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Assessment of Cutaneous Wound Healing Potential of Hyaluronic Acid and Chitosan in Dogs

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Abstract

Several new topical products have been applied to enhance wound healing and reduce scar formation. The present study aimed to evaluate the potential of the topically applied hyaluronic acid (HA) serum and chitosan (CH) gel for skin wound healing in dogs. Ten adult mongrel dogs were divided into two groups (n = 5 for each). Experimental skin wounds were created in all animals. Eight full-thickness round skin wounds of 2 cm diameter were made on both sides (four for each side) over the dog's dorsal area, where the left side wounds were kept as a control and treated with saline solution only. HA serum and CH gel were used to treat the right-side wounds in the first and second groups, respectively. Wound healing was evaluated clinically and histologically at the 7th, 14th, and 21st days post-treatment. In addition, the tensile strength was measured in both treated wound groups and compared with the control wounds and the normal skin. The results showed that HA-treated wounds exhibited higher wound contraction and better tensile strength compared with CH-treated group. In addition, the histological findings of HA-treated wounds showed marked improvement in histological repair scores compared with CH-treated ones. We concluded that topical HA in a serum formulation provided better skin wound healing compared to CH-treated group.

KEYWORDS
Cutaneous wound healing, Histologic score, Hyaluronic acid, Chitosan, canine, Wound contraction, Tensile strength.

INTRODUCTION

As the largest organ in the body, the skin makes up around 15% of the weight of an adult. The best way to explain it is as a cutaneous membrane with two clearly separated layers: the epidermis and dermis. In addition to protecting the body against external physical, chemical, and biological threats, it also prevents excessive water loss from the body and aids in thermoregulation. The skin is continuous with the mucous membranes lining the body cavities (Kolarsick *et al*., 2011; Garland, 2013).

Skin damage disrupts the natural continuity of skin, causing the wounds that are followed immediately by the physiological wound healing process, which seeks to return the skin's natural structure and function (Dhivya *et al*., 2015). Wound healing is dynamic, complex, and well-organized to allow the re-establishment of the skin's structural integrity. Inflammation, re-epithelialization, wound contraction, and remodeling are the four steps of this process. They are moving forward without a dividing line between them (Miguel *et al*., 2019). Although the skin is remarkably capable of healing from a small cut, when the injury is severe or involves large area of skin, proper and prompt dressing application is all that is necessary to prevent infection and hasten the healing process (Niyas Ahamed and Sastry, 2011). The primary goal of wound-healing agents is to hasten healing and scar formation (Oryan *et al*., 2018).

Chitosan (CH) is a linear polysaccharide of natural origin composed essentially of β-(1, 4)-linked glucosamine units (2-amino-2-deoxy-β-D-glucopyranose) along with some N-acetyl-glucosamine units (2-acetamino-2-deoxy-β-D-glucopyranose) (Metwally *et al*., 2023). It is often produced by alkaline deacetylation of chitin, the primary constituent of crustaceans' exoskeletons as shrimps (Ahmed and Ikram, 2016). CH is characterized by its biocompatibility, biodegradability, and non-toxicity (De Queiroz Antonino *et al*., 2017). Numerous studies have demonstrated the advantages of using CH as a biologically active dressing for wound healing. The wound dressings made of CH hydrogel are hemostatic, bacterially resistant, biocompatible, and degradable (Inas and Kawkab, 2012; Matica *et al*., 2019). When CH is applied to open wounds, causes enhanced growth factor activity, inflammatory cell infiltration, granulation tissue production, and angiogenesis (Feng *et al*., 2021). The extracellular matrix (ECM) is made up largely of hyaluronic acid (HA), a naturally occurring non-sulfated glycosaminoglycan that is composed of N-acetyl glucosamine and D-glucuronic acid. HA formulations can be classified as low molecular weight (500–1000 kDa), medium molecular weight (1200–4500 kDa), and high molecular weight (6000- 7000 kDa) (Iturriaga *et al*., 2021). HA can be found in various forms, including hydrogels, scaffolds, creams, films, foams, gels, serums, lotions, and implants, etc (Bukhari *et al*., 2018). HA and its derivatives possess favorable biocompatibility and visco-

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elasticity, resulting in its frequent application in the cosmetics and pharmaceutical industries (Su *et al*., 2014). Many in vitro and in vivo investigations have shown that HA plays an important role in fibroblast proliferation, granulation tissue development, and angiogenesis, as well as keratinocyte proliferation and migration during wound healing (Su *et al*., 2014; Ferrari *et al*., 2015; Sharma *et al*., 2018; Zhu *et al*., 2018).

The current study used clinical and histopathological evaluation in mongrel dogs to determine the therapeutic effects of HA and CH in the quickening of cutaneous wound healing.

MATERIALS AND METHODS

Ethics approval

All procedures of the present study were carried out according to the guidelines of the ethical committee of the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt (VM-2022-0035).

