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The Efficacy of Medical Honey on Achilles Tendon Injury in Dogs (Biochemical Study).

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ABSTRACT

Objective: The goal of this study is to assess clinically and biochemicaly (Hydroxyproline content) the effect of medical honey on tendon injury. Materials and methods: 18 adult dogs were used for this study. Under general anesthesia and following aseptic technique, core lesions (defects) in the all animals left Achilles tendons were created by multiple perforations of needle gauge 18. All animals were classified into three groups according to local treatment; negative control (normal tendon from the right Achilles tendons), positive control (injured tendon treated with 0.9 % NaCl) and medical honey treated groups. Clinical evaluation and hydroxyproline content of the injured tendons were performed in different times post tendon injury. Results: after 6- and 9-days' post-surgery, there were significant decreasing lameness scores in the group that received medical honey as opposed to the control group. There was no difference in the operative site swelling between the honey-treated group and the positive control after the third- and sixth-days following surgery. However, the positive control group significantly showed decrease in swelling more than treated group at 12-, 15-, and 18-days post tendon injury. After 45 days' post-surgery, there was notable elevation in hydroxyproline levels in the honey-treated medical group as opposed to the control group., however, the negative control group still significantly have the higher content. Conclusion: Medical honey had analgesic, cost effective and positive impact on tendon tissue regeneration; the healing process periods was minimally reduced due to a maximal amount of hydroxyproline formation, in compare with positive control..

Keyword: *Achilles tendon, dog, honey, hydroxyproline*

1. INTRODUCTION

Tendon is a complexly organised connective tissue that efficiently conveys muscular force within the skeletal system. The shape, function, and physiology of tendon tissues demonstrate the significant, recurrent mechanical stresses they endure. The mechanical demands contribute to elevated clinical incidence of tendon illnesses and pose significant obstacles for their management (Snedeker and Foolen, 2017).

Tendons generally exhibit low cellularity, comprising mature fibroblasts or tenoblasts, as well as fibrocytes or tenocytes. Consequently, over 90% of the dry mass of tendons is composed of collagen, with minimal cellularity and other tissue elements including glycosaminoglycans and elastic fibres (Moshiri and Oryan, 2013).

The principal load-bearing The main component of tendons is type I collagen., protein distinguished by a systematic arrangement of amino acids, particularly glycine, proline, and hydroxyproline, along with significant modifications to its molecular structure throughout manufacture (Zabrzyński, et al. 2018).

One necessary amino acid is hydroxyproline (Hyp), which is found in collagen, elastin, and gelatin are examples of connective tissue proteins. Hydroxyproline is located at the Y position of the collagen structure, where it stabilises forming hydrogen bonds with nearby collagen α-chains to form the triple helix (Durairaj, et al. 2020).

Hydroxyproline is significantly concentrated in dehydrated collagen. Consequently, Hydroxyproline can be utilised to estimate the quantities of collagen in tissues. The quantification of Hydroxyproline in wound healing may be used in an indirect manner to measure the amount of collagen present in the afflicted areas. This measurement can signify the degree of new cell regeneration at damaged locations. Increased Hydroxyproline levels indicate the state of regeneration of tissue in wounds, reflecting tissue repair and healing (Durairaj, et al. 2020 and Al-Hussein, et al. 2023).

Tendon injuries can be categorised as tendinopathy or tendon rupture, with the latter presenting significant treatment issues for orthopaedic surgeons. Tendon injuries may result from hyperpronation, severe loading, and microtrauma. The Achilles tendon is one of the most frequently torn or damaged tendon (Lim, et. al. 2019; Abd Al-Hussein et al., 2023).

A damaged tendon can significantly impair normal body mobility and may result in several consequences if not treated*:*

swiftly and appropriately. Conventional treatments, including surgical repair and tissues grafting, are ineffectual and exhibit a high recurrence rate (Lim, et. al. 2019).

Tissue engineering provides several alternative treatment modalities, including biological tissue structures, stem cell therapy, cell-secreted products, and other therapy materials. A bioreactor with mechanical stimulation is essential for enhancing tendon damage (Lim, et. al. 2019).

