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Comparative Efficacy of Chitosan with or Without Honey on Cutaneous Wound Healing in Donkeys

Areesha Arkan[1*], Sadaf Aslam[1], Sohail Aslam[2], Sohaib Safdar[1], Ali Qaisar[1], Danish Jeelani[1], Amber Fatima[1], Obaid Muhammad Abdullah[1]

[1]Department of Veterinary Surgery, University of Veterinary and Animal Sciences (UVAS), Lahore.
[2] University of Veterinary and Animal Sciences Sub-Campus CVAS Jhang.

**Corresponding author's E-mail: esha.arkan007@gmail.com*

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Abstract

The domesticated donkey, derived from the African wild ass, has played a crucial role in human history for over 5,000 years, serving as a working and pack animal. However, donkeys often suffer from skin wounds and injuries due to various factors, including equipment use, road accidents, and lack of veterinary care. Wound healing is a complex process involving inflammation, proliferation, and maturation phases, with impaired cell proliferation potentially delaying healing. Equines, including donkeys, are particularly susceptible to traumatic skin wounds, with limb wounds healing more slowly due to factors such as tissue loss, contamination, and excessive skin tension. In such cases, wound healing by second intention is common but can lead to complications. Chitosan, a biopolymer derived from the shells of crustaceans, has shown promise in promoting wound healing. It helps with tissue granulation, collagen deposition, and tissue regeneration, while also preventing wound contamination and maintaining a sterile environment. Honey, with its antimicrobial, anti-inflammatory, and antioxidant properties, is another natural remedy that accelerates wound healing and is often used in combination with chitosan for optimal results. This biologically-based approaches hold potential for improving the healing of donkey wounds and preventing infections, offering safer and more effective alternatives to traditional wound care.

Keywords: *Donkey, Wound healing, Chitosan, Equines, Hone*

1. Introduction

The donkey (Equus asinus) is a domesticated subspecies of the ass. Being a member of the equine family, the donkey is considered to be derived from an African wild ass (Equus africanus). The first-time domestication of the ass was done in Egypt and Mesopotamia around 3000 BC. It has been used by humans as a working, draught, and pack animal, for at least 5000 years (Burden and Thiemann 2015). Currently, there are over more than 44 million donkeys worldwide, playing an important role in the economics of underdeveloped countries. Donkeys are often subjected to various minor skin wounds by draft craft hooks, metal bites, harnesses, etc., and major injuries by road accidents, fights, and close stabling with other animals (Tawdrous 1998). The wounds, injuries, or diseases affect the viability of donkeys and lower their ability to work, due to ignorance and lack of veterinary care (Burden and Thiemann 2015).

A wound is defined as any breakage in skin integrity due to injury or illness. A wound follows a cascade of events that begins with the trauma/injury and ends up with the complete and organized closure of the wound with the scar formation. Normal wound healing implies a network of synchronized biological processes that reinstate the integrity of tissue/skin after the injury (Harman et al. 2021). The basic principles of wound healing are debriding non-viable tissue, minimizing tissue damage, maximizing tissue perfusion, adequate oxygenation of affected tissue, proper nutrition, and a moist wound healing environment.

Wound healing is generally divided into three stages: inflammation, proliferation, and maturation/remodeling phase (Kumari et al. 2010). A wound heals primarily by the process of epithelialization and wound contraction, determining the closure of an open wound by inward movement of the surrounding tissue or skin. The process of re-epithelialization and wound contraction chiefly depends upon the proliferation of keratinocytes and fibroblasts, respectively.

The impaired proliferation of keratinocytes and fibroblasts may delay or complicate wound healing (Lee and Moon 2003).

The incidence of traumatic skin wounds is more prevalent in equines as compared to other species. Wounds on limbs, especially on the metacarpus and metatarsus are common in equines. These wounds form an excessive granulation tissue, contract, and epithelize at slower rates, thus healing after a prolonged time as compared with the other wounds in the body (Wilmink et al. 1999b). In equines, wounds at this site are often left to heal by the second intension, because of the considerable amount of tissue loss, contamination, extended inflammatory phase, excessive skin tension, greater retraction of margins, and inability to primary wound closure. Thus, wound healing by second intention in equines is related to such complications which are specie specific, particularly in the wounds which are present on the distal limb (Wilmink et al. 1999c).

Wound healing by second intension depends on the formation of granulation tissue to fill the wound bed, restoration, and reinstatement of the epithelial barrier through re-epithelization and wound contraction. In clinical practice, poor wound healing along with its delaying pathologies is ranked as the 2nd most common cause of death or euthanasia in equine patients (Sparks et al. 2020).

Appropriate methods and suitable approaches are the keys to promoting the efficient healing of wounds by restoring the disturbed functional status of the skin and repairing the damaged anatomical continuity of the tissue. Due to the increased drug resistance and cytotoxic effects of antibiotics and antiseptics, the role of bioactive materials in wound healing has been investigated by researchers. Among the biomaterials, chitosan is currently attracting much interest in medical and pharmaceutical preparations because of its biocompatibility, Biodegradability, bio-adhesion, hemostasis, and bacteriostatic and fungicidal properties, making it a safe and non-toxic drug. It is a biopolymer, obtained by the deacetylation of chitin (the second most abundant polysaccharide found in nature) and is mainly present in the shells of crustaceans. Chitosan is considered the most studied naturally occurring polymer for skin healing and repair, so is utilized in wound dressings or preparations. It helps in recovering the original tensile strength of the wound by promoting tissue granulation, organization and correct deposition of collagen fibers, and normal tissue regeneration (Zaid et al. 2017).

