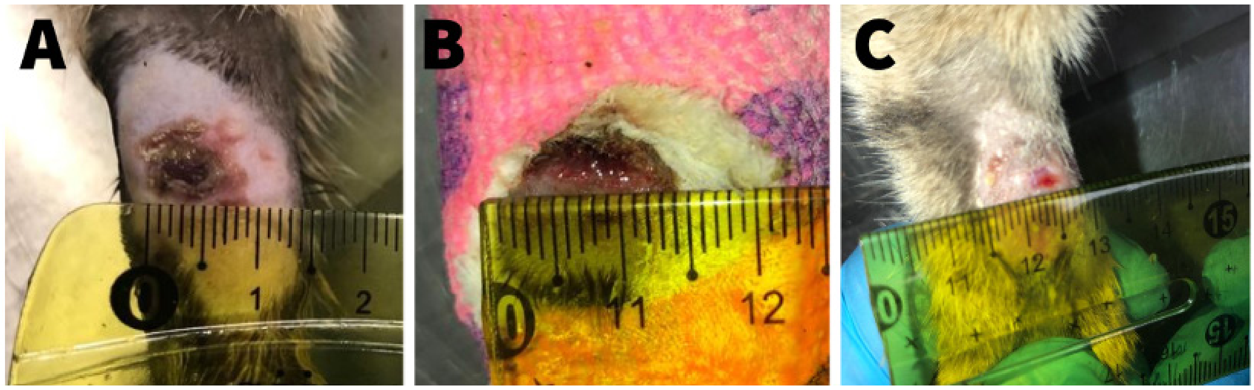

Figure 4. *Case G2F1 (Dakdevir): Appearance of the wound on Day 1 (A), Day 7 (B), and Day 13 (C).*

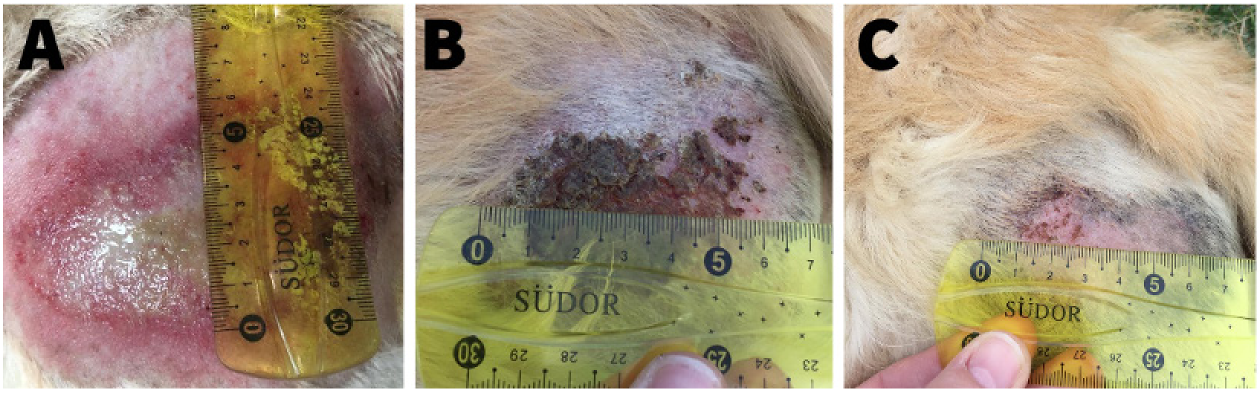

Figure 5. *Case G2F2 (Max): Appearance of the wound on Day 1 (A), Day 7 (B), and Day 14 (C).*

studies (Anderson, 1996; Davidson, 2015). To achieve debridement, irrigation is recommended for mechanically removing surface bacteria, foreign material, and necrotic debris. Balanced electrolyte solutions are preferred for irrigation due to their reduced risk of damage to healthy tissue. Irrigation has been reported to reduce bacterial load and prevent infection (Atiyeh et al., 2009; Fernandez & Griffiths, 2012). Although previous studies lack strong evidence that wound cleansing improves healing or reduces infection risk, it is a universally accepted and recommended practice. Therefore, in this study, all wounds were irrigated with 0.9% isotonic solution and mechanically debrided using gauze. This approach was intended to reduce bacterial load and accelerate healing.

Many topical products are recommended to accelerate wound healing. Most wounds heal using basic wound care techniques with appropriate dressing applications (Fahie & Shettko, 2007). Topical treatments should provide broad-spectrum microbial reduction. To minimize contamination, additional topical agents can be employed early in wound management and are often preferred over systemic antibiotics. The potential benefits of topical drugs must outweigh their cytotoxic effects, and various topical antimicrobial agents are available for this purpose (Davidson, 2015). HOCl is a relatively newly formulated, pH-neutral topical antimicrobial with strong disinfectant activity. It does not harm living tissues and is effective in treating wounds, skin infections, and ulcers. Its antibacterial effect reduces bacterial load without impeding wound healing and prevents biofilm formation. Studies have confirmed that HOCl exhibits strong antimicrobial activity against bacteria and viruses, while being non-irritating (Gunaydin et al., 2014). HOCl is recommended due to its non-irritating properties, lack of staining, ease of use, and painless application. It also reduces scarring, prevents crust formation, and possesses anti-inflammatory effects that alleviate wound-related pain (Gold et al., 2017; Gunaydin et al., 2014; Sampson & Muir, 2002; Selkon et al., 2006). In this study, HOCl was applied to wounds, and no irritation or allergic reactions were observed during treatment. Healing was ac-