Chemicals and Reagents

Hyaluronic acid serum® powder was purchased from Bos Essentials, TSR Exchange LLC (New Hampshire, USA). High Molecular weight chitosan was procured from Alpha Chemika Co (Mumbai, India). Glycerol® was purchased from Research Product International, USA. Glacial acetic acid® and NaOH were obtained from ReAgent chemicals, UK. Drontal plus® and Amitraz 20® (formamidine pesticide) were purchased from Bayar, India, and Afrimash, Nigeria, respectively. Atropine Sulfate® (1 mg), XylaMed® (Xylazine HCL, 100 mg), and Ketamax® (Ketamine HCL, 50 mg) were procured from Geevet Remedies, India, Bimeda, USA, and Troikaa Pharmaceuticals Ltd, India, respectively. All chemicals and reagents were used as received from the supplier without any further modification.

Preparation of HA serum

In an airtight container and at room temperature, HA was added to filtered water and blended until fully dissolved (1% HA solution). The mix was shaken vigorously and refrigerated to chill until gelled (usually within 4-8 h.), according to the manufacturers' instructions. The final serum formulation was held at room temperature and evaluated further.

Preparation of CH gel

Briefly, the solvent was prepared by mixing 1% glacial acetic acid and glycerol at the ratio of 1: 3. One gram of finely powdered chitosan was dissolved in 100 ml of the previous solvent under stirring with a magnetic stirrer at room temperature for 1-2 h. to form a clear, pale-yellow solution of 1% CH. The pH of the solution was adjusted by adding 5N NaOH until it reached 7 (Moussaoui *et al*., 2012).

Animals and experimental design

The current study included ten adult female stray mongrel dogs (1-2 years old) with average body weight ranging from 15-20 Kg. All animals were kept in individual cages for two weeks to acclimate and were checked to be free from signs of infection. All dogs were dewormed by oral administration of Drontal® plus (praziquantel/pyrantel/pamoate/febantel) chewable tablets, one tablet /10 kg BW, and dipped in diluted Amitraz® (0.005%) before the initiation of the experimental study. Dogs were fed on a commercial standard meal and plenty of water throughout the experimental period. Experimental animals were divided equally into two groups (n=5); HA- and CH-dressed groups.

Full-thickness wounding model

Initially, all enrolled dogs were restrained in ventral recumbency and underwent a cephalic intravenous (IV) catheter placement. A 20-gauge, 1.25-inch IV catheter (Surflo 1.25 inch; Terumo Medical Corporation, Elkton, MD, USA) was placed in the right cephalic vein and secured with tape and light bandage for premedication with Atropine sulfate 1% (0.04 mg/kg IV) and Xylazine HCL 2% (1.1 mg/kg IV). The anesthesia was induced and maintained with Ketamine HCL 5% (10 mg/kg IV). A jugular catheter (Jor-Vet; Jorgensen Laboratories, Inc, Loveland, CO, USA) was placed aseptically using the Seldinger technique. A suture was used to keep the catheters in place, and to prevent displacement, a light bandage was placed. Dogs were positioned in sternal recumbency and the hair on the dorsolateral region of each dog was clipped and shaved, and the area was cleaned with 70% ethanol. Under aseptic measures, two cm diameter full-thickness circular wounds were surgically created bilaterally (four on each side) using a 2 cm diameter biopsy punch (Biopsy Punch, IndiaMART InterMESH., Ltd., Noida, Uttar Pradesh, India), two cm ventrolateral to the dorsal midline, and three cm apart from each other. The day on which the wounds were created is considered as Day zero. The size, depth, and location of the wounds were all consistent. Incision of the muscular layer was avoided, and tension of skin was kept constant during the procedure. The right-side wounds were treated twice daily with HA (in the first 5 dogs) and CH gel (the other 5 dogs) for four weeks, respectively, and the left-side wounds in both groups were treated daily with normal saline solution (0. 9 % NaCl) for four weeks and were kept as control.

Wound healing assessment

Clinical examination

The cranial wounds from different groups were imaged at days 7, 14, and 21 post-treatments to clinically evaluate the wounds. Throughout the whole period of the experiment, the animal's health was monitored daily for any abnormalities. The following formulas were used to measure the wound parameters and the healing time:

Wound Surface area (WSA) = πr^2 (cm^2). where r was 1 cm

(WSA %) = (Wound Surface Area*100)/3

Wound contraction % (WC %) = 100 % - WSA %

Histopathological examination

For the histopathological examination, biopsies were obtained from the caudal wounds at days 7, 14, 21 post-treatments then embedded in paraffin wax after being preserved in 10% neutral buffered formalin. Sections with a thickness of around 5 µm were prepared and stained with Harries hematoxylin and eosin (H&E) (Ricca Chemical Company, Arlington, TX, USA) and Masson's trichrome stain (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and examined under a light microscope (LEICA microsystem GmbH, Wetzlar, Germany) (Suvarna *et al*., 2018). Also, histologic acute inflammation score (HAIS) was qualitatively evaluated. For the histological assessment, we used a modified scoring system as a semi-quantitative method, including three parameters of acute inflammation; polymorphonuclear cells

(PMNC), edema, and hemorrhage, as for the calculation of the total histological acute inflammation score (HAIS), while for the calculation of the histological repair score (HRS), four parameters of tissue repair (epithelialization, angiogenesis, collagen disposition, and fibroblast proliferation) were used.

