Research Article

BASRAH JOURNAL OF VETERINARY RESEARCH, 2024, 23(4):76-86.
https://bjvr.uobasrah.edu.iq/

Clinical Evaluation of The Use of PRP as A Treatment for Achilles Tendon Defects in Dog Model

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DOI: https://doi.org/10.23975/bjvr.2024.152941.1131
Received: 27 August 2024 Accepted: 27 September 2024.

Abstract

The Achilles tendon is the most resilient in the musculoskeletal system of canines. It extends the hock, allowing the animal to balance on its toes. Its primary role is to propel the rear limb forward and support the hock. Platelet-rich plasma (PRP): Platelets store growth factors and are crucial in various physiological functions like blood clotting, blood vessel formation, immune defense, and tissue healing. To analyze and contrast platelet-rich plasma's impacts on Achilles tendon regeneration. Ten dogs were divided into two groups; 5 received treatment with Platelet-Rich Plasma (Group A), and 5 served as control (Group B) and were left untreated. Results scoring the degree of lameness in dogs that were not treated throughout the entire experiment The control group showed lameness scores, resulting in the animals being unable to lift their legs and take a few steps. The group that was treated with PRP had notably lower levels of lameness and pain throughout the study period. PRP treatment resulted in better healing of the injured tendon compared to the untreated group, and it showed gradual improvement after 7 days. The conclusion proved that PRP can greatly enhance tendon repair and growth and minimize inflammation.

Keywords: Achilles Tendon, platelet-rich plasma, Tendon defect, Dogs.

Introduction

Tendons are classified as thick, fibrous, and connecting tissues that link muscles to bones in order to enable joint movement (1- 3). Mechanical bridges made of sturdy fibrous structures connect muscles to bones and convert the force produced by muscle contractions into movement (4). The layer of connective tissue known as epitenon covers the surface of the tendon and seamlessly transitions into the endotenon. Epitenon's role is to aid in the movement of various structural components and house blood, lymphatic vessels, and nerve structures. Distant from the joint, the paratenon is a loose connective tissue layer that encloses the tendon and facilitates its movement beneath the skin (5). Injuries and degeneration of tendons and ligaments can lead to limited healing ability and a high likelihood of reoccurrence because both tendons and ligaments have a low metabolic rate (6). The Achilles Tendon is the most powerful in a dog's musculoskeletal system. It extends the hock and enabling the animal to stand on its toes (7). An Achilles tendon tear is the most frequent sudden condition. Achilles tendon tear is a common tendon that can rupture in the lower part of the body (8). Healing of tendon injuries is a slow process, and the resulting tissue is typically not as effective as healthy tendon tissue, causing a high re-injury rate. The natural healing of damaged tendons depends on the tenocytes' capability to multiply and create an extracellular matrix (ECM) mainly containing collagen and proteoglycans. The high ratio of ECM to cells is believed to cause tendons' poor healing ability (9,10). Platelet rich Platelets (PRP) The platelet-rich plasma contains various essential growth factors like PDGF, VEGF, TGF-β1, EGF, b-FGF, and HGF, which enhance its biological effectiveness and healing properties (11). These growth factors are essential for stimulating local blood vessel formation, controlling cell functions, guiding stem cells to specific locations, increasing stem cell numbers, and encouraging the production of proteins that help with tissue repair (12- 14). These activate the cell membrane receptors and internal signaling proteins that regulate repair and restructuring processes (15,16).

Materials and Methods

Experimental Animals: For this study, ten adult dogs, who were 1 year old and had an average weight of 23kg, were employed. The dogs spent 7 days getting used to the surroundings before the study began. They were kept in separate cages at the Veterinary Medicine University of Basrah's animal house and experienced the same environment. The dogs received a rabies vaccination and were examined to verify their well-being. Next, the dogs were divided evenly into groups with a size of 5.

