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Effects of Platelets Rich Plasma and N Acetyl Cysteine on Achilles Tendon Regeneration in Dog

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: The Achilles tendon is one of the most important tendons in the body. When any problem or defect occurs, such as (cut, tear or puncture), it may hinder the motor functions of the foot. The recent study highlights the effective role of N_acetyl cysteine and Platelet_rich plasma in accelerating the healing process of the tendon.
Aim of the Study: The present study aimed to study and compare the effects of Platelets rich plasma and N acetyl cysteine on Achilles tendon regeneration after tendon defect inducing in dog**.**
Materials and Methods: Fifteen adult dogs were utilized in this study , the dogs were divided into three groups(5 dogs for each one): Group A(PRP group) was treated with PRP topically on the

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tendon, Group B(N-AC group) in which tendon defect was treatedby topical application of N-acetylcysteine , while in Group C(control group) the tendon defect was left without treatment.The animal was aneshtetized and the right hind limb was prepared for aspetic surgery , Achilles tendon defect was created by using needle 18 guage (core lesion) and the site of operation was closed routinally Achilles tendon healing and histopathological monitoring were evaluated at 42 days post-injury.

Results: Throughout the study, the participants' general health was assessed daily. Results showed that tendon healing was more successful in the PRP- and NAC-treated tendon than in the untreated tendon, with significant improvement in the treated groups after 42 days.

Discussion: Our study has shown that utilizing PRP and NAC for treating tendons is successful in preserving certain structural characteristics during the initial stage of tendon remodeling due to the growth factors in PRP and the NAC's capability in assisting TSPCs to survive and differentiate helps with tendon recovery after damage.

Keywords: Tendon injury; achilles tendon; platelet-rich plasma; N-acetylcysteine; canines.

1. INTRODUCTION

1.1 Tendons

Tendons are dense fibrous connective tissues that attach muscles to bones in order to aid in joint movement (Abou Neel et al., 2013).

The epitenon, a connective tissue layer, shields the outer part of the tendon and smoothly links with the endotenon. The epitenon's primary function is to facilitate the movement of various structural units and provide pathways for blood vessels, lymphatic vessels, and nerve structures. The paratenon is located away from the joint and is a loose connective tissue that surrounds the tendon, aiding in its smooth movement under the skin (Lopez & Jung, 2015).

Limited healing ability and higher chances of recurrence in tendon and ligament injuries and degeneration may be due to the slow metabolic rate of these tissues (Andia & Abate, 2013).

The Achilles Tendon is the strongest tendon in the musculoskeletal system of canines and plays a crucial role in controlling hock extension for movement and balancing on the balls of the feet. Its main function is to propel forward, providing passive support for the hind limb and hock (Rafat et al., 2022).

The most frequent sudden injury is a torn Achilles tendon Achilles Tendon is often the most frequently ruptured tendon in the lower part of the body (Shamrock et al., 2023).

Tendon injuries take a long time to heal, and the repaired tissue is typically not as functional as normal tendon tissue, resulting in a high rate of re-injury the natural healing process of damaged tendons depends on tenocytes' capacity to multiply and generate extracellular matrix (ECM), containing primarily collagen and proteoglycans the reason for tendons having a poor healing tendency is believed to be due to the high ratio of ECM to cells (Raikin et al., 2013).

platelet rich Platelets (PRP): A particular component in blood containing powerful growth factors has sparked international attention as a potential biological therapy for tendinosis and tendon tears (Kajikawa et al., 2008).

Research in the clinical field has suggested that growth factors extracted from platelets in platelet rich plasma (PRP) may have the ability to assist in treating different disorders and improve the healing process of tendons and ligaments under traumatic and degenerative circumstances (Kaux et al., 2020).

They trigger the cell surface receptors and intracellular signaling proteins regulate the maintenance and regeneration processes (Molloy et al., 2003).

Additionally, PRP is able to stimulate tendon stem/progenitor cells (TSCs) to differentiate into functional tenocytes, resulting in higher collagen synthesis and assisting in the recovery of injured tendons (Zhang & Wang, 2010).

Calcium chloride ($CaCl_2$): Calcium chloride ($CaCl_2$) is a frequently utilized activator in PRP (platelet-rich plasma) therapy. Nevertheless, there are possible side effects linked to its use: such as (Pain and discomfort, Hematoma, Infection, and Allergic reaction). It's essential to recognize that these side effects are usually mild and short-lived (Margono et al., 2022).