The above-mentioned parameters were assessed using the following scoring system for each one: 0, none present/minimal; 1, mild; 2, moderate; 3, marked. The interpretation of collagen density was based on the intensity and depth of distribution within tissue sections according to the following scoring values: 0, none; 1, superficial; 2, superficial to mid-layers; 3, all layers. The HAIS (range=0-6) and HRS (range=0-5) were calculated for each specimen.

Tensile strength test

The tensile strength test was carried out according to Kwan *et al.* (2011) to investigate the effects of post-treatment on tensile properties of healed skin. Wounded skin tissues were harvested from dogs on day 28 after wound creation. The tensile test was performed using the Instron 5848 MicroTester (Instron, Norwood, Massachusetts, USA) with a 100N load cell at a constant strain rate of 1 mm/sec at room temperature. To ensure that breakage occurred at the gauge length of 7.5 cm (the region with a reduced cross-sectional area where elongation was measured), the excised healed skin was sliced into a dumbbell shape with the aid of a custom-made metal template. Specimens were put into the load cell using a paper frame and kept in place by the grips, and when the skin sample was broken, the test was over. Before loading, the Vernier Caliper was used to measure the thickness of the skin and the width of the gauge zone. Displacement was measured by an encoding sensor. The following formula was used to calculate the tensile strength of wounds (Shukla *et al.*, 1999):
Tensile strength = (Breaking load (force))/(Cross-sectional area)
Where Cross-sectional area = thickness × width of skin strip.

The cross-sectional area of the wound was determined, and the force used to break the wound was automatically recorded using computer software (Instron® Bluehill Lite Software) Tensile stress is one of the tests used to evaluate the mechanical properties of topical agents.

Statistical analysis

The findings of the study were presented as mean values ± standard deviations (SD) through One-way analysis of variance (ANOVA) using SPSS (Ver. 16) software. The value of $p \leq 0.05$ was considered statistically significant.

RESULTS

Planimetric studies

The WSA and WSA% of treated wounds decreased significantly compared to that of control wounds at the 7th, 14th, and 21st days post-treatment (Table 1). Also, the WC% of treated wounds was increased significantly in comparing with the control wounds at the 7th, 14th, and 21st days post-treatment (Table 2). Additionally, the WSA and WSA% decreased significantly in HA-dressed wounds compared with CH-dressed wounds at the 14th and 21st days post-treatment (Table 1), and the WC% of HA-dressed wounds was significantly higher than that of CH-dressed wounds at the 7th and 14th days post-treatment (Table 2) when p was ≤ 0.05. No evidence of infection was observed visually on the incisional

The histopathological findings

At the first-week post-wounding

Generally, at this stage the total histologic acute inflammato-

Table 1. WSA (cm^2), and WSA (%) in full-thickness skin wounds in control and treated groups.

Parameter / Days	Control		HA group		CH group	
	WSA (cm^2)	WSA (%)	WSA (cm^2)	WSA (cm^2)	WSA (cm^2)	WSA (cm^2)
7	2.7±0.22	90.4±7.34	1.4±0.345	52.8±9.98 [a]	1.9±0.3 [a]	64.0±9.83 [a]
14	0.95±0.0087	31.7±2.86	0.2±0.079 [a]	5.9±2.6 [a]	0.4±0.12 [a,b]	13.7±4 [a,b]
21	0.2±0.09	6.5±3.0	0.002±0.004 [a]	0.05±0.12 [a]	0.022±0.03 [a]	0.7±0.98 [a]

Data are expressed as Mean ±SD
([a]): Significant changes when compared with control group when $p \leq 0.05$. ([b]): Significant changes when compared with Group 3 group when $p \leq 0.05$.

Table 2. WC% in full-thickness skin wounds in control and treated groups.

Days	Control	HA group	CH group
7	9.6±7.3	52.8±9.98 [a]	36.0±9.8 [a, b]
14	68.0±2.9	94.0±2.6 [a]	86.3±4.0 [a,b]
21	93.5±3.0	99.9±0.12 [a]	99.3±0.98 [a]

Data are expressed as Mean ±SD
([a]): Significant changes when compared with control group when $p \leq 0.05$. ([b]): Significant changes when compared with Group 3 group when $p \leq 0.05$.

Table 3. Max load (N/mm^2), Elongation%, and Breaking time (sec) of full thickness skin wounds in normal skin, control, and treated skin groups.