Preparation of autologous PRP: PRP was made during surgery by drawing 20 ml of blood from the jugular vein, dividing it into two test tubes (10 ml each), and adding 0.5 ml of 3.2% sodium citrate to each tube. After spinning at 1500 rpm for 10 minutes, the top layer was removed, and the rest of the blood is considered the PRP (2 ml). This fraction was centrifuged once more at a speed of 1500 rpm for 15 minutes and subsequently activated with two drops of 10% calcium chloride. (17).

Surgical procedure: An aseptic preparation was performed on the surgical site of the right hind limb for the procedure (figure 1). Before administering anesthesia, the patient was fasted from food for 12 hours and from water for 3 hours. Anesthesia was induced through intramuscular injection of xylazine HCl and ketamine HCl at a dosage of 5 mg per kg. B.W. and 15 mg per kg. B.W. in that order (18).

Surgical operation: The procedure was carried out with the animal presently on the right side with the hind limb. The upper side incision on the longitudinal right hind limb was made about 3 cm below the gastrocnemius muscle and 3 cm higher than the calcaneus. After the Achilles tendon was revealed. To pinpoint the central injury (defect), we inserted stay sutures at both the proximal and distal ends of the tendon (figure 2). The imperfections were produced by making 2 cm (defect size) frequent punctures in the Achilles tendon using an 18-gauge needle (see figure 3). After that, animals in group A had their damaged tendons treated locally with Platelet Rich Plasma. In contrast, group B's tendons were not treated before the skin was closed in the standard manner. The cut involved the muscles. The closure of each was done with simple continuous sutures using 2-0 USP Vicryl chromic, followed by skin closure using a cross-mattress technique pattern of stitches.

Fig 1: The surgical site was aseptically prepared for the operation.

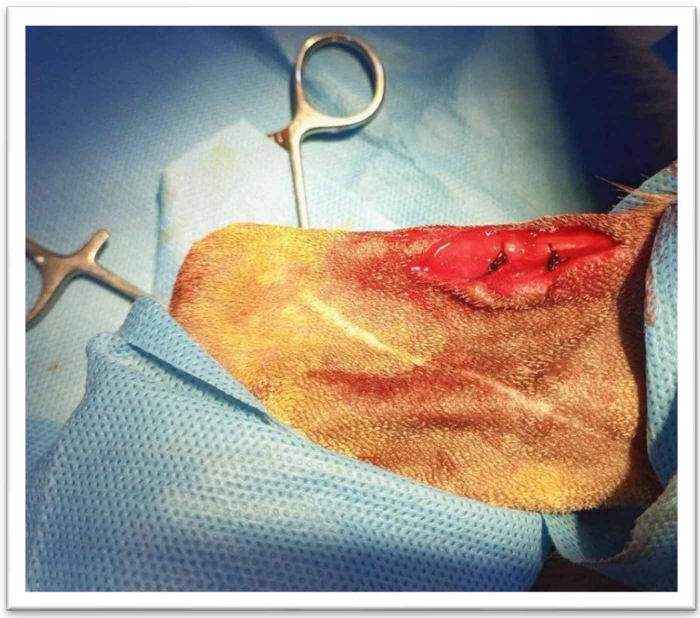

Fig 2: Stay sutures were placed at the proximal and distal ends of the tendon.

