Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 4, https://carcinogenesis.com/

Hemp Oil as a Potential Agent to Promote Achilles Tendon Healing: An In Vivo Study in