Parameter	Normal skin	Control group	HA group	CH group
Max load (N/mm^2)	250±12.6	81.78±8.45 [a]	269.16±27.6 [b]	164.5±41 [a, b, c]
Elongation%	73.6±5.12	18±4.4 [a]	64.87±21.4 [b]	79.75±33.3 [b]
Breaking time (sec)	59.2±3.2	15±3.8 [a]	39.4±13.9	58.6±22.99 [b]

Data are expressed as Mean ±SD
([a]): Significant changes when compared with normal skin control group when $p \leq 0.05$. ([b]): Significant changes when compared with control group when $p \leq 0.05$. ([c]): Significant changes when compared with hyaluronic group when $p \leq 0.05$

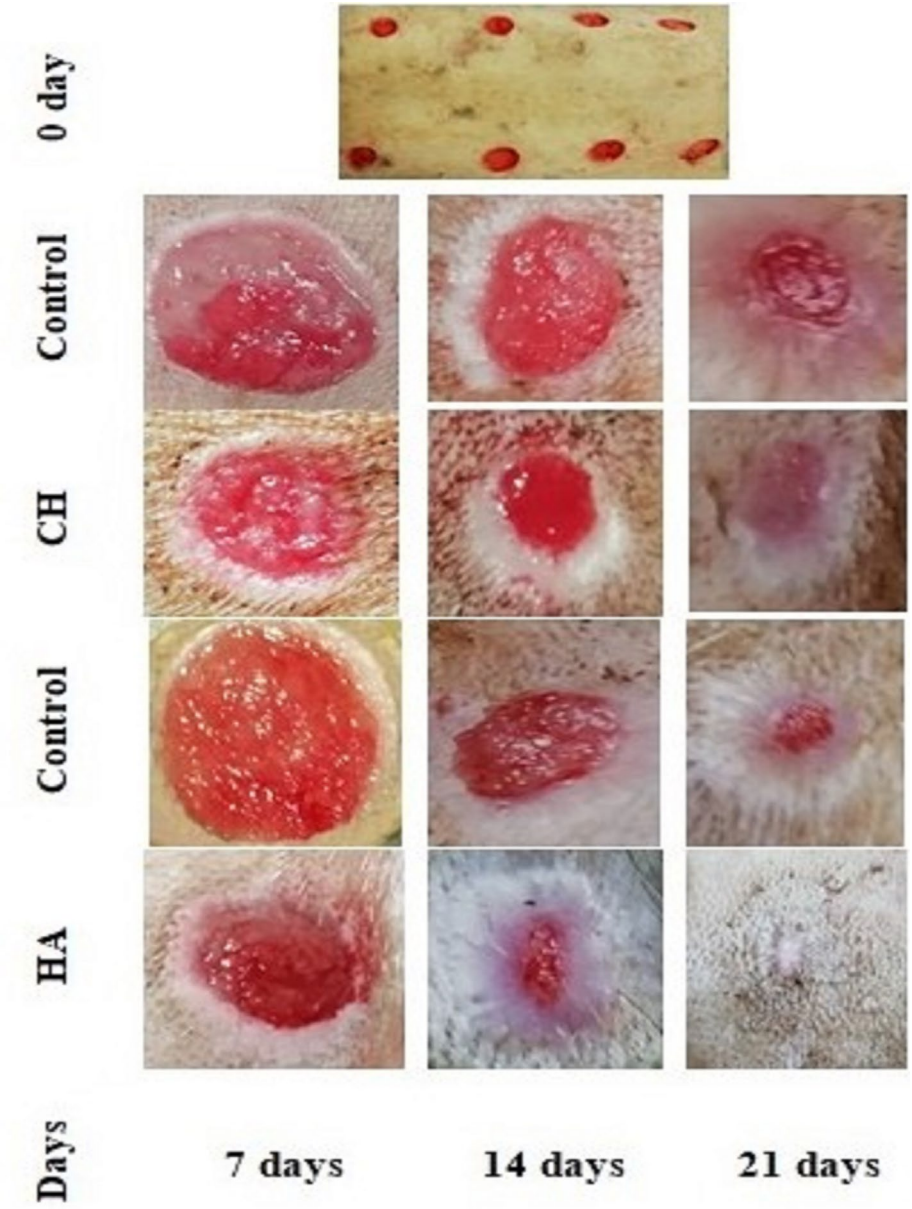

Fig 1. The wound healing in Control, HA, and CH treated groups at the 3rd, 7th, and 14th days post-treatment.

ry score (HAIS) was high compared with histological repair score (HRS), at this stage the wound was covered by upper granulation tissue that was rich in inflammatory cells with neovascularization and lower deep granulation tissue with more fibroblast proliferation. The histological lesion score for wound healing and histological hallmarks of wound tissue in different treated groups were illustrated in (Fig. 1 and 2) respectively. The microscopic examination of wound in control undressed group revealed increased inflammatory reaction with intense polymorphonuclear cells infiltration mixed with few mononuclear cells and mild angiogenesis in the upper larger layer of granulation tissue. There was hemorrhage and edema of the underlying dermis with few fibroblasts proliferation. The free wound edges showed mild hyperplastic proliferation of epidermal epithelium that showed vacuolization and necrosis; there was degenerated fragmented tissue debris covering the epidermal epithelium indicating failure of epithelization. There was smaller deep granulation tissue proliferation in deeper dermal tissue, the granulation tissue consisted of mild fibroblast proliferation with mild angiogenesis but there was no evidence of collagen deposition with low HRS and high HAIS.