N-acetylcysteine (NAC): NAC is able to effectively reduce the buildup of ROS and reduce inflammation,making it valuable for tendon repair and preventing injury formation (Hsiao et al., 2019). Studies on animals have demonstrated that NAC has a strong ability to protect against oxidative stress and inflammation in various situations, such as enhancing recovery from brain damage caused by temporary cerebral ischemia, as well as managing pain and inflammation during infections (Crupi et al., 2020).

2. MATERIALS AND METHODS

Ethics statement: The animal ethics committee The University of Basrah's College of Veterinary Medicine has approved the study at and experiments in accordance with the approved number (16/37/2024). All steps outlined in the research were conducted in accordance with the public guidelines for animal experiments set by the institution the animals were kept in standard housing. Furthermore, the tasks specified in the report were carried out in compliance with regulations, including administering the rabies vaccine to the dogs and using drugs such as Ivermectin 1% for parasite control the medication was injected subcutaneously at a dose of 0.4 mg for each kg of body weight (Abdulmawjood & Thanoon, 2022).

Experimental Animals: This study involved fifteen adult dogs, each 1 year old and weighing an average of 23kg. The dogs were given time to adjust to the surroundings for a week prior to the beginning of the research. They were kept in separate cages at the College's animal facility within Basrah's veterinary medicine university and were subjected to the identical conditions. The dogs received rabies shots and were medically inspected for their well-being. Afterwards, they were split into three separate groups with each group having 5 dogs.

Preparation of autologous PRP: PRP was generated during the operation by collecting 20 ml of blood from the cephalic vein, which was subsequently split into two test tubes (10 ml each) containing 0.5 ml of 3.2% sodium citrate. Following a 10-minute spin at 1500 rpm, the liquid on top was discarded, and the remaining blood now represents the PRP (2 ml). Once more, the section was rotated at 1500 rpm for 15 minutes prior to being activated with two drops of 10% calcium chloride (Anitua et al., 2012).

Surgical procedure: A sterile procedure was carried out on the surgical area of the back right leg. Prior to giving anesthesia, the patient refrained from eating for 12 hours and drinking for 3 hours. The dogs were given anesthesia using Administer xylazine-hydrochloride 2% at a dose of 5 mg per kg of body weight via intramuscular injection, along with ketamine-hydrochloride 10% at a dose of 10 mg per kg of body weight via intramuscular injection (Landry & Maza, 2020).

Surgical operation: involved positioning the animal on its right side with the hind leg facing up, conducting a skin incision on the longitudinal right hind limb about 3 cm below the gastrocnemius muscle and 3 cm above the calcaneus to reveal the Achilles tendon. Sutures were placed at both ends of the tendon to identify the central injury, creating a 2 cm defect by puncturing the tendon using a needle. Group A received local treatment with Platelet Rich Plasma, group B with NAC, and group C received no treatment before muscle closure with simple continuous sutures and skin closure with a cross mattress technique using 2-0 USP Vicryl chromic stitches.

Histopathology assessments: The Achilles tendon, including the defect site, was cut approximately 3 cm above and below the Achilles tendon defect for histological examination. The tissue was then placed in 10% formalin for 24 hours, mounted on a glass slide, and stained with hematoxylin and eosin (H&E) The number of examiners for the sample was one person.

3. RESULTS

histopathological Features of the Achilles Tendon: The histopathological evaluation of the Achilles tendon revealed notable disparities between the treatment groups and the positive control group. The Achilles tendon in the control positive group had signs of chronic tendinopathy, specifically chronic Achilles tendinitis. These signs included collagen fibers that were scattered, weak, and disorganized, fibroblast cells that had lost their spindle shape, tenocytes that were missing or clustered, vacuolation, fibrin deposition, and congestion (1. 1). In addition, there was a significant presence of inflammatory cells at the site of inflammation Moreover, the majority of the inflammatory cells that entered the damaged area were lymphocytes, and there were also some noticeable giant cells present (Fig. 2). Additionally, we detected fibrin deposition in the location where infiltrated inflammatory cells had accumulated (Fig. 2). The distorted spindle shape of fibroblasts and vacuolation of tenocytes were very obvious in the affected area.