Chitosan prevents wound dehydration and contamination by forming a semi-permeable membrane sheet upon the wound that maintains a sterile environment beneath the dry scab. Porous membranes of chitosan control water loss through evaporation provide excellent permeability to oxygen and promote drainage of exudate from the wounds. The main biochemical effects of chitosan are the activation of fibroblasts, improving the function of polymorphonuclear leucocytes and macrophages, acceleration of the migration of macrophages, production of cytokines, and stimulation of type IV collagen synthesis. As chitosan has antibacterial and potent topical analgesic action, so there is no need to use other substances while treating the wound (Silva and Pighinelli 2017).

Honey has been used since ancient times by different populations, both for nutritional and biomedical uses. Honey, having approximately 17% of water, is a natural solution of supersaturated sugars including fructose, glucose, sucrose, maltose, and other types of carbohydrates. It exhibits antimicrobial, debriding, and deodorizing action, along with anti- inflammatory, antioxidant, and wound-healing properties. Honey is increasingly used as biological therapy in clinical practice such as acceleration of wound healing and care of ulcers, bed sores, and other infected skin wounds (Abd El-Malek et al. 2017). Honey aids in wound healing by keeping the wound moist while its high viscosity prevents wound infections. Its anti-inflammatory action helps in the growth of new epidermal tissue and the removal of exudate from the affected area. Because of its suitability in all the stages of wound healing, honey is commercially used for wound dressings and preparations. Many studies propose the combination of chitosan and honey for more effective wound healing and to get significant advantages in preventing the wound from infections (Wang et al. 2012).

Literature Review:

Donkeys

Donkeys (Equus asinus), descendants of African wild asses (Equus africanus), have played a vital role in human history, particularly as working and pack animals. Unfortunately, donkeys often suffer from skin wounds and injuries, primarily due to the rigors of agricultural work and transport activities. Factors such as limited resources, overwork, overloading, rough handling, and the use of ill-fitting equine accessories like harnesses and saddles contribute to the occurrence of skin injuries. Poor welfare and limited access to veterinary care can further exacerbate these issues. In developing countries, donkeys are essential for rural livelihoods, serving as crucial draught and transport animals (Mota-Rojas et al. 2021).

Skin

Skin, the body's largest organ, serves as a protective barrier against the external environment. Any injury or illness that disrupts skin integrity can result in a wound. Wound healing is a dynamic and complex process that aims to restore the skin's original structure and function. When wounds become chronic and fail to heal using standard treatments, they pose significant medical, social, and economic challenges (Cukjati et al. 2001).

Wound Healing in Equines

Equines, including horses and donkeys, often face challenges in healing wounds, particularly in the lower limb regions. Slow wound healing can lead to secondary infections, chronic bleeding, and plasma protein exudation, rendering the affected equine unproductive and devalued. The success of wound healing is closely linked to the regional blood supply in horses. Wounds in the distal limb region, which is sparsely covered with tissue and close to the underlying bone, tend to heal more slowly. The development of exuberant granulation tissue, resembling tumor growth, is a common clinical issue (Knottenbelt 1997).

Biomaterials

To address the challenges of chronic non-healing wounds, significant research has focused on developing safe and effective wound dressings. Traditional medicinal preparations that use biomaterials are often favored due to their increased bioavailability, biodegradability, efficacy, cost-effectiveness, and reduced side effects (Al-Musawi et al. 2020).

Chitosan

Chitosan, a biodegradable compound derived from the shells of crustaceans, has gained attention as an effective, biocompatible, and non-toxic material for wound healing. It exhibits antibacterial and hemostatic properties, promotes cell adhesion and proliferation, and accelerates wound healing (Patrulea et al. 2015). Chitosan helps inhibit the formation of excessive granulation tissue and promotes tissue growth through proper epithelialization (Silva and Pighinelli 2017).

Honey

Honey is known for its various bioactivities, including antioxidant and osmotic effects, antibacterial properties, analgesic properties, and the ability to modulate the initial inflammatory response in wound healing (Tsang et al. 2017). Its bioactive components help in sterile and effective wound healing in equines (Bischofberger et al. 2011).

Chitosan combined with Honey

The combination of chitosan and honey has shown promising results in wound healing. Hydrogel sheets prepared by combining chitosan and honey have proven effective for treating burn wounds (Wang et al. 2012). The positive charge on chitosan amido groups increases when combined with honey, enhancing its adsorption ability onto cells, making it an excellent wound-healing preparation (Radoor et al. 2021). The synergistic effects of honey and chitosan make their combination an ideal candidate for wound dressings (Sarhan and Azzazy 2015). This combination has also been used effectively for treating chronically infected wounds (Abd El-Malek et al. 2017).

In conclusion, donkeys, as essential working animals, often suffer from skin injuries. Wound healing is a complex process, particularly in equines, and wounds in the distal limb region can be challenging to treat. Biomaterials like chitosan and natural substances like honey have shown great promise in promoting wound healing. When used in combination, these materials have proven effective in improving wound closure, reducing complications, and enhancing the overall healing process in equines. The combined use of chitosan and honey appears to be a valuable approach for wound dressings and wound care in equines, offering potential benefits for both veterinary and human medical practices.

2. Materials And Methods

Study Area

The study was conducted at the Surgery Clinic and Indoor Hospital, the University of Veterinary and Animal Sciences (UVAS) in Lahore.

Ethical Statement

The study was approved with Institutional Guidelines of Ethical Review Committee, Office of Research Innovation and Commercialization at the University of Veterinary and Animal Sciences (UVAS), Lahore vide No: DR 82/ Dated: 22-02-2022.

Study Animals

The study was conducted on 12 donkeys presented with clinical wounds on limbs, irrespective of age, breed, and gender.