The HAIS was reduced significantly in HA-dressed group compared with control undressed one with numerical non-significant improvement of HRS. The upper granulation layer showed

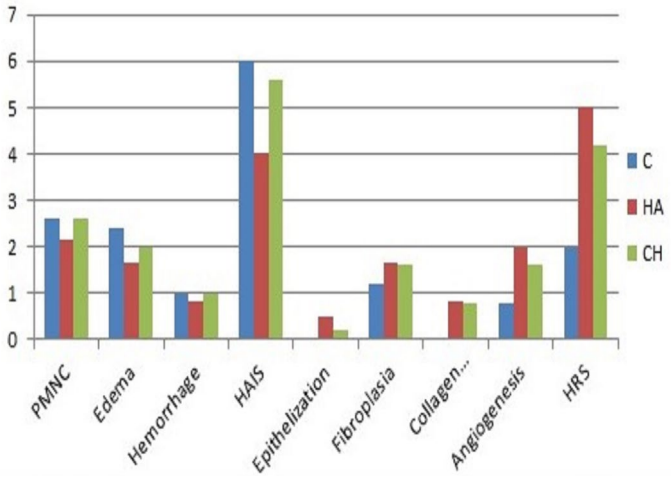

Fig 2. Histological lesion score at the 1st week post wounding

hemorrhage with more mononuclear cells' infiltration, enhanced angiogenesis and more fibroblast proliferation were observed at this layer. The deep granulation tissue layer was large that characterized by moderate fibroblast proliferation with mild collagen deposition. The epithelization was observed in this group and characterized by epithelial migration, thickening and hyperplasia

of epidermal epithelium at wound edge with significant reduction in HAIS and increased HRS compared with the undressed group.

The HAIS in chitosan dressed group was lower than control undressed group but not significant as the upper granulation tissue layer showed infiltration of macrophages and neutrophils more than the HA group while the HRS was much similar to the HA group. Failure of epithelization was detected in this group at this stage.

At the second week post-wounding

The histological lesion score for wound healing and histological hallmarks of wound tissue in different treated groups were illustrated in (Fig. 3 and 4) respectively. In the control undressed group, there was hyperplastic proliferation of epidermal cells at the wound free edges. The underlying dermis showed less polymorphonuclear cells but more macrophages infiltration, less edema and hemorrhages with significant reduction in HAIS and increased in HRS compared with 7 days period. There was moderate fibroblast proliferation, mild collagen deposition in loose oriented bundles and more angiogenesis. While in HA dressed group there was significant increase in HRS and slight reduction in HAIS. There was hypertrophied and thickening of epidermal cells with partial differentiation covering large area of the wound granulation tissue. The exposed upper granulation tissue showed little reduction of inflammatory reaction compared with control undressed group but enhanced abundant fibroblast proliferation and excessive angiogenesis while the underlying dermis that cover with neo-epidermis exhibited enhanced fibroplasia and collagen deposition that arranged in wavy parallel less interconnecting bundles (more light blue stained immature collagen with little dark blue stained mature collagen) and few macrophages infiltration and moderate angiogenesis. On other hand, the HAIS in chitosan dressed group was high and characterized by intense polymorphonuclear cells infiltration of the dermal tissue on the contrary the HRS was increased and characterized by enhanced angiogenesis and fibroblast proliferation and more collagen deposition.

At the third week post-wounding

There was enhanced repair and remodeling of wound area. The histological lesion score for wound healing and histological findings of wound tissue in different treated groups were illustrated in (Fig. 5 and 6) respectively. In the control group, the epithelization was complete but with thin undifferentiated epidermal cells while the underlying dermis showed moderate collagen deposition but in loose parallel lightly stained immature collagen bundles with moderate angiogenesis. While in HA dressed group showed abundant collagen bundles deposition in compact parallel interconnected pattern that stained dark blue indicating its maturity. Neutrophils were not detected but few macrophages were observed in deep dermal tissue. The angiogenesis and fibroblast proliferation were reduced compared with control group and epithelization differentiation was detected.

The microscopic picture of Chitosan dressed group revealed complete epithelization with undifferentiated epidermal cells. There were few polymorphonuclear cells in the dermal tissue and the collagen bundles were moderate but arranged in parallel less interconnected bundles light blue immature collagen with mild deposition of mature dark blue collagen compared with control and HA dressed groups with moderate angiogenesis and fibroplasia.