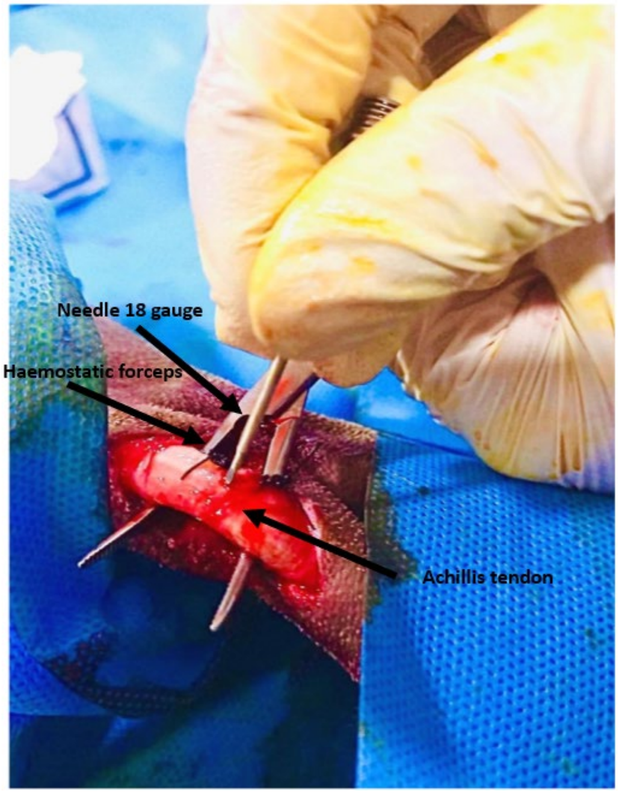

Fig 3: The defects were created by perforations frequent of Achilles tendon using needle gauge 18.

Clinical Evaluation: All surgeries were completed without any complications during or after the procedures. After the surgery, the dogs were observed and supervised to assess their lameness. They were seen freely moving and running in the meadow to assess their gait. At the same time, the lameness evaluation scores were established by (19) (table 1). In this situation, three clinical parameters - standing posture grade, lameness while walking, and lameness while trotting - were assessed on days 0, 3, 5, and 7. (20).

Histologically Evaluation: All animals were euthanized 42 days after surgery using a high dose of general anesthesia, as directed by the veterinary medicine college's ethical committee, for histopathological analysis. The Achilles tendon was cut about 3cm at the defect site, and the tissue was placed in 10% formalin for fixation. The sections were mounted on glass slides and stained with Hematoxylin and Eosin (H&E).

Table 1: The lameness assessment grades.

	Standing posture
1	Normal stance
2	A slightly unusual position with partial weight bearing on the limb.
3	Severely abnormal stance (non-weight-bearing)
	Lameness at walk
1	No signs of lameness; consistent weight bearing was noticed at every step.
2	Slight lameness with some weight placed on the affected limb
3	Obvious lameness with partial weight-bearing Marked lameness with no weight-bearing
	Lameness at trot
1	Absence of lameness
2	Slight lameness
3	lameness with partial weight, bearing
4	Marked lameness with no weight, bearing

Results

After surgery, the clinical parameters suggested that all animals that underwent the operation were in good health, attentive, and exhibiting regular activity with a strong appetite. There was no indication of any significant issues like infection or the wound opening up at the leg that was operated on. Nevertheless, animals in the two groups showed lameness several days after the surgery. Lameness was categorized based on the lameness scoring system. In the group that did not receive PRP, lameness symptoms lasted for 7 days, while in the PRP group, they lasted for 5 days. Interestingly, a lower level of lameness that lasted for 5 days was reported in the PRP group.

Lameness Analysis Results: A clinical trial demonstrated lameness in the control group and the group treated at different intervals (0, 3, 5, and 7 days after surgery). Significant differences ($P<0.05$) were noted among groups with different letters.