Inclusion Criteria / Exclusion Criteria

All donkeys with fresh cutaneous wounds (1-3 inches) on limbs were included in the study. All the animals that develop any other systemic illness were excluded from the study.

Pre-treatment Evaluation

A general examination was conducted to examine the general health condition of all the donkeys presented for treatment. Before treatment, the following information was recorded:

- Cause of Wound
- Nature of Wound (infected or non-infected)
- Wound Size

Consent of the Client

A written consent form for the study was designed to be signed by the owners of all the donkeys presented for the treatment.

Preparation of Site

The hair surrounding the wound site was shaved off. All the wounds were properly washed with Normal Saline before applying the respective treatment protocol.

Anesthesia

Local anesthesia (Lidocaine 2%) was used on the wound site before collecting the biopsy sample for histopathology (Kennedy 2004).

Preparation of Biomaterial

Chitosan gel was prepared (in PG Laboratory, IPS-UVAS) by using Chitosan powder (Chemsavers, Pakistan). Firstly 1% Acetic Acid solution is mixed with Chitosan powder, to prepare Chitosan solution 0.1%(w/v), by stirring it with a magnetic stirrer for 24 hours until completely dissolved. To make gel out of Chitosan Solution (liquid form), 1g of Carbopol-940 was added to the solution. The solution was mixed properly, to make 0.1% Chitosan Gel (Zaid *et al.* 2017).

In Group B, Chitosan Gel with Honey was used as the treatment protocol for wound healing. For this purpose, Chitosan Gel was mixed with local indigenous honey in equal proportions to make a topical dressing for the wounds.

Fig 1: Weighting of Chitosan Powder

Fig 2: Preparation of Chitosan Solution

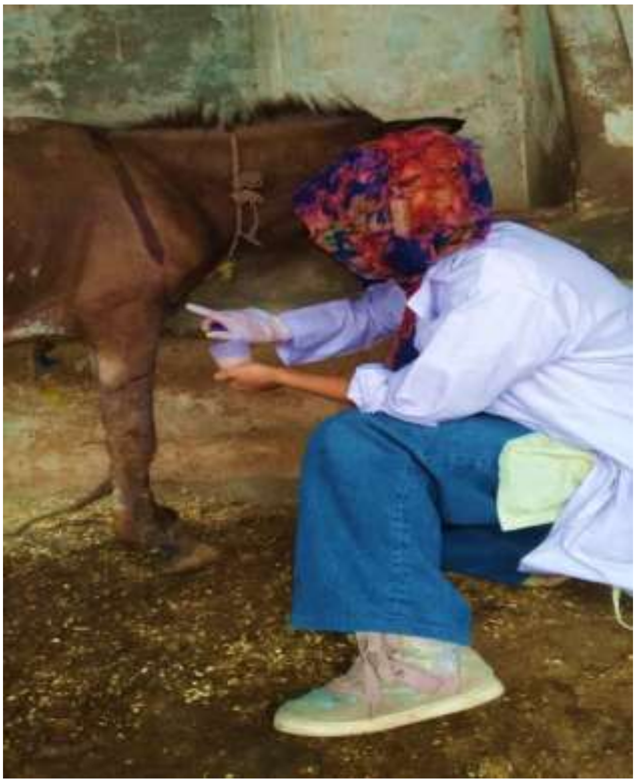
Fig 3: Magnetic Stirrer (12 Hours)

Fig 4: Chitosan Solution + Carbopol = Chitosan Gel

Experimental Design

12 donkeys were randomly divided into 2 groups i.e. A and B with 6 donkeys in each group. Group A was cured with chitosan gel without honey while Group B was treated by applying chitosan gel with honey.

Fig 5: Application of Chitosan Gel



Group A

The Donkeys from group A were named A1, A2, A3, and so on. Group A was a classical group in which Chitosan Gel without Honey was used to treat the affected donkeys. The surrounding hair was shaved off and the wound site was properly washed with Normal Saline. Then a direct application of Chitosan Gel was done on the wounds, until complete healing.

Group B

The Donkeys from group B were named B1, B2, B3, and so on. Group B was a classical group in which Chitosan Gel with Honey was used to treat the affected donkeys. The surrounding hair was shaved off and the wound site was properly washed with Normal Saline. Then Chitosan Gel mixed with Honey was directly applied to the wounds until completely healed.

Treatment Plan

The division of animals in Group A and B with their respective treatment protocols is given in the table below;

Table 1 Treatment Plan

Sr. No	Groups	No. of Animals	Treatment Protocol	Application	Frequency	Readings
1.	Group A	6 Donkeys A1, A2, A3, A4, A5, A6,	Chitosan Gel	Direct application with gauze	After 24 hours	Record Data till 28 Days
2.	Group B	6 Donkeys B1, B2, B3, B4, B5, B6	Chitosan Gel with Honey	Direct application with gauze	After 24 hours	Record Data till 28 Days

Post-operative Management

Daily wound dressing was done after 24 hours until complete wound healing. The wounds were recorded till the 28^{th} day of healing.

Parameters of Study

Macroscopic Evaluation (Wound Contraction)

Wound healing was evaluated by measuring the wound contraction rate at days 0, 7, 14, 21, and 28. The wound contraction rate was calculated by the following formula (Pawar et al., 2013).

$$\%Wound\ Contraction = \frac{initial\ wound\ size - specific\ day\ wound\ si}{initial\ wound\ size} \times 100$$

Wound surface area was measured by using Vernier Caliper, and applying the given formula:

$Wound\ Surface\ Area = Length(mm) \times Width(mm) =$ ______ mm^2

Fig 6: Measurement of Wound Size

Microscopic Evaluation (Histopathology)

Histopathology of wound samples of both groups was done to observe and compare the re-epithelization, granulation, angiogenesis, and fibrous connective tissue of the skin. Samples for histopathology were taken by biopsy punch (4mm), before the start of treatment (day 0) and on the 28th day. The tissue samples were fixed in 10% formalin for 48 hours. After rinsing and dehydration with alcohol, samples were embedded in paraffin. These 4-7µm thick sections were stained with hemotoxylin-eosin (H&E) stain and then photographed under 100X magnification (Nazar *et al.* 2015).