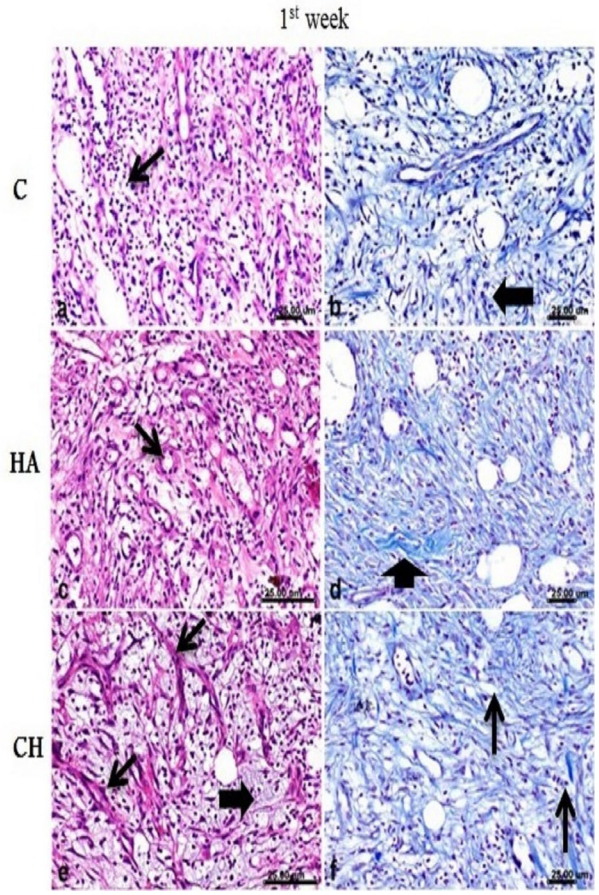

Fig 3. Photomicrograph histological section at 1st week post wounding, stained by H&E (a, c, e) and Masson`s trichrome (b, d, f).

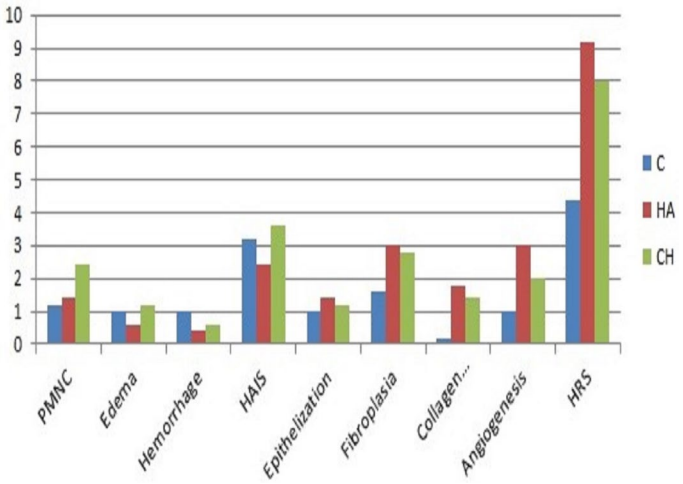

Fig 4. Histological lesion score at the 2nd week post wounding.

The wound tensile strength

In the tensile strength test, the healed wounds after CH application showed significantly lower load when compared with HA-healed wounds, wounds of the control group, and the normal skin. While a load of HA-healed wounds was virtually identical to that of the normal skin, implying the high strength present in wounds treated with HA (Table 3). The mean elongation of HA- and CH-healed wounds was significantly higher than the wounds of the control group and was comparably identical to the normal skin (Table 3). Moreover, the breaking time of HA- and CH-healed wounds was insignificantly shorter than that of normal skin. The breaking time of CH-healed wounds was significantly longer than that of the wounds of the control group (Table 3).

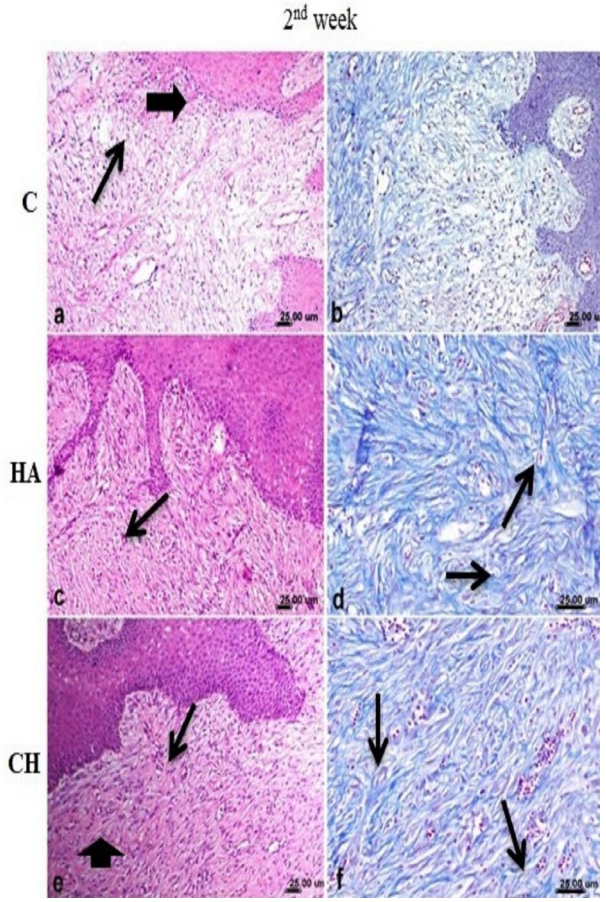

Fig 5. Photomicrograph histological section at the 2nd week post wounding, stained by H&E (a, c, e) and Masson`s trichrome (b, d, f).