The histological impact of the ANC-treated group on Achilles tendon damage can include several key changes and improvements in the tissue structure and cellular composition. After ANC treatment, collagen fibers may be better organized and aligned, there may be more collagen in the extracellular matrix, and tenocytes may multiply more quickly. We observed nearly normal fibroblasts, along with thin, disorganized collagen fibers and vacuolated tenocytes (Figs. 3). Furthermore, newly formed blood vessels were evident in the affected part. The ANC group's treatment results demonstrated varying levels of response. Some animals in this group exhibited significant underdevelopment of tendon tissue, characterized by a dense presence of inflammatory cells and disorganized fibers. However, there was an increase in blood supply, as well as the formation of new blood vessels at the site of inflammation, accompanied by mild bleeding (Fig. 4). Also, collagen fibers that are spread out, weak, and disorganized, fibroblast cells that have lost their spindle shape, and tenocytes that are missing or have empty spaces inside them are all signs of chronic tendonitis. However, there was an increase in blood supply, as well as the formation of new blood vessels at the site of inflammation (Fig. 4). Furthermore, inflammatory cell infiltration coincided with increased blood supply at the inflammation site

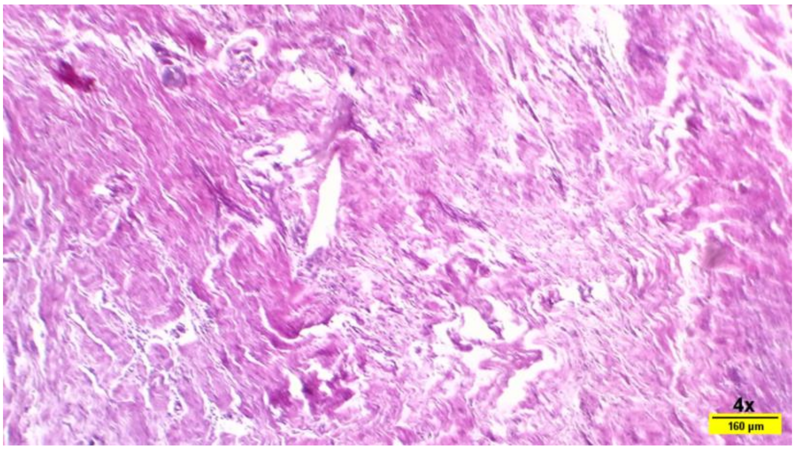

Fig. 1. A section of the Achilles tendon of the positive control group showed chronic tendonitis characterized by scattered, weak, and disorganized collagen fibers, loss of spindle shape in fibroblast cells, missing or clustered tenocytes, vacuolation, and fibrin deposition. H&E, 4x (Control)

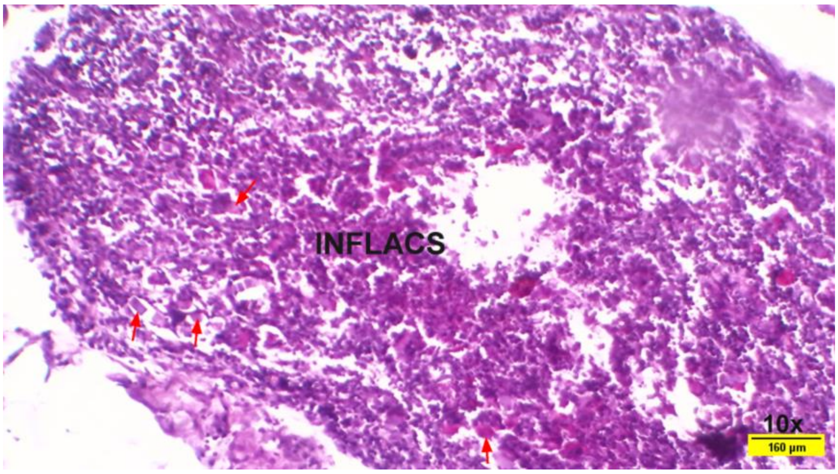

Fig. 2. A section of the Achilles tendon of the positive control group showed chronic tendonitis that characterized by presence of severe infiltration of inflammatory cells (INFLACs) and deposition of fibrin (red arrows) in the inflammation site. H&E, 10x (Control)