Table 3. *Wound areas (mm²) over days in wounds treated with Crystalin and Froximun*

	Crystalin Sprey			Froximun Powder			Z	p
	X± SS (mm2)	Median	Min-Max	X± SS	Median	Min-Max		
1.day	517.27 ± 666.41	295.71	100.55-1851.37	705.29 ± 875.43	256.61	70.26-2179.41	-0.16	0.87
2.day	366.81 ± 435.48	224.23	86.62-1240.37	484.23 ± 581.17	242.72	58.97-1561.61	0.00	1.00
3.day	318.47 ± 439.75	129.74	73.46-1207.14	404.09 ± 486.40	232.58	42.51-1329.13	-0.16	0.87
4.day	297.56 ± 436.27	108.94	63.14-1180.47	375.44 ± 469.56	223.36	34.07-1285.84	-0.32	0.74
5.day	270.56 ± 387.18	98.33	57.83-1050.93	325.52 ± 414.27	204.72	1138-297.82	-0.16	0.87
6.day	242.82 ± 378.47	77.59	43.83-1008.19	300.48 ± 398.01	195.34	25.52-109.92	-0.48	0.63
7.day	219.92 ± 357.69	69.94	32.68-945.05	233.11 ± 347.46	114.05	24.11-926.90	-0.16	0.87
8.day	176.60 ± 275.90	58.54	21.76-732.68	155.30 ± 222.55	79.26	0.00-587.83	-0.64	0.52
9.day	165.34 ± 268.17	51.48	15.53-706.80	131.28 ± 181.96	69.84	0.00-478.69	-0.48	0.63
10.day	142.21 ± 231.55	46.43	8.10-609.72	120.72 ± 167.99	63.17	0.00-439.46	-0.48	0.63
11.day	138.18 ± 230.74	40.11	5.29-603.94	98.12 ± 135.07	44.52	0.00-346.37	-0.48	0.63
12.day	124.86 ± 210.56	34.63	0.00-549.30	68.11 ± 86.27	33.08	0.00-213.76	-0.32	0.74
13.day	98.99 ± 156.97	31.45	0.00-412.60	42.87 ± 60.87	7.73	0.00-127.56	-0.89	0.37
14.day	90.49 ± 151.42	28.18	0.00-395.87	35.94 ± 53.08	6.58	0.00-125.43	-0.89	0.37
15.day	79.34 ± 145.91	20.69	0.00-373.59	27.54 ±40.96	5.79	0.00-99.21	-0.66	0.50
16.day	71.87 ± 135.73	15.53	0.00-345.83	23.24 ± 36.63	5.58	0.00-92.41	-0.49	0.61
17.day	62.25 ± 118.97	14.28	0.00-302.89	16.47 ± 35.48	0.00	0.00-88.40	-1.02	0.30
18.day	58.31 ± 115.75	11.54	0.00-292.95	15.42 ± 32.97	0.00	0.00-82.19	-1.02	0.30
19.day	53.27 ± 111.67	9.25	0.00-280.37	14.34 ± 30.69	0.00	0.00-76.52	-0.71	0.47
20.day	50.81 ± 110.45	5.86	0.00-275.79	12.61 ± 27.16	0.00	0.00-67.67	-0.71	0.47
21.day	39.91 ± 87.73	2.12	0.00-218.51	11.47 ± 24.62	0.00	0.00-61.37	-0.53	0.59
22.day	36.03 ± 81.80	0.00	0.00-202.66	9.48 ± 20.88	0.00	0.00-51.92	-0.19	0.84
23.day	34.35 ± 79.94	0.00	0.00-197.38	8.27 ± 18.59	0.00	0.00-46.13	-0.19	0.84
24.day	29.63 ± 72.58	0.00	0.00-177.79	5.84 ± 14.31	0.00	0.00-35.06	-0.12	0.90
25.day	16.75 ± 41.03	0.00	0.00-100.52	4.57 ±11.21	0.00	0.00-27.46	-0.12	0.90
26.day	13.82 ± 33.85	0.00	0.00-82.93	3.81 ± 9.34	0.00	0.00-22.89	-0.12	0.90
27.day	8.47 ± 20.75	0.00	0.00-50.84	2.42 ± 5.94	0.00	0.00-14.56	-0.12	0.90
28.day	5.94 ± 14.56	0.00	0.00-35.68	0.00 ± 0.00	0.00	0.00-0.00	-1.00	0.31
29.day	0.00 ± 0.00	0.00	0.00-0.00	0.00 ± 0.00	0.00	0.00-0.00	0.00	1.00
30.day	0.00 ± 0.00	0.00	0.00-0.00	0.00 ± 0.00	0.00	0.00-0.00	0.00	1.00