Honey has been used in wound management throughout many civilisations globally for ages; nonetheless, it is regarded as "alternative healing" by numerous professionals (Lukanc, et al. 2018).

The antimicrobial qualities of honey are ascribed to a number of factors, such as its acidity, osmolarity, hydrogen peroxide concentration, and phytochemical components. Honey reduces pain because oedema-induced pressure on tissues and prostaglandins produced during inflammation lead nerve endings to become sensitized, causing discomfort in wounds. (Lukanc, et al. 2018)

The application of honey has lately attained clinical prominence for potential utilisation in wound therapy and regenerative medicine (Wani, et al. 2020). Honey is a harmless, natural ingredient that effectively inhibits bacterial growth and treats various sorts of wounds. Honey has a diverse array of active chemicals, such as flavonoids, phenolic acids, organic acids, enzymes, as well as vitamins, which may enhance the wound healing process (Tashkandi, 2021; Tobias and Johnston, 2012). Thus, our study's objective was to look into how well medical honey might treat wounds to tendons in canines clinically and biochemically (hydroxyproline concentration)

2. MATETERIALS AND METHODS:

Approval of experiment

The Research Committee of the Faculty of Veterinary Medicine of the University of Basra gave its clearance for the current work. For the duration starting from the outset, all animal procedures followed the rules set forth by the University of Basra's Faculty of Veterinary Medicineof (17 September 2024) to (11 November 2024).

Experimental animals

18 adult dogs, were used in this study weighing 17-24 kg. The animals were housed in designated separate kennels within the animal facility for a duration of two weeks before surgery for acclimatization. The dogs were injected with ivermectin at dose 0.1 mg/ kg B.W. (Plumb, 2018; Saleh et al.,2020), clinically examined, and provided with a food and water.

Surgically inducing tendon injury:

The site of operation was prepared surgically, and surgery was done at safety general anesthesia (by administration 2.2 mg/kg xylazine intramuscularly, followed by 11 mg/kg ketamine 10 minutes later IM had been injected (Plumb, 2018; Abduljaleel, 2024).

A midline incision was performed through the skin, subcutaneous tissue, and fascia, located distal of the gastrocnemius muscle and proximal to the calcaneus (Figure 1). Core lesions (defects) in the all animals left Achilles tendons were created by multiple perforations of needle gauge 18 (Figure 2). A stay suture knot was applied distal to the defect as a marker of the lesion (Figure 3). All animals were classified into 3 groups according to local treatment as the following: negative control (normal tendon from the right Achilles tendons), positive control (injured tendon treated with 0.9 % NaCl) and medical honey treated group. Eventuallythe skin was closed with simple interrupted pattern and Robert-Jones bandages were applied for a week to rehabilitate moving the leg gradually (allows gradual loading of the tendon) (DeCamp, et al. 2016). Animals transferred to the animal house and were given antibacterial agent Cefteriaxone 22 mg/Kg b. w. for 3 days post operatively.

Figure 1: showed the left leg was prepared surgically

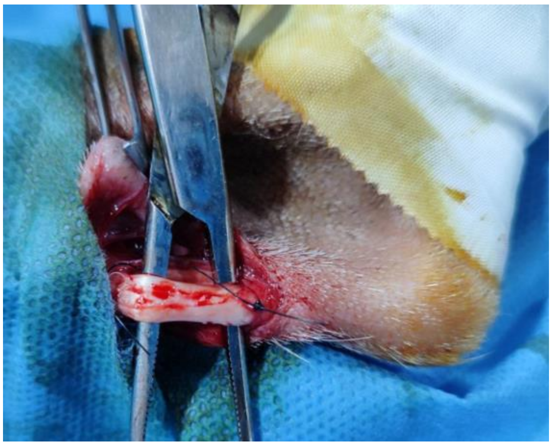


Figure 3: Multi penetration in the tendon were performed by using an 18-gauge needle

Figure 4: stay suture were placed proximal to tendon defects

CLINICAL EVALUATION

A clinical assessment of the post-surgical wound was performed, taking into account the animal's overall health, evaluations of oedema, as well as lameness ratings.

The swelling scores were categorized as evident 3, mild 1, and non 0 (Muhammad & Naeem, 2022; Saleh et al.,2023). The lameness scores classified from the best to the worse 1-4 as we mentioned it in table 1.