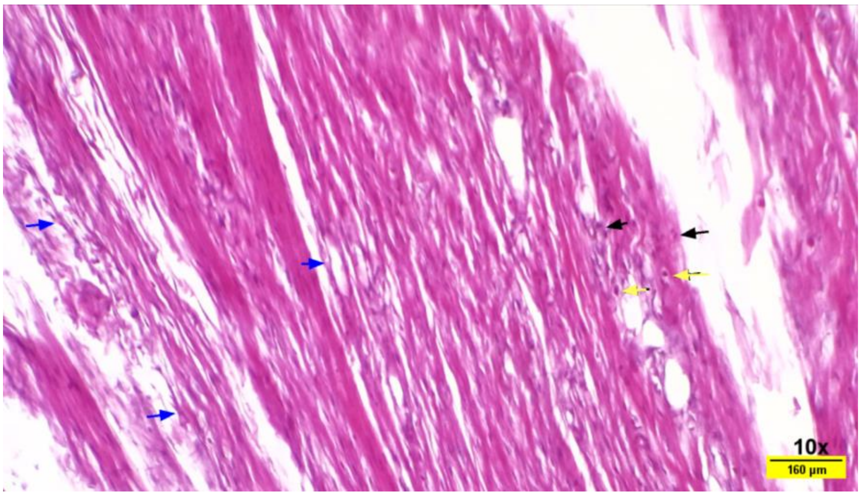

Fig. 3. A section of the Achilles tendon from the ANC-treated group exhibited regenerative features including improved organization and alignment of collagen fibers, increased collagen content within the tendon matrix, and heightened proliferation of tenocytes (black arrows). We observed nearly normal fibroblasts, along with thin, disorganized collagen fibers (blue arrows) and vacuolated tenocytes (yellow arrows). H&E, 10x

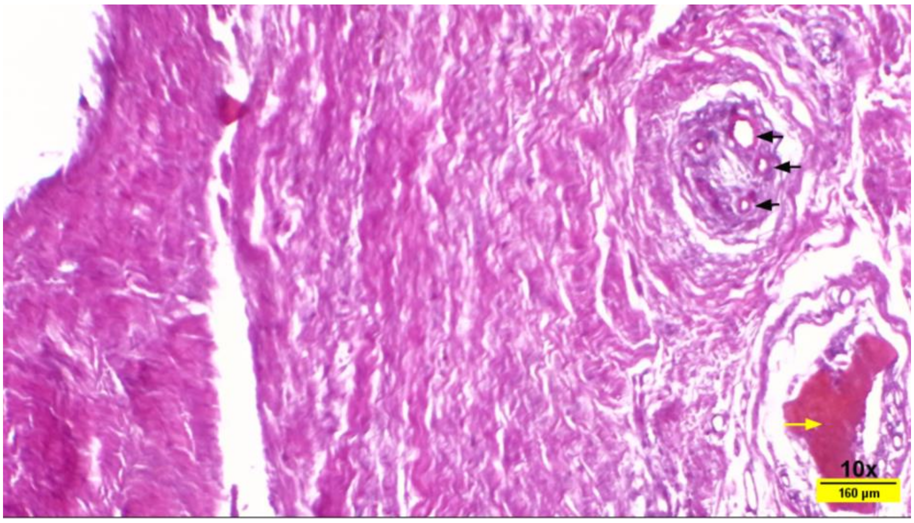

Fig. 4. A section of the Achilles tendon from the ANC-treated group exhibited chronic tendonitis including disorganized collagen fibers. However, there was an increase in blood supply and the formation of new blood vessels (black arrows) at the site of inflammation, accompanied by mild bleeding (yellow arrow). H&E, 10x

The PRP-treated group showed significant improvement, with the tendon appearing mostly natural in composition. Consistent and dense collagen was observed, along with dense external material filling the tendon. The shape of the arch cells and tenocytes appeared normal, and a blood bias pad was present in the tendon. While most animals in the group showed a good response, there was some variability. In a few cases, weak scattered fibers were noted, along with new blood vessels and a few inflammatory cells. Parallel, slightly wavy collagen bundles was observed, along with enhanced extracellular matrix production (Fig. 5). Furthermore, slender, elongated fibroblast cell nuclei, normal tenocytes, and blood vessels were evident (Fig. 6). Although this group exhibited several favorable histological changes and regeneration, there was also an evident loss of nuclei in a small number of collagen bundles (Fig. 7).