hieved within 14–19 days without excessive scarring or crust formation, consistent with previous studies (Gold et al., 2017; Gunaydin et al., 2014; Sampson & Muir, 2002; Selkon et al., 2006). The lack of scarring and crust formation was attributed to the anti-inflammatory effects of HOCl. The pH-neutral nature of HOCl likely contributed to preventing bacterial infections and accelerating wound healing. Various HOCl formulations with different concentrations and pH levels are available on the market, and their effects have been reported to vary. In this study, statistical significance in wound healing was observed on Day 19, with clinical healing taking longer in some cases. This variation in healing time aligns with earlier findings (Sampson & Muir, 2002; Selkon et al., 2006) and may be attributed to differences in HOCl concentration and pH levels, as only one product, Crystalin®, was used in the study.

Froximun® powder, containing the natural zeolite clinoptilolite mineral, includes clinoptilolite silicate and aluminum tetrahedron in its structure (Kraljević Pavelić et al., 2018). It has antiviral, antibacterial, antifungal, and absorbent properties and is reported to be non-toxic (Grce & Pavelić, 2005; Ivkovic et al., 2004; Maeda & Nosé, 1999). In this study, no toxicity or allergic reactions were observed in animals treated with clinoptilolite. Previous studies have indicated that the substance forms a protective layer over the wound, absorbs exudates/transudates, and enhances platelet concentration alongside coagulation factors (Alam et al., 2005). In our study, no exudate/transudate formation was observed in wounds treated with clinoptilolite, and the wounds were covered with the powder containing the active ingredient. Following mechanical debridement, the powder adhered well to the moist wound surface by absorbing moisture, forming a protective layer. Consistent with previous studies, this ensured prolonged retention of the active ingredient on the wound surface. Moreover, its antifungal, antiviral, and antibacterial properties, along with its effects on cytokines, were reported to accelerate wound healing. The observed wound healing is facilitated through platelet aggregation, coagulation and hemostasis, neovascularization, cell activation, growth factor release, and subsequent granulation tissue formation. Clinoptilolite has been shown to significantly enhance wound healing, particularly by promoting granulation tissue formation in open wounds (Aksoy et al., 2019; Ivkovic et al., 2004). In our study, Froximun® powder was used as a clinoptilolite source, filling wound cavities and promoting granulation tissue formation, thereby accelerating wound healing. The selection of this active ingredient, like hypochlorous acid, was influenced by its non-cytotoxic properties. In addition to wounds, clinoptilolite has been used in conditions such as abomasal displacement (Erdoğan et al., 2018) and canine parvoviral enteritis (Akdağ, 2014), where it demonstrated accelerated recovery times and positive outcomes without adverse effects.

Wound healing and its monitoring can be conducted using various methods, with histopathological applications being among the most frequently employed. Histopathological evaluations are predominantly applied in studies involving experimental animals. In contrast, monitoring wound healing in owned companion animals is often limited to recording the day the wound closes, without numerical data for statistical analysis. In this study, wound healing was monitored using Image J software (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, https://imagej.nih.gov/ij/) (Suarez-Arnedo et al., 2020). This software allowed for a more objective digital evaluation of the wounds. The wound perimeters were defined, and the areas within these perimeters were measured in mm^2 daily. The data obtained were subjected to statistical analysis using the Friedman test, revealing significant reductions in wound areas by Day 19 in both groups compared to baseline values. These findings align with the existing literature and confirm that both medications are effective in promoting wound healing (Aksoy et al., 2019; Gold et al., 2017; Gunaydin et al., 2014; Ivkovic et al., 2004; Selkon et al., 2006). The results demonstrate the utility of Image J for objective wound tracking and highlight the efficacy of the topical agents used in this study.

CONCLUSION

In the study, wounds were treated using HOCl and clinoptilolite. Wound healing occurred with both active ingredients without any toxic or allergic reactions. No significant difference in wound healing duration was observed between the two substances. This lack of superiority could be attributed to factors such as the different forms of the preparations containing these active ingredients and the localization of the wounds on the body. Although both preparations are widely available on the market, HOCl-based products offer a more cost-effective solution for sustained treatment. They can also be applied easily and effectively by both veterinarians and pet owners. On the other hand, while the powdered form of clinoptilolite can be efficiently applied by veterinarians in clinical settings and secured with appropriate bandaging, its application and the continuity of treatment may present challenges for pet owners. Future studies should investigate the long-term effects of these treatments using larger sample sizes.

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Conflict of interest

The authors declared that there is no conflict of interest.

Ethical statement or informed consent

This study was approved by the Balıkesir University Animal Experiments Local Ethics Committee, Balıkesir, Türkiye (Approval no: 2020/1-9).

Author contributions

All authors read and approved the final version of the manuscript.

Availability of data and materials

The data used to prepare this manuscript are available from the corresponding author when requested.

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