Table 1: Dogs with clinical lameness rating system (Nganvongpanit, et al. 2013)

Gauge	Scale	Clinical assessment
Lameness	1	walks in a typical manner
	2	Walking a little halting
	3	Walking with a moderate lack of energy
	4	Very sluggish when walking

HYDROXYPROLINE CONTENT EVALUATION

Tendon samples were collected at 15-, 30-, and 45-days' post-surgery from the negative control, positive control, as well as medical honey-treated groups, and were preserved in a buffer solution. The analysis of collagen and cross-links was conducted using the measurement of hydroxyproline. Samples were obtained as previously stated, and Their level of hydroxyproline was measured with a modified spectrophotometric approach.

The samples underwent hydrolysis in 6 molar HCl at 105°C for a duration of 14 to 16 hours. Hydroxyproline underwent oxidation with chloramines T, followed by treatment with A chromophore is created by incubation at 60°C with Ehrlich's reagent. By extracting the hydroxyproline product from the alkaline medium into toluene and subsequently into the acidic phase, 47 interfering chromophores were removed. The absorbance of the acid phase was measured at 543 nm, and the hydroxyproline percentage was computed using a calibration curve created from reference solutions that were examined similarly to the samples.

Each tendon sample weighing 50–100 mg was placed on a plate and dried for three hours at 100°C in an oven. while hydroxyproline assay sampling was taking place in in order to determine each sample's dry matter percentage. The hydroxyproline concentration in tendon samples was measured in mg/g DM (Al-Hussein, et al. 2023; Al-Khalifah, et al. 2019).

Statistical analysis

The mean values plus standard errors were used to present the results. The data was statistically analyzed using the Independent Samples Test and One-Way ANOVA with multiple comparison tests using the statistical software program SPSS for Windows version 22, USA. Significant differences were defined as $P < 0.05$.) Jasim et al.,2025)

3. RESULTS

During period of this study, general health status was examined daily and it remained stable. Lameness was assessed on a hard surface onlyThree days post-surgery, all animals exhibited a same level of lameness, however, gait gradually improved in the medical honey treated group and it was significant after 6 days' post tendon injury. In addition, at the 9th days, there was significant improvement in decreasing lameness scores in treated group in compare to the positive control group (figure 4 table2).

After the 3rd and 6th day of surgery, the sites of injury showed a slight increase swelling in medical honey treated group more than positive control, but it was not significant. In addition, there was increasing in injury swelling in both groups. However, there was no significant difference between them. At the 12, 15, and 18 days post tendon injury, the local swelling decreased gradually, however, the positive control group significantly showed decrease in swelling more than treated group (figure 5, table 3).

Biochemical result of the hydroxyproline examination of the tendons after 15 and 30 days of surgery showed no difference between positive control and medical honey treated group. However, there was a significantly high hydroxyproline content in the normal tendon of negative control (normal tendons) in compare with other groups.

After 45 days post-surgery, There was a notable rise in hydroxyproline content in medical honey treated group in compare to positive control group, but the negative control group still significantly have the higher content of this amino acid in compare with other groups (figure 6 table 4).