Analysis of the Achilles Tendon Healing based on Histopathological Changes: Distinguishing between the positive control, ANC, and PRP groups can be achieved by analyzing the documented histological characteristics. To identify the group that exhibited the most favorable response to therapy, it was necessary to examine and compare the observed qualities within each group. In the absence of therapeutic intervention, the positive control group was displayed the inherent or untreated structure of the tendon. In the control positive group, the Achilles tendon showed chronic tendinopathy, especially tendinitis. The collagen fibers were weak, spread out, and disorganized; the fibroblast cells had lost their spindle shape; the tenocytes were either missing or grouped; and there were empty spaces, fibrin deposits, and swelling. In addition, inflammatory cells were abundant at the inflammation site. Lymphocytes made up the majority of the inflammatory cells that infiltrated the injured area, while some large cells were also visible. Additionally, we found fibrin deposition where the infiltrated inflammatory cells had gathered. Disrupted fibroblast spindles and tenocytes vacuolation were evident. ANC-treated groups show variations in response levels, with some animals demonstrating significant underdevelopment. Furthermore, the existence of dense inflammatory cells and disorganized fibers signifies a substandard tendon composition. Moreover, the elevated quantity of inflammatory cells indicates the presence of continuous inflammation and a deficiency in the healing process. Furthermore, the absence of substantial neovascularization in the presence of inflammation suggests an inadequate healing response. The group demonstrated a noteworthy enhancement in platelet-rich plasma (PRP) treatment, characterized by a predominantly natural composition. Furthermore, the presence of continuous and compact collagen, along with dense external material filling the tendon, suggests improved tendon repair. Furthermore, the typical structure of arch cells and tenocytes demonstrates a sound cellular morphology. Moreover, the slight presence of inflammatory cells indicates a regulated inflammatory reaction. Moreover, an increased blood circulation and the development of fresh blood vessels signify ongoing healing and regeneration. While weakly scattered fibers may show some fluctuation in a few cases, the majority of animals exhibit a positive overall response.

Comparative Histopathological Scoring of Achilles Tendon Healing: A grading system has been developed to compare the degree of healing between the positive control, ANC treated, and PRP treated groups based on their histological findings. The scoring system was devised in the following manner.

1. **Organized Collagen Fibers (OCF):**

- The Positive Control group had scattered, weak, and disorganized collagen fibers, scoring 1.
- The ANC Treated group showed thin and disorganized collagen fibers, scoring 2.
- The PRP Treated group exhibited consistent and dense collagen fibers, scoring 5.

2. **Shape of Fibroblasts (SF):**

- The Positive Control group had fibroblasts that lost their spindle shape and were vacuolated, scoring 1.
- The ANC Treated group had nearly normal fibroblasts with some distortion, scoring 3.
- The PRP Treated group had normally shaped spindle fibroblasts, scoring 4.

3. **Shape of Tenocytes (ST):**

- The Positive Control group had missing or clustered tenocytes that were vacuolated, scoring 2.
- The ANC Treated group had increased proliferation with some vacuolation of tenocytes, scoring 2.
- The PRP Treated group had normally shaped tenocytes, scoring

4. **Extracellular Matrix (ECM):**

- The Positive Control group showed congestion and fibrin deposition in the ECM, scoring 1.
- The ANC Treated group exhibited increased collagen and better organization in the ECM, scoring 3.
- The PRP Treated group showed enhanced ECM production, scoring 4.

5. **Severity of Infiltration of Inflammatory Cells (SIIC):**

- The Positive Control group had severe inflammation with lymphocytes and giant cells, scoring 1.
- The ANC Treated group had moderate inflammation and newly formed blood vessels, scoring 3.

- The PRP Treated group had few inflammatory cells and new blood vessels, scoring 4.

6. **Thickness of Collagen Bundles (TCB):**

- The Positive Control group had thin, weak collagen bundles, scoring 2.
- The ANC Treated group had moderately thick collagen bundles, scoring 3.
- The PRP Treated group had dense, thick collagen bundles, scoring 5.

7. **Deformity of Fibroblasts and Tenocytes (DFT):**

- The Positive Control group had severe deformity and vacuolated tenocytes, scoring 1.
- The ANC Treated group had moderate deformity of fibroblasts and tenocytes, scoring 2.
- The PRP Treated group had minimal deformity of fibroblasts and tenocytes, scoring 5.