Statistical Design

Collected data regarding wound size and wound contraction were analyzed through Factorial ANOVA using PROC GLM in SAS software (Version 9.1). Significant treatment means were compared through the DMR test (Daniel and Cross 2018).

3. Results and Discussion

This study was conducted with the purpose of access the comparative efficacy of Chitosan Gel, with and without honey for cutaneous wound healing in donkeys. The following parameters were studied on each donkey:

Evaluation Parameters

Macroscopic Evaluation

a) Wound Size (cm)

b) Wound Contraction Rate (%)

Microscopic Evaluation

c) Histopathological Findings

- Inflammation
- Granulation
- Fibrous Connective Tissue
- Re-epithelization
- Angiogenesis.

Wound Size (cm)

The treatment protocol of the respective groups was applied from day 0 till the 28th day. Wound size and contraction in each donkey were measured by Vernier Calipers and the readings were taken on days 0, 7th, 14th, 21st, and 28th.

Collected data regarding wound size and wound contraction were analyzed through Factorial ANOVA using PROC GLM in SAS software (Version 9.1). Significant treatment means were compared through the DMR test. Statistical analysis showed that data is significant at $p \leq 0.05$.

Wound size of donkeys in Group A (Chitosan)

In Group A, 6 donkeys with skin wounds were treated with the Chitosan gel. The wounds were treated and observed till the 28th day. In Group A, 3 out of 6 wounds were completely healed by the 28th day. The mean wound size of donkeys on day 0 was 2.57 ±0.42, while on day 7 it was 1.91 ± 0.36. till day 14, wound size was reduced up to 1.22 ± 0.28. on day 21 size of the wound was found to be 0.51 ± 0.22. till day 28, half f the wound population was completely healed and the mean wound size was 0.51 ± 0.22.

Table 2: Mean and Standard Deviation of Wound Size (cm) included in Group A

Wound Size (cm)	A1	A2	A3	A4	A5	A6	Mean ± STD
Day 0	3.22	3.30	3.87	2.00	1.60	1.40	2.57 ±0.42
Day 7	2.50	2.56	2.98	1.36	1.09	0.96	1.91 ± 0.36
Day 14	1.75	1.69	2.07	0.71	0.58	0.50	1.22 ± 0.28
Day 21	1.05	0.66	1.18	0.07	0.05	0.04	0.51 ± 0.22
Day 28	0.40	0.08	0.28	healed	healed	healed	0.13 ± 0.07

Wound size of donkeys in Group B (Chitosan+Honey)

In Group B, 6 donkeys having cutaneous wounds were treated with the Chitosan gel and Honey. The mean wound size of donkeys on day 0 was 2.98 ±0.40, while on day 7 it was 2.08 ±

0.36. The reduced wound size on day 14 was 1.16 ± 0.32. Till day 21, out of 6 total wounds 1 wound was completely healed, and the mean wound size was found to be 0.38 ± 0.22. When measurements were taken on day 28, the mean wound size was 0.04 ± 0.02, with 4 wounds completely healed when treated with chitosan+honey in Group B.

Table 3: Mean and Standard Deviation of Wound Size (cm) included in Group B

Wound Size (cm)	B1	B2	B3	B4	B5	B6	Mean ± STD
Day 0	3.99	3.91	3.54	2.73	1.90	1.83	2.98 ± 0.40
Day 7	3.01	2.97	2.44	1.83	1.28	0.94	2.08 ± 0.36
Day 14	2.02	2.04	1.25	0.94	0.65	0.07	1.16 ± 0.32
Day 21	1.04	1.08	0.08	0.03	0.03	healed	0.38 ±0.22
Day 28	0.15	0.06	healed	healed	healed	healed	0.04 ± 0.02

Comparison of Mean Wound Size of all groups

The mean wound size of Group A and B on day 0 was 2.57±0.42 and 2.98 ± 0.40 respectively. It shows wounds included in Group B were comparatively of greater size than Group A. Mean wound size of Group A and B on day 7 was 1.91 ± 0.36 and 2.08 ± 0.36 respectively. On day 14, the mean wound size of group A was 1.22 ± 0.28 while in group B it was 1.16 ± 0.32. Till day 21, the mean wound size of group A was reduced to 0.51 ± 0.22 while in group B it decreased up to 0.38 ±0.22. Reaching the 28th day, the mean wound size of group A was 0.13 ± 0.07 while the mean wound size of group B showed that the majority of the wounds were healed with a mean wound size of 0.04 ± 0.02. Superscripts written on the mean value, stands for A1=a, A2=b, A3=c, A4=d, A5=e, and A6=f in Group A, while in Group B it is B1=a, B2=b, B3=c, B4=d, B5=e. The number of individuals mentioned in the superscripts were beings compared accordingly, by applying Factorial ANOVA using PROC GLM in SAS software, and data is significant at $p \leq 0.05$.

Table 4 Comparison of Mean Wound Size (cm) of Group A and B

Size (cm)	Day 0	Day7	Day 14	Day 21	Day 28
Group A	2.57^{ab} ±0.42	1.91^{bcd} ± 0.36	1.22^{cde} ± 0.28	0.51^{ef} ± 0.22	0.13^{f} ± 0.07
Group B	2.98^{a} ± 0.40	2.08^{bc} ± 0.36	1.16^{ed} ± 0.32	0.38^{ef} ±0.22	0.04^{f} ± 0.02

*Significant at $p \leq 0.05$.