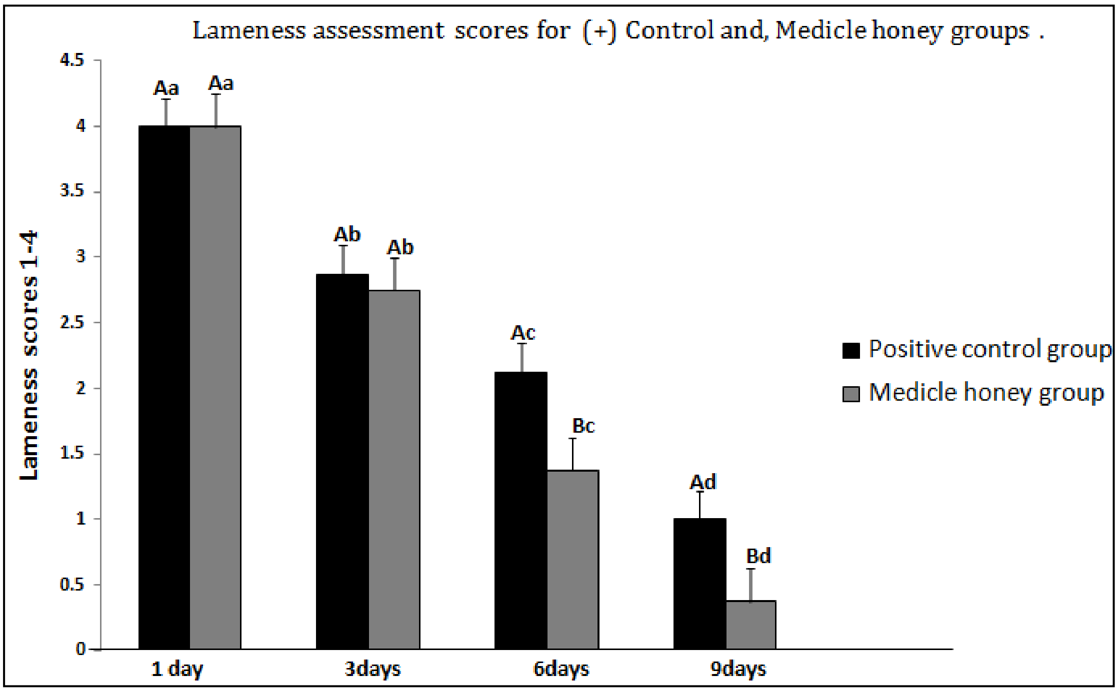
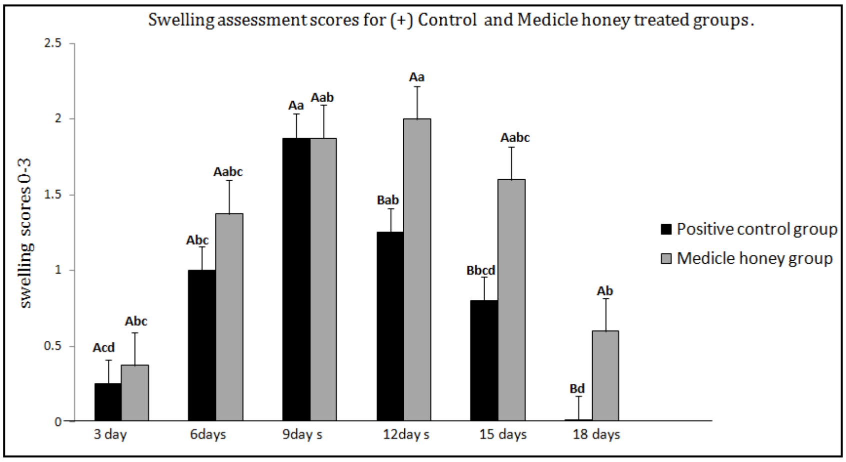


Figure 4: Clinical research, evaluation of the lameness score for positive control, and medical honey at 1-, 3-, 6-, and 9-days following tendon damage. ABC Different letters between groups indicate significant differences ($P<0.05$). ABCD Within the group, different letters indicate significant differences ($P<0.05$).

Table 2: Clinical study, lameness score assessment for positive control, medical honey and PRF groups at 1, 3, 6 and 9 days after tendon injury (means and standard errors).

Groups	1day	3 days	6 days	9 days
Positive Control	4± 0.00 ^{Aa}	2.875±0.22 ^{Ab}	2.125±0.12 ^{Ac}	1±0.00 ^{Ad}
Medical honey	4±0.00 ^{Aa}	2.75±0.25 ^{Ab}	1.375±0.18 ^{Bc}	0.375±0.18 ^{Bd}

ABC Different letters between groups indicate significant differences (P<0.05). ABCD Within the group, different letters indicate significant differences (P<0.05).



Group. Figure 1.Clinical study, swelling wound assessments for control (+), medical honey groups after 3, 6, 9, 12, 15 and 18 days after tendon injury. ABC. Different letters indicate significant differences (P<0.05) between groups. ABCD Various letters within the group indicate significant differences (P<0.05).