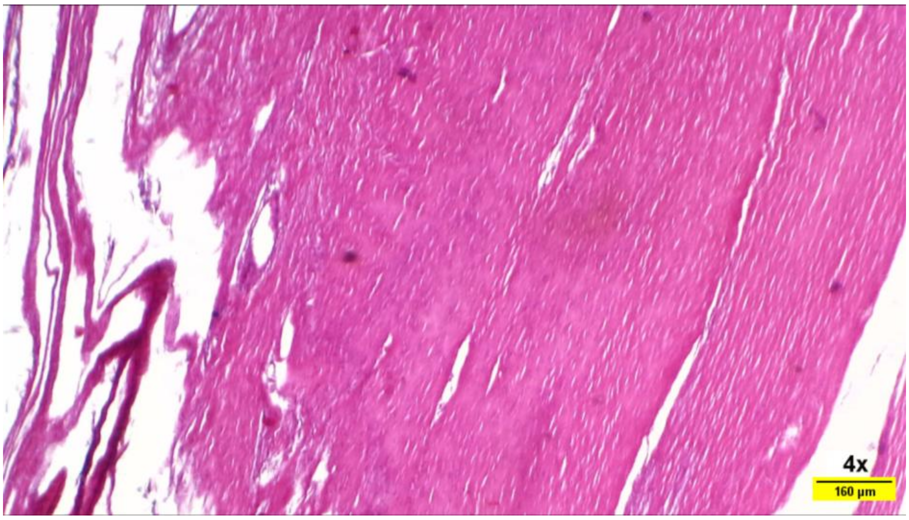

Fig. 5. A section of the Achilles tendon from the PRP-treated group exhibited significant improvement, the tendon appearing mostly normal in composition. Parallel, slightly wavy collagen bundles was observed, along with enhanced extracellular matrix production. H&E, 4x

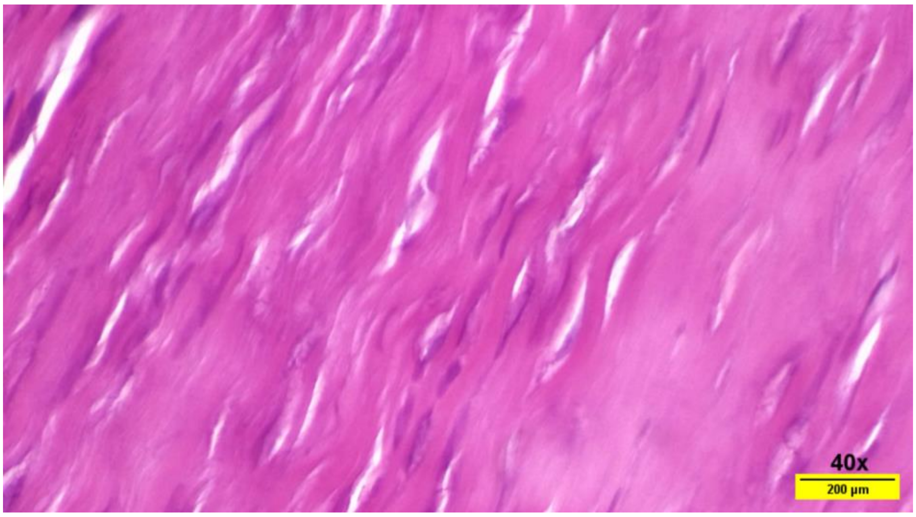

Fig. 6. A section of the Achilles tendon from the PRP-treated group exhibited significant improvement, the tendon appearing mostly normal in composition. Parallel, slightly wavy collagen bundles was observed, along with enhanced extracellular matrix production. Slender, elongated fibroblast cell nuclei, normal tenocytes, and blood vessels were evident. H&E, 40x