Wound Contraction Rate (%)

Wound Contraction was measured in percentage and evaluated by the formula given in Materials and Methods (Chapter 3).

Wound Contraction Rate of Group A (Chitosan)

In group A (Chitosan gel), the mean wound contraction rate of all 6 wounds on day 7 was

27.18 ± 2.05, while on day 14, the mean wound contraction percentage was 39.50 ± 3.62. Till day 21 wounds of group A were 69.58 ± 6.45 percent healed. While, 3 wounds out of 6 wounds, were 100% healed till day 28, with a mean contraction rate of 87.68 ± 6.45.

Table 5 Wound Contraction Rate (%) of Group A (Chitosan)

Wound Contraction Rate (%)	A1	A2	A3	A4	A5	A6	Mean ± STD
Day 7	22.36	22.42	22.99	32.00	31.88	31.43	27.18 ± 2.05
Day 14	30.00	33.98	30.54	47.80	46.79	47.92	39.50 ± 3.62
Day 21	40.00	60.95	42.99	90.14	91.38	92.00	69.58 ± 6.45
Day 28	61.90	87.89	76.27	100	100	100	87.68 ± 6.45

Wound Contraction Rate of Group B (Chitosan+Honey)

In group B the wounds were treated with chitosan combined with honey. The mean wound contraction rate of all 6 wounds on day 7 was 32.32 ± 3.64, while on day 14, the mean wound contraction percentage was 50.56 ± 9.05. It means that till day 14, all the wounds were 50% healed when chitosan gel and honey were used as a treatment protocol. Till day 21, 1 out of 6 wounds is 100% healed with a mean contraction percentage of 80.23 ± 10.30. measurements showed that 4 out of 6 wounds were 100 % healed till day 28, with a mean contraction rate of 96.67 ± 2.40.

Table 6: Wound Contraction Rate (%) of Group B (Chitosan gel + Honey)

Wound Contraction Rate (%)	B1	B2	B3	B4	B5	B6	Mean ± STD
Day 7	24.56	24.04	31.07	32.97	32.63	48.63	32.32 ± 3.64
Day 14	32.89	31.31	48.77	48.63	49.21	92.55	50.56 ± 9.05
Day 21	48.51	47.05	93.60	96.81	95.38	100	80.23 ± 10.30
Day 28	85.58	94.44	100	100	100	100	96.67 ± 2.40

Comparison of Mean Wound Contraction Rate of all the groups

The mean wound contraction% of Group A and B on day 7 was 27.18 ± 2.05 and 32.32 ± 3.64, respectively. Till day 14, wounds of group A were contracted up to 39.50% ± 3.62, while wounds of group B were contracted up to 50.56% ± 9.05. wound contraction rate in group A till the 21st day was 69.58% ± 6.45, while in group B it is 80.23% ± 10.30. Reaching the 28th day wounds of group A were healed up to 87.68$^{\%}$ ± 6.45, while the wounds included in group B were efficiently healed up to 96.67$^{\%}$ ± 2.40. Superscripts written on the mean value, stands for A1=a, A2=b, A3=c, A4=d, A5=e, and A6=f in Group A, while in Group B it is B1=a, B2=b, B3=c, B4=d, B5=e. The number of individuals mentioned in the superscripts were beings compared accordingly, by applying Factorial ANOVA using PROC GLM in SAS software, and data is significant at $p \leq 0.05$.

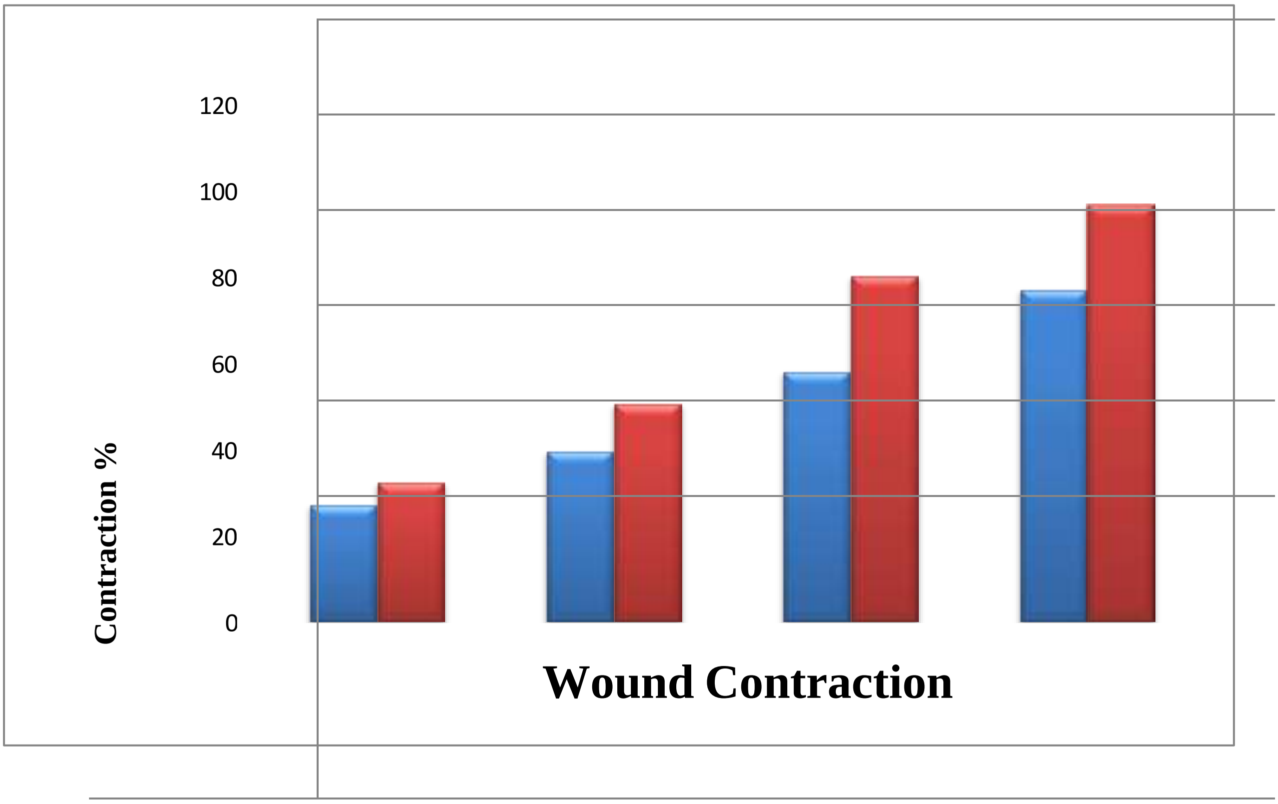
Table 7: Comparison of Mean Wound Contraction rate (%) of Group A and B