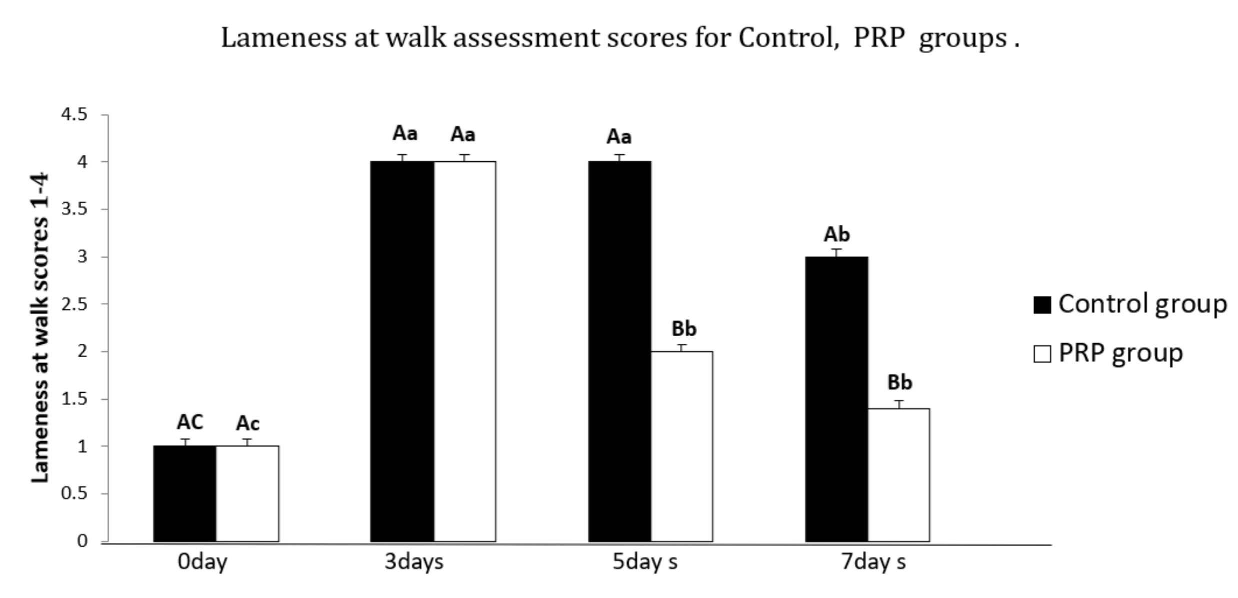

Chart 1: Lameness evaluation at walking

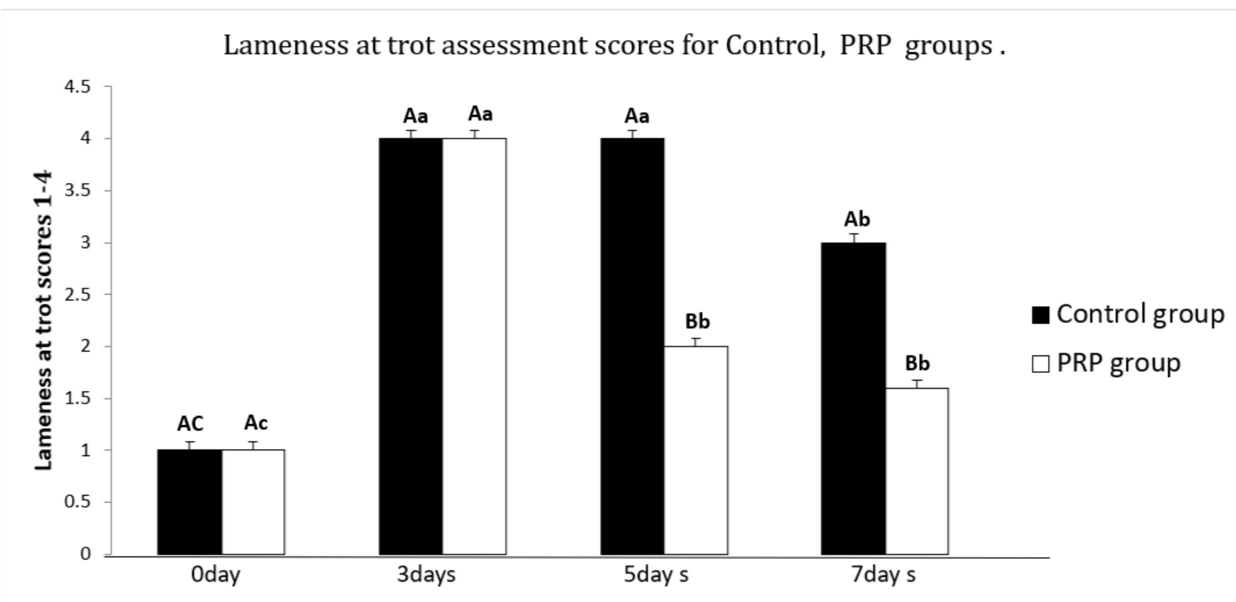

Chart 2: Lameness evaluation at a trot

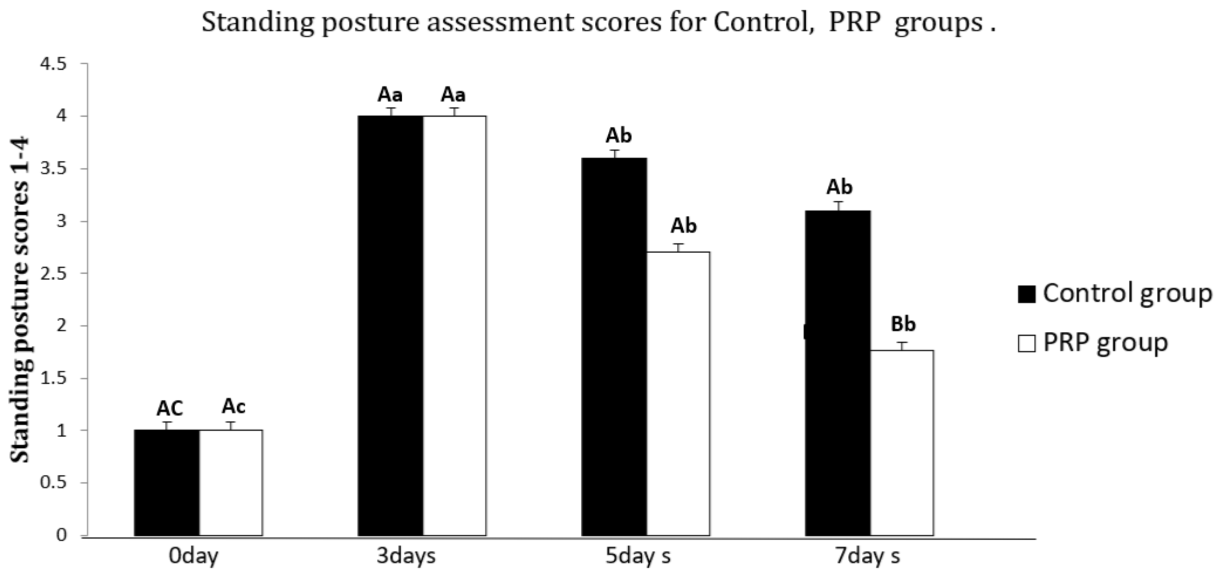

Chart 3: Lameness evaluation at a posture

Histologically Results

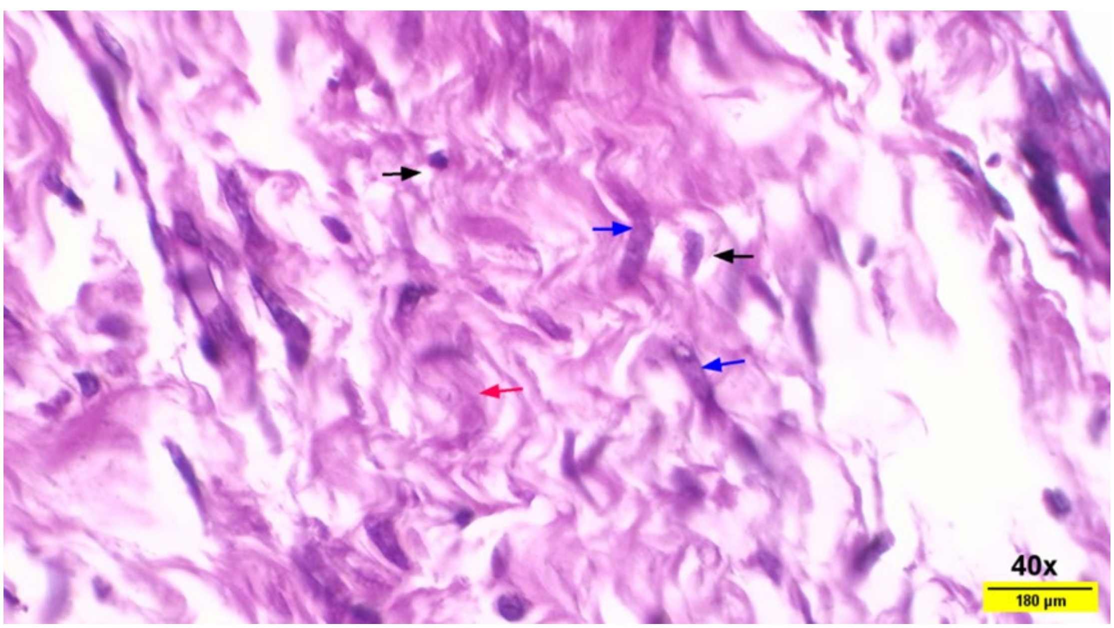