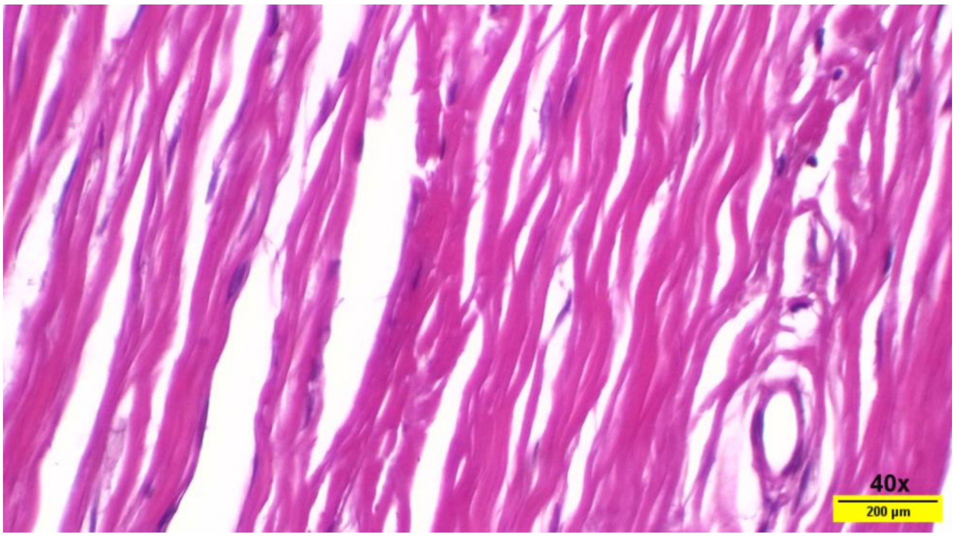

Fig. 7. A section of the Achilles tendon from the PRP-treated group exhibited significant improvement, the tendon appearing mostly normal in composition. Parallel, slightly wavy collagen bundles was observed, but decrease extracellular matrix was observed. Slender, elongated fibroblast cell nuclei, normal tenocytes, and increased blood vessels supply (black arrows) were evident. H&E, 40x

The ANC Treated group had a moderate response to treatment with a total score of 18, showing some improvements but not as significant as the PRP group. The Positive Control group was severely affected with a total score of 9, indicating severe damage and disorganization across all evaluated criteria. Therefore, the PRP Treated group demonstrated the best histopathological improvements and the most effective treatment response.

4. DISCUSSION

The positive control group's Achilles tendon showed evident signs of chronic tendinopathy with empty spaces, absence of spindle-like fibroblasts, insufficient, scattered, and broken collagen fibers, and tenocytes that were either gone or clustered together. The continued inflammatory response was highlighted by the accumulation of fibrin, congestion, and a notable presence of inflammatory cells. In their study on untreated tendinopathy (Smith et al., 2019). Observed comparable findings of disorganized collagen fibers, vacuolated tenocytes, and infiltration of inflammatory cells (Lu et al., 2021). Also observed that various animals exhibited varied responses to ANC. Certain animals showed disorganized collagen fibres and a persistent presence of inflammatory cells (Nguyen et al., 2022). Noted that ANC treatment yielded improved and more uniform outcomes, including structured collagen and faster reduction of inflammation. They argued that due to the increased dosages or longer durations of treatment in their study, they did not observe continuous inflammatory cells or disordered fibers, unlike the findings at present. The group treated with PRP showed significant progress with dense and well-arranged collagen fibers, typical fibroblast and tenocyte morphology, and improved vascularization. There were fewer inflammatory cells, indicating that the inflammatory reaction was being managed. However, some cases showed frail, scattered fibers and the disappearance of some nuclei in collagen bundles (Ren et al., 2021). Along with Ren et al. (2021) validated PRP's effectiveness in promoting collagen restructuring, increased blood vessel formation, and tendon renewal.

5. CONCLUSION

We demonstrated that PRP and NAC can effectively improve tendon healing, stimulate tendon growth, and reduce inflammation in tendon injuries, but PRP treatment promotes tendon tissue regeneration and subsequent collagen deposition in the defect area faster than NAC. PRP application appears to be a promising method for accelerating tendon healing.

ETHICS APPROVAL

The animal ethics committee The University of Basrah's College of Veterinary Medicine has approved the study at and experiments in accordance with the approved number (16/37/2024).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Abdulmawjood, Y. F., & Thanoon, M. G. (2022). A comparative study of nano magnesium oxide versus platelets rich fibrin to repair the induced radial fracture in dogs. *Iraqi Journal of Veterinary Sciences, 36*(2), 451–458. https://doi.org/10.33899/ijvs.2021.130500.1836

Abou Neel, E. A., Bozec, L., Knowles, J. C., Syed, O., Mudera, V., et al. (2013). Collagen—emerging collagen-based therapies hit the patient. *Advanced Drug Delivery Reviews, 65*, 429-456.

Andia, I., & Abate, M. (2013). Platelet-rich plasma: Underlying biology and clinical correlates. *Regenerative Medicine, 8*(5), 645-658.

Anitua, E., Prado, R., Sánchez, M., & Orive, G. (2012). Platelet-rich plasma: Preparation and formulation. *Operative Techniques in Orthopaedics, 22*(1), 25–32.