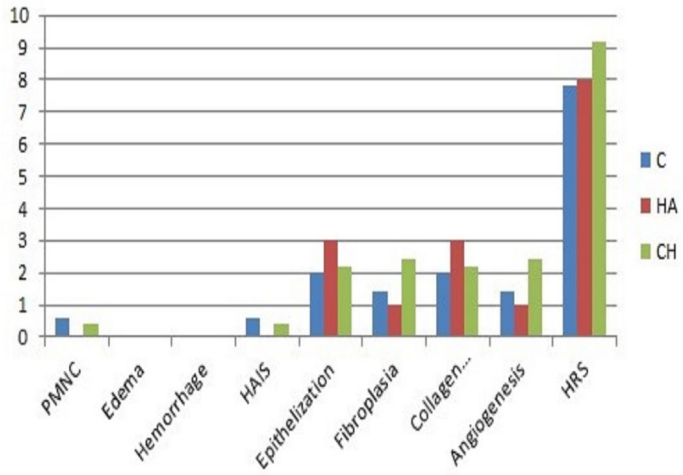

Fig 6. Histological lesion score at the 3rd week post wounding

DISCUSSION

Developing novel agents with wound-healing properties is urgently needed because wound healing is one of the critical medical issues in human and animal medicine (Metwally *et al.*, 2022). Our study aimed to evaluate the effects of HA and CH in the healing of full-thickness cutaneous wounds, where wounds treated with topical exogenous HA showed accelerated re-epithelialization and wound-healing effect compared with wounds treated with CH or saline solution.

Hyaluronic acid (HA) has obtained increased attention among other biomaterials for wound care as it is a significant extracellular matrix (ECM) component and is essential for the process of tissue regeneration and wound healing (Litwiniuk *et al.*, 2016). It has been demonstrated that HA can influence the three main stages of wound healing, including inflammatory responses, cell migration, and angiogenesis, through interactions with certain cell receptors (Aya and Stern, 2014). In vivo preliminary studies aimed at defining HA's function in the healing of various tissues show that topically applied HA can promote rat skin wound healing (Abatangelo *et al.*, 1983) and hamsters (King *et al.*, 1991) and perforated tympanic membrane healing in rats (Longinotti, 2014). HA enhanced re-epithelialization by stimulating the migration and proliferation of keratinocytes (Nyman *et al.*, 2013, 2019), thus resulting in accelerated microvascular density and more elastic soft tissue formation (Zhao *et al.*, 2013; Shimizu *et al.*, 2014; Neuman *et al.*, 2015). It has been established that HA participates in each stage of the wound healing process (Iocono *et al.*, 1998; Litwiniuk *et al.*, 2016) not only as a component of the wound environment but as a factor that actively influences tissue regeneration (Litwiniuk *et al.*, 2016), which includes cell migration, cell proliferation, organization of the granulation tissue matrix, robust the inflammatory response, and angiogenesis (Voinchet *et al.*, 2006). The CH-treated wounds showed delayed healing that was attributed to excessive granulation, which caused a delay in epithelialization (Johnston, 1990; Ueno *et al.*, 1999). It promotes fibroblast, lymphocyte, and polynuclear neutrophil cell migration at the site of lesions during the inflammatory phase due to its rheological properties. HA also controls inflammation by generating proinflammatory cytokines and promoting angiogenesis. In addition, HA speeds up repair processes such as fibroblast migration and growth, collagen synthesis, and endothelial cell growth during the granulation phase (Liguori *et al.*, 1997).