Figure 4: control. A section of the Achilles tendon of the positive control group showed chronic tendonitis characterized by scattered, weak, and disorganized collagen fibers(red arrow), loss of spindle shape in fibroblast cells (blue arrows), missing or vacuolated tenocytes (black arrows), and edema.

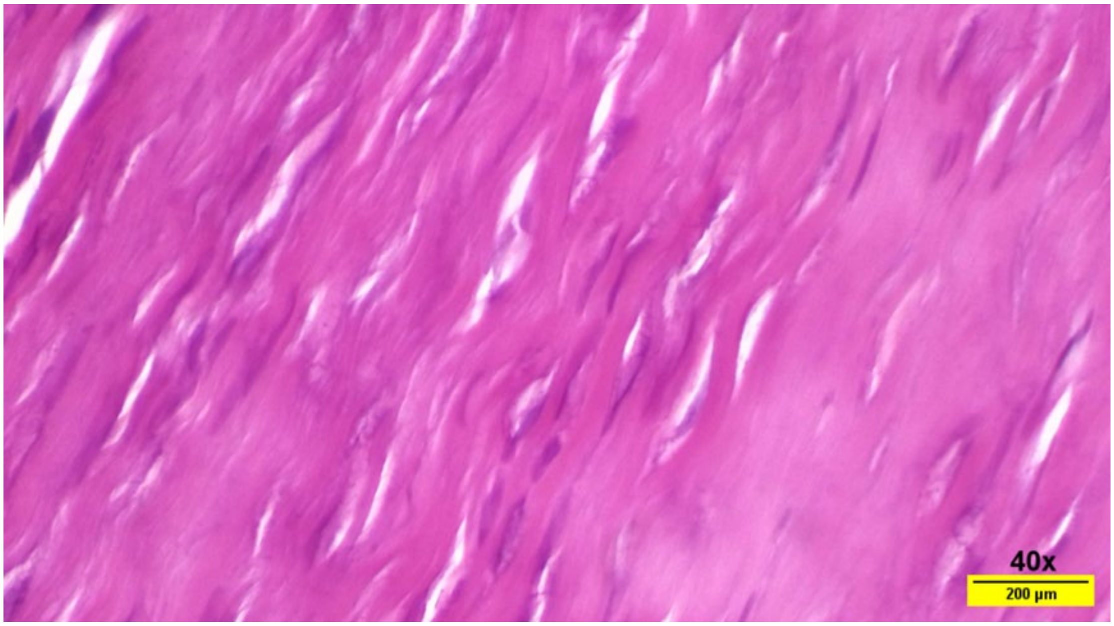

Figure 5: PRP. A section of the Achilles tendon from the PRP-treated group exhibited significant improvement, the tendon appearing mostly normal in composition. Parallel, slightly wavy collagen bundles and enhanced extracellular matrix production were observed. Slender, elongated fibroblast cell nuclei, normal tenocytes, and blood vessels were evident.