Crupi, R., Gugliandolo, E., Siracusa, R., Impellizzeri, D., Cordaro, M., Di Paola, R., & Cuzzocrea, S. (2020). N-acetyl-L-cysteine reduces *Leishmania amazonensis*-induced inflammation in BALB/c mice. *BMC Veterinary Research, 16*(1), 1-12.

Hsiao, M. Y., Lin, P. C., Liao, W. H., Chen, W. S., Hsu, C. H., He, C. K., et al. (2019). The effect of the repression of oxidative stress on tenocyte differentiation: A preliminary study of a rat cell model using a novel differential tensile strain bioreactor. *International Journal of Molecular Sciences, 20*, 3437.

Kajikawa, Y., Morihara, T., Sakamoto, H., et al. (2008). Platelet-rich plasma enhances the initial mobilisation of circulation-derived cells for tendon healing. *Journal of Cellular Physiology, 215*, 837-845.

Kaux, J. F., Libertiaux, V., Dupont, L., Colige, A., Denoël, V., Lecut, C., Hego, A., Gustin, M., Duwez, L., Oury, C., Gothot, A., Greimers, L., & Drion, P. (2020). Platelet-rich plasma (PRP) and tendon healing: Comparison between fresh and frozen-thawed PRP. *Platelets, 31*(2), 221-225. Epub 2019 Mar 27. PMID: 30915890. https://doi.org/10.1080/09537104.2019.1595563

Landry, J., & Maza, P. (2020). Effectiveness of the anaesthetic combination of tiletamine, zolazepam, ketamine, and xylazine for the sterilisation of street dogs in field clinics. *Veterinary Record Case Reports*. https://doi.org/10.1136/vetreccr-2019-000953

Lopez, R. G., & Jung, H. G. (2015). Achilles tendinosis: Treatment options. *Clinical Orthopaedics and Surgery, 7*, 1–7.

Lu, V., Tennyson, M., Zhang, J., & Khan, W. (2021). Mesenchymal stem cell-derived extracellular vesicles in tendon and ligament repair—A systematic review of in vivo studies. *Cells, 10*(10), 2553.

Margono, A., Bagio, D. A., Julianto, I., & Suprastiwi, E. (2022). The effect of calcium gluconate on platelet-rich plasma activation for VEGF-A expression of human dental pulp stem cells. *European Journal of Dentistry, 16*(2), 424–429. https://doi.org/10.1055/s-0041-1735930

Molloy, T., Wang, Y., & Murrell, G. (2003). The roles of growth factors in tendon and ligament healing. *Sports Medicine (Auckland, N.Z.), 33*, 381–394.

Nguyen, P. K., Jana, A., Huang, C., Grafton, A., Holt, I., Giacomelli, M., & Kuo, C. K. (2022). Tendon mechanical properties are enhanced via recombinant lysyl oxidase treatment. *Frontiers in Bioengineering and Biotechnology, 10*, 945639.

Rafat, A., Gadallah, S. M., Misk, T. N., Fadel, M. S., Abdallah, A. N., & Sharshar, A. (2022). Potential regenerative effect of mesenchymal stem cells-derived microvesicles on healing of the ruptured Achilles tendon in a dog model. *Journal of Current Veterinary Research, 4*(2), 140-151.

Raikin, S. M., Garras, D. N., & Krapchev, P. V. (2013). Achilles tendon injuries in a United States population. *Foot & Ankle International, 34*(4), 475-480. Epub

2013 Feb 5. PMID: 23386750. https://doi.org/10.1177/1071100713477621

Ren, B., Lv, X., Tu, C., & Li, Z. (2021). Research trends of platelet-rich plasma application in orthopaedics from 2002 to 2020: A bibliometric analysis. *International Orthopaedics, 45*, 2773–2790.

Shamrock, A. G., Dreyer, M. A., & Varacallo, M. (2023, August 17). Achilles tendon rupture. In *StatPearls [Internet]*. Treasure Island (FL): StatPearls Publishing. Retrieved January 2024, from https://www.statpearls.com

Smith, M. V., Castile, R. M., Brophy, R. H., Dewan, A., Bernholt, D., & Lake, S. P. (2019). Mechanical properties and microstructural collagen alignment of the ulnar collateral ligament during dynamic loading. *The American Journal of Sports Medicine, 47*(1), 151-157.

Zhang, J., & Wang, J. H. (2010). Platelet-rich plasma releasate promotes differentiation of tendon stem cells into active tenocytes. *The American Journal of Sports Medicine, 38*, 2477–2486.

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