%	Day 7	Day 14	Day 21	Day 28
Group A	$27.18^{e} \pm 2.05$	$39.50^{de} \pm 3.62$	$69.58^{bc} \pm 6.45$	$87.68^{ab} \pm 6.45$
Group B	$32.32^{de} \pm 3.64$	$50.56^{dc} \pm 9.05$	$80.23^{ab} \pm 10.30$	$96.67^{a} \pm 2.40$

*Significant at $p \leq 0.05$.

Graphical Representation of Wound Contraction Rate of all groups

The wound size of Group A was compared with the wound size of Group B. The comparison of wound contraction rates of both treatment protocols is represented in Figure 4.1. In the graphical representation, Blue bars are denoting the wound contraction% of Group A (chitosan) while red bars are denoting the wound contraction% of Group B (chitosan+honey) at regular intervals. It is visible from the graph that wounds that were treated with chitosan gel combined with honey (group B) were contracted at a greater percentage and healed more effectively as compared to the wounds which were treated with chitosan gel without honey (group A).

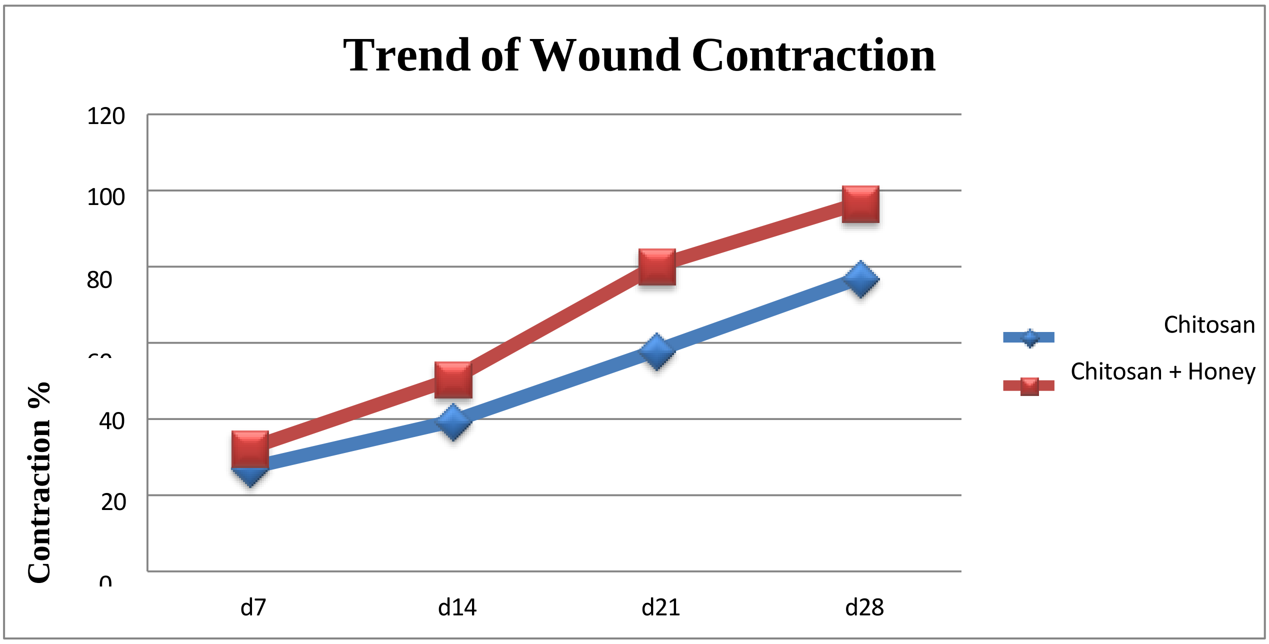


	d7	d14	d21	d28
Chitosan	27.18	39.5	57.99	77.01
Chitosan + Honey	32.32	50.56	80.23	96.67

Graph 1: Wound contraction rate (%) of Group A and Group B

The trend of Wound Contraction

The trend of graph 4.2 shows that in group B (chitosan + honey), there is a sudden increase in wound contraction percentage from day 14 to 21, i.e. 51% to 80%, respectively, as compared to the wound contraction percentage of Group A (chitosan) i.e. 40% to 58%, at day 14 and 21 respectively.



Graph 2: Trend of wound contraction rate (%) of group A (Chitosan) and group B (Chitosan+Honey) at regular intervals

Histopathological Findings

Histology was done to evaluate the granulation, inflammation, angiogenesis, and re- epithelization of wounds, before and after the treatment protocol of the respective groups. Hemotoxylin and Eosin staining was used for the histology of tissue samples.