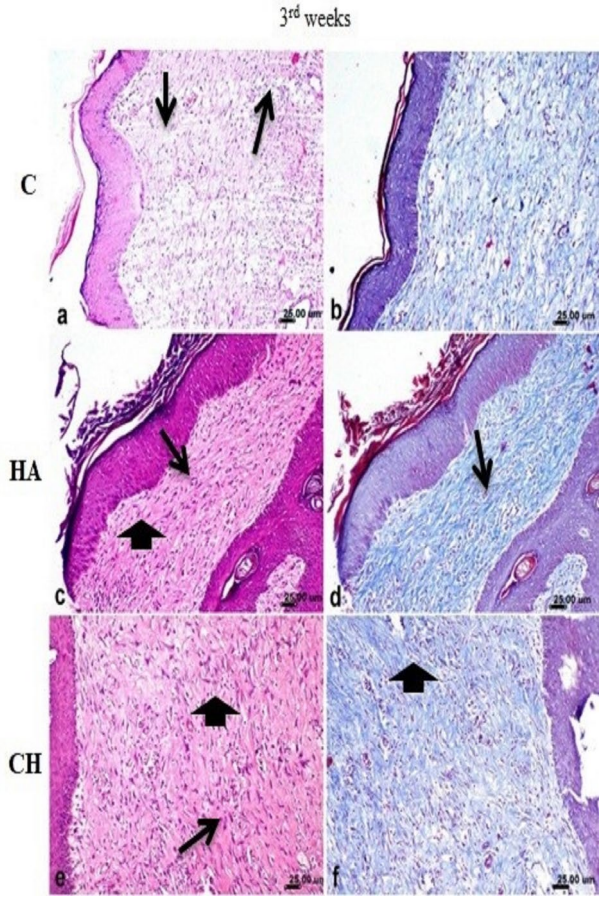

Fig 7. Photomicrograph histological section at the 3rd week post wounding, stained by H&E (a, c, e) and Masson`s trichrome (b, d, f).

During morphogenesis, HA stimulates the proliferation and migration of keratinocytes and dermal collagen remodeling; however, it also suppresses inflammatory response, resulting in decreased fibrosis, and consequently fibrous tissue (Koller, 2004). The degradation products from the HA-fibrin matrix, act as regulator molecules of the wound-healing process (Prathiba and Gupta, 2000; Fathi, 2012). These effects explain why the rate and quality of wound healing of HA-treated skin is better than that of

those treated with CH. HA dressing accelerates the healing process by maintaining a tissue-moist environment and protects the wound from harmful alterations, such as bacterial colonization. Therefore, the application of HA leads to the quick healing of acute wounds (Voinchet *et al*., 2006). The HA increased the tensile qualities of the restored skin, giving it a near likeness to healthy skin, additionally speeding up the wound healing process (Pinkus and Perry, 1953). Interestingly, HA's hydrophilic qualities make the fibrin clot softer and simpler for cells to colonize. Fetal wound healing has been studied as an ideal tissue healing condition for designing effective regenerative therapeutic treatments since it is characterized by the absence of fibrotic scar formation. Surprisingly, it has been found that fetal tissue regeneration results in reduced scarring when HA is present for a longer period of time (West *et al*., 1997; Chen, 2002). As a result of this observation, it was proposed that an HA-rich environment inhibits the matrix cells responsible for scar formation (Longinotti, 2014).

Clinical observation of the treated and controlled wounds indicated that hemostasis was clear directly after the topical application of chitosan (Inas and Kawkab, 2012). Chitosan accelerates the infiltration of inflammatory cells (Okamoto *et al*., 1995). The efficacy of chitosan and its derivatives for wound healing is due to the acceleration of the infiltration of the peripheral mononuclear cells (PMNC) (Ueno *et al*., 2001; Santos *et al*., 2007; Dai *et al*., 2011), macrophages, and fibroblasts (Ueno *et al*., 2001) or osteoblasts (Klokkevold *et al*., 1996) into the wound area, increase in effusion which forms thick fibrin and activates the migration of the fibroblasts into the wound area, stimulation of the migration of the macrophages and stimulation of proliferation of fibroblasts and the production of type III collagen (Ueno *et al*., 1999). Additionally, chitosan also has antimicrobial properties (Dai *et al*., 2011).

It has also been found that chitosan could increase the tensile strength of wounds (Dai *et al*., 2011). The wound-healing effects of chitosan could be affected by the factors of molecular weight (Minagawa *et al*., 2007; Alsarra, 2009), de-acetylation degree, as well as the state of chitosan (Azad *et al*., 2004). The skin's tensile strength, continuity, and function are all determined by type I collagen. In order to revert back to normal skin, type III collagen is expected to decrease in contrast to type I collagen while remodeling and maturation occur (Cheng *et al*., 2011). CH promotes fibroblast proliferation and type III collagen synthesis (Ueno *et al*., 1999). Furthermore, a decrease in type I collagen and an increase in type III collagen are linked to collagen fibers that are thinner and more flexible, resulting in a reduction in tensile strength (Eriksen *et al*., 2002). Chitin and its derivatives may help wounds heal faster by speeding up the fibroblastic synthesis of collagen in the first few days of healing (Chen *et al*., 2008).

CONCLUSION

Topical HA serum accelerates the healing of the experimentally induced full-thickness skin wounds in dogs with higher epithelialization compared with topical CH gel and control wounds, respectively. When compared to the control, wounds treated with CH and HA resulted in better fibril alignments and tensile features in the repaired skin. We concluded that topical HA in a serum formulation provides good skin wound healing, as demonstrated through clinical, gross, histologic, and tensile strength assessments.

CONFLICT OF INTEREST

The authors declare that there is no competing interest.

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