Discussion

Regenerating tendons is a slow and intricate process. Multiple biological or synthetic methods were employed for tendon augmentation to improve treatment outcomes and boost healing rates. (21). A successful restoration with a positive result must possess the appropriate strength, tension, and durability to withstand the varying workload (22). During the recent experiment, findings indicated that using self-derived PRP has effectively sped up the healing process of the damaged tendon. This result could show that PRP can potentially act as temporary support for the host tissue during growth and repair. Moreover, using PRP to enhance the transacted tendon could benefit the tendon's mechanical properties. Observation during clinical evaluation shows progress in walking and a gradual reduction in lameness, particularly noticeable on the seventh day after surgery in the PRP group. Additionally, the PRP could potentially have healing benefits by promoting the growth and movement of fibroblasts. For example, besides producing the extracellular matrix, the treated groups showed an early enhancement in the tendon's mechanical performance, and lameness symptoms quickly disappeared (23). Additionally, incorporating PRP functions as a fibrin tissue glue, enhancing the tendon's tensile strength and gait (24). In the same way, a recent research study showed the positive impact of utilizing PRP for repairing digital flexor tendons in rabbits (25).

Conclusion

PRP can effectively enhance tendon healing and growth and decrease inflammation in tendon injuries.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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التقييم السريري لاستخدام البلازما الغنية بالصفائح الدموية كعلاج لعيوب وتر العرقوب في الكلاب

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الخلاصة

وتر العرقوب هو الوتر الأكثر مرونة في الجهاز العضلي الهيكلي لذوات الأنياب. لوتر العرقوب القدرة على التمدد ، مما يسمح للحيوان بالتوازن على أصابع قدميه. دورها الأساسي هو دفع الطرف الخلفي إلى الأمام. تخزن البلازما الغنية بالصفائح الدموية والصفائح الدموية عوامل النمو وهي ضرورية في الوظائف الفسيولوجية المختلفة مثل تخثر الدم وتكوين الأوعية الدموية والدفاع المناعي والأنسجة ومقارنة آثار البلازما الغنية بالصفائح الدموية على تجديد وتر العرقوب. تم تقسيم 10 كلاب إلى مجموعتين ، 5 كلاب تلقت العلاج بالبلازما الغنية بالصفائح الدموية، 5 كلاب كانت بمثابة عنصر تحكم تركت غير معالجة ، وسجلت درجة العرج في الكلاب التي لم يتم علاجها طوال التجربة بأكملها أظهرت المجموعة التحكم درجات العرج ، مما أدى إلى عدم قدرة الحيوانات على رفع أرجلها واتخاذ خطوات قليلة ، المجموعة التي عولجت بالبلازما الغنية بالصفائح الدموية كانت لديها مستويات أقل بشكل ملحوظ من العرج والألم طوال فترة الدراسة ، أدى العلاج بالبلازما الغنية بالصفائح الدموية إلى شفاء أفضل للوتر المصاب مقارنة بالمجموعة غير المعالجة, إظهار التحسن التدريجي بعد 7 أيام. من الناحية النسيجية ، عززت البلازما الغنية بالصفائح الدموية الشفاء مع زيادة تكاثر الخلايا والهجرة وإنتاج الكولاجين للخلايا المشتقة من الأوتار بما في ذلك زيادة الخلايا والأوعية الدموية لأنسجة الأربطة ، مقارنة بتلك الموجودة في مجموعة التحكم أظهر وتر العرقوب في مجموعة التحكم التهاب الأوتار المزمن. اثبت الدراسة أن البلازما الغنية بالصفائح الدموية يمكن أن تعزز بشكل كبير إصلاح الأوتار ونموالأوتار وتقليل الالتهاب.

الكلمات المفتاحية: وتر العرقوب ، بلازما غنية بالصفائح الدموية ، عيب الوتر ، كلاب.