Group A

Histopathological findings showed an influx of neutrophils on day 0 as evidence of acute inflammation and dead tissue was also seen microscopically. Following the treatment protocol of Chitosan Gel, 3 out of 6 wounds was completely healed before the 28th day, remaining 3 were in the process of healing. Histology was done on the 28th day. Those wounds which were completely healed before the 28th day, had a complete epidermal layer with scar formation, while the wounds which were in the process of healing had neutrophil and polymorphonuclear cells seen during histology, and also epidermal outgrowth on the wound edge was in progress.

Group B

Histopathological findings showed an influx of neutrophils on day 0 as evidence of acute inflammation and dead tissue was also seen microscopically. Following the treatment protocol of Chitosan Gel with honey, 1 out of 6 wounds were completely healed before the 21st day, 3 out of 6 wounds were completely healed before the 28th day, remaining 2 were in the process of healing till the 28th day. Histology was done on the 28th day. Those wounds which were completely healed before the 28th day, had a wound bed fully covered by new epidermal cells with scar formation, while those that were in the process of healing had neutrophil and polymorphonuclear cells seen, and epidermal outgrowth on the wound edge was in progress.

On histopathological examination, Group B seemed to have a higher number of fibroblast cells in the epidermal layer as compared to Group A. The wound-healing process in this Group B reached the third phase in almost 20 days (from inflammation to proliferation to maturation), while Group A's wounds were in transition from inflammation to proliferation in the same period.

Table 8: Comparison of Histopathological Findings of Group A and Group B

Treatment Groups	Inflammation	Granulation	Fibrous Connective tissue	Re- epithelization	Angiogenesis
Group A	+++	+++	++	++	+++
Group B	++	++	+++	+++	+++

Day 0 **Day 28**

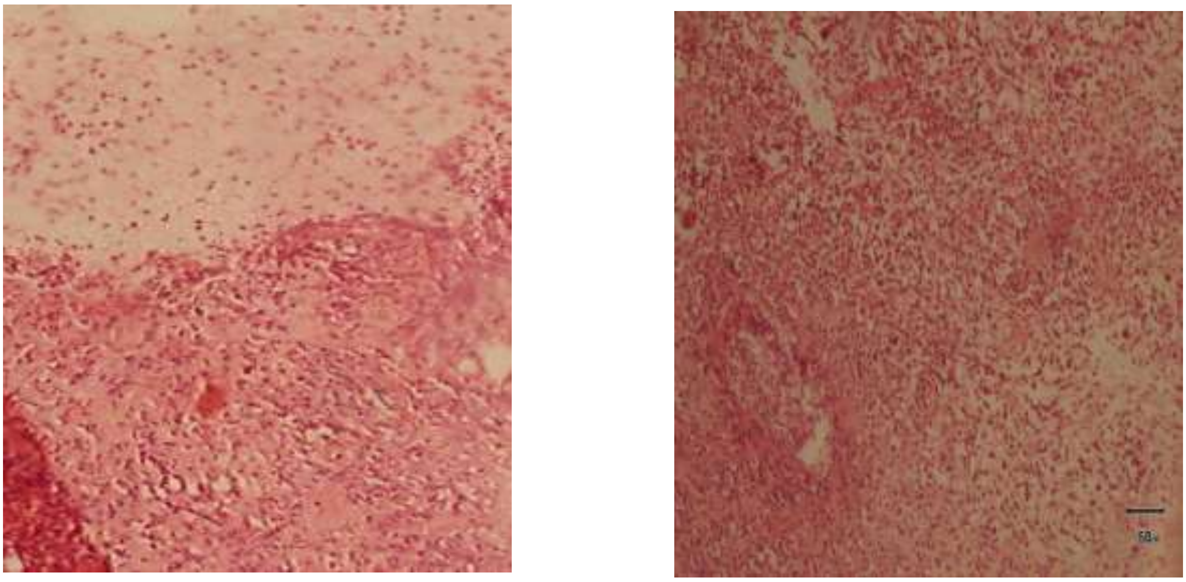


Fig 7: Histological view of wound at day 0 and day 28 (Group A-Chitosan)