Figure 1: Clinical study, swelling wound assessments for control (-), medical honey and PRF groups after 3, 6, 9, 12, 15 and 18 days after tendon injury (means and standard errors).

groups	3days	6 days	9 days	12 days	15 days	18 days
positive Control	0. 25 ±0.16 ^{Acd}	1.00 ±0.26 ^{Abc}	1.875 ±0.12 ^{Aa}	1.25 ±0.16 ^{Bab}	0.80 ±0.20 ^{Bbcd}	0.00 ±0.00 ^{Bd}
Medical honey	0.375 ± 0.22 ^{Abc}	1.50 ±0.37 ^{Aabc}	1.875 ±0.29 ^{Aab}	2.00 ±0.26 ^{Aa}	1.6 ±0.24 ^{Aabc}	0.6 ±0.24 ^{Ab}

ABC Different letters between groups indicate significant differences (P<0.05). ABCDE Within the group, different letters indicate significant differences (P<0.05).

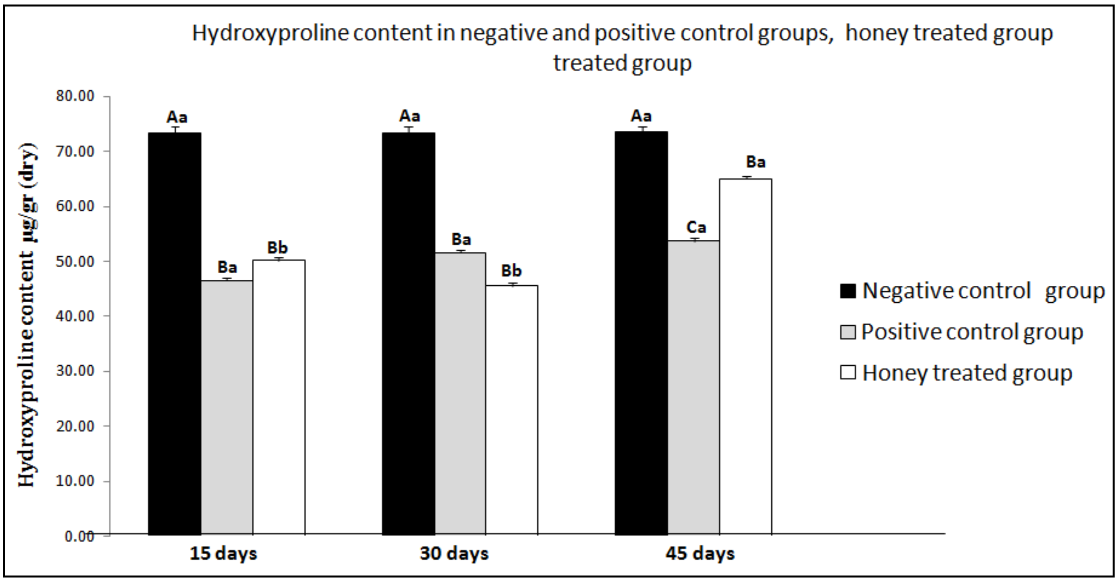


Figure 1: Biochemical study, hydroxyproline concentration assessment for normal negative control and positive control and medical honey groups at 15, 30 and 45 days after tendon injury. ABC Significant differences (P<0.05) are shown by different letters between groups. Significant differences (P<0.05) are indicated by different letters within a group.

table 1: Biochemical study, hydroxyproline concentration assessment for (-) control, (+) control and medical honey groups at 15, 30 and 45 days after tendon injury. (Means and standard errors).

groups	15day	30 days	45 days
Negative Control	73.46 ±1.05^{Aa}	73.40±1.09^{Aa}	73.53±1.01^{Aa}
Positive Control	46.483± 0.46^{Ba}	51.529±3.3^{Ba}	53.716±1.24^{Ca}
Medical honey	50.16±0.55^{Bb}	45.551±2.12^{Bb}	64.995±0.94^{Ba}

ABC Different letters between groups indicate significant differences (P<0.05). Within a group, different letters indicate significant differences (P<0.05).

DISCUSSION

Tendons, a connective tissue primarily made of collagen fibers, have certain mechanical characteristics that are necessary for functional movements. Growing interest in Regenerative medicine and tissue engineering is correlated with an increase in tendon injuries and ruptures. Enhancing our Animal models are necessary to understand how the tendon heals after severe trauma or acute transections. Thus, Preclinical model selection is crucial for successful translation of safe and efficacious novel pharmaceuticals into clinical application (Bottagisio and Lovati, 2017; Naeem et al.,2020).

This experiment's goal is to assess clinically and biochemicaly (Hydroxyproline content) the effect of medical honey on tendon injury.

Following the induction of tendon damage in the positive control group, we employed Sterile saline at 0.9% for lavage of wounds of the defects. It is utilised for wound cleansing and has minimal impact on diminishing bacterial load. Isotonic fluids are regarded as the optimal selection, because they are non-toxic and don't cause electrolyte imbalance or cellular lysis (Lukanc, *et al.* 2018).

The clinical examinations after 6 days post tendon injury showed that the lameness gradually improved in the medical honey treatedgroup, after 9 days; there was significant improvement in decreasing lameness scores in this treated group

The body's inflammatory response initiates the healing process; however, an extended reaction can impede healing, resulting in further tissue damage and complicating wound management. An extended inflammatory response is frequently linked to elevated exudate levels. Mitigating inflammation and alleviating discomfort for the patient diminishes vasodilation, hence decreasing oedema and exudate. The capacity of honey to eradicate infections and debride wounds is believed to enhance its anti-inflammatory properties. The mechanism via which honey alleviates excessive inflammation remains unidentified, but it has been proposed that it may be associated with the antioxidants in honey neutralising free radicals (Wani, *et al.* 2020; Ibrahim et al., 2020). Consequently, Mu-receptor blockers can experimentally counteract the anti-nociceptive and analgesic effects of honey and some flavonoids,indicating their resemblance to opioid analgesics (Pleeging, *et al.* 2022).

After the 3rd and 6th day of surgery, there were increasing in injury swelling in both groups. However, after 12 days of the tendon injury, the local swelling decreased gradually and the positive control group significantly showed decrease in swelling more than treated group.

Honey exerts immunomodulatory effects on injured tissue due to its c cytokine-releasing characteristics that impact the cells surrounding the wound, including monocytes/macrophages, keratinocytes, endothelial cells, neutrophils, and fibroblasts. During the inflammatory phase of wound healing, the initial inflammatory response is exacerbated by the release of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, which is essential for wound repair (Pleeging, *et al.* 2022; Alrafas et al.,2023).

After 15- and 30-days' post-surgery, the hydroxyproline content showed the same low level in both injured tendons of positive control and medical honey treated group in compare with negative control group. However, there was a significant increasing in hydroxyproline content in medical honey treated group after 45 days in compare with positive control. The negative control still significantly had a higher level of hydroxyproline more than other groups at this period.

Honey may help heal wounds because it contains a number of active components, including vitamins, enzymes, organic acids, phenolic acids, and flavonoids. Honey's high amino acid content may encourage the formation of collagen and fibroblast deposition (Tashkandi, 2021).

In the phase of proliferation, myofibroblasts that are actin-positive in α-smooth muscle synthesise extracellular matrix constituents, as fibronectin, collagens, Elastin, proteoglycans, and other substances are necessary for effective wound healing. The persistent presence of myofibroblasts, caused by oxidative and inflammatory stressors, results in severe scarring because of the ongoing creation of extracellular matrix components and contraction (Klingberg, et al. 2013; Berglund, et al. 2010; Jassim et al., 2023).

Honey may encourage myofibroblasts to undergo apoptosis. Having a sufficient vascular structure is necessary to supply nutrients and oxygen,as well as promoting swift tissue repair. Honey consistently exhibits pro-angiogenic activity in wounds and can promote the development of VEGFR-II and VEGF, two important angiogenic mediators. Moreover, honey has been demonstrated to facilitate successive phases of wound healing, encompassing both the healing process and the deposition of collagen I and III (Pleeging, *et al.* 2022).

CONCLUSION

Medical honey had analgesic, cost effective and positive impact on tendon tissue regeneration; the healing process periods was minimally reduced due to a maximal amount of hydroxyproline formation, in compare with positive contro

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