Day 0 **Day 28**

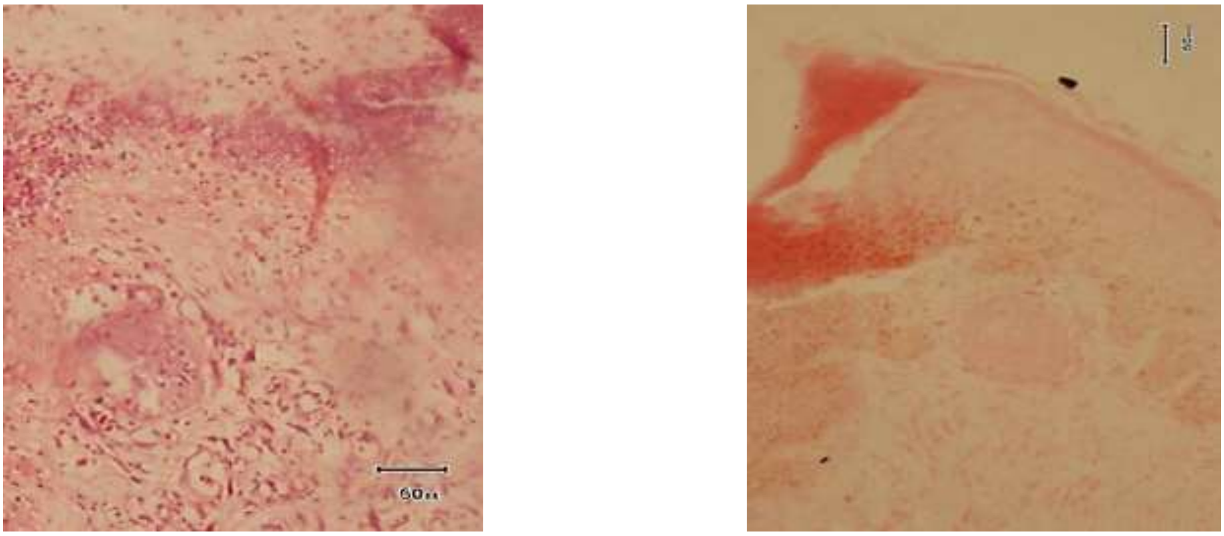


Fig 8: Histological view of wound at day 0 and day 28 (Group B-Chitosan+Honey)

The study aimed to evaluate the wound healing potential of chitosan-gel alone and in combination with honey on cutaneous wounds in donkeys. The results demonstrated that wounds treated with chitosan and honey (Group B) showed more effective healing compared to wounds treated with chitosan gel alone (Group A).

Factors Affecting Equine Wound Healing

Equine wounds, especially in the limbs, can be challenging to heal due to factors such as wound surface area contraction, epithelization, granulation tissue formation, and contamination. Hindrances in the wound healing process can lead to delayed healing and an increased risk of infection (Dart et al. 2009).

Exuberant granulation tissue formation can further delay the healing process, and factors that stimulate collagenase production or inhibit collagen synthesis can contribute to this issue (Schwartz et al. 2002). To improve equine wound healing, it is essential to promote wound contraction, epithelization, and the reduction of scar tissue formation, ultimately enhancing the chances of complete athletic effectiveness (Jørgensen et al. 2021).

Chitosan as a Wound Healing Agent

In Group A, chitosan was used in the form of a gel to promote tissue regeneration. Chitosan, known for its hemostatic, biocompatible, and biodegradable properties, acted as an appealing substrate for wound healing. Its antimicrobial properties not only prevented the spread of infection but also acted as a blood clotting accelerator (Al-Musawi et al. 2020). This study's findings are consistent with other research on the wound-healing properties of chitosan (Nooshabadi et al. 2020).

Honey as a Wound-Healing Agent

In Group B, chitosan gel was combined with honey to assess the effectiveness of both biomaterials. Honey, with its antimicrobial, anti-inflammatory, and wound-healing properties, has been recognized as a significant wound-healing booster (Bischofberger et al. 2011). The combination of chitosan and honey in this study proved to be a successful approach to enhancing wound healing in donkeys.

Comparing Group A and Group B

The results of the study indicated that wounds treated with chitosan and honey (Group B) showed better healing outcomes compared to wounds treated with chitosan gel alone (Group A). Similar findings have been reported in other studies, where the combination of chitosan and honey resulted in enhanced wound healing (Askari et al. 2022). The enhanced wound contraction observed in Group B is in line with previous research findings (Sarhan and Azzazy 2015).

Histopathological Findings

Histopathological analysis further supported the superior wound healing in Group B. The absence of exuberant granulation tissue and the presence of aligned dermis and proper re-epithelization in the histology samples of Group B indicate that wounds treated with chitosan-honey gel underwent a more organized healing process. The microscopic findings confirmed the efficacy of chitosan-honey gel in promoting tissue regeneration and stimulating fibroblast proliferation (Khodja et al. 2013).

Cosmetic Appearance and Re-epithelization

The wounds in Group B exhibited a more cosmetic appearance, aligned dermis, and proper re-epithelization. Fibrous connective tissue, angiogenesis, and minimal inflammation were observed, highlighting the effectiveness of chitosan-honey gel in promoting wound healing. This aligns with the findings of previous studies (Zaid et al. 2017).

In conclusion, the combination of chitosan and honey proved to be an effective approach to enhancing wound healing in donkeys. The wounds treated with chitosan-honey gel showed better wound contraction, histopathological findings, and cosmetic appearance compared to wounds treated with chitosan gel alone. The antimicrobial properties of both chitosan and honey contributed to preventing infection, and the combination of these natural substances is a promising option for wound dressings in equines. This study provides valuable insights into the use of biomaterials and natural agents for promoting wound healing in domesticated donkeys, with potential applications in equine and veterinary medicine.

4. Conclusion

In equines, particularly domesticated donkeys, cutaneous wounds are a common occurrence due to their role as working animals. However, the healing of such wounds can be challenging, characterized by slow progression, massive tissue loss, and the development of exuberant granulation tissue. To address these issues, this study investigated the potential of chitosan and honey, used individually and in combination, as natural wound-healing agents.The hypothesis that chitosan gel combined with honey would be more efficient in promoting antiseptic wound healing was tested. The study involved 12 donkeys divided into two groups: Group A treated with chitosan gel alone, and Group B treated with a combination of chitosan gel and honey. Wound contraction rates and histopathological changes were assessed over a 28-day period. The results clearly demonstrated the superiority of Group B, treated with chitosan gel and honey, in facilitating faster and more effective wound healing. Wounds in Group B exhibited a significantly higher rate of contraction and a more organized wound bed, with no exuberant granulation tissue formation. In contrast, Group A, treated with chitosan gel alone, showed less effective wound contraction and delayed healing. This research underscores the potential of chitosan and honey as a combined treatment for equine cutaneous wounds, offering a safe, natural, and efficient alternative to traditional synthetic medications. The study's findings have practical implications for enhancing the wound healing process in domesticated donkeys and, by extension, in other equines, contributing to improved animal welfare and economic benefits in regions where these animals play a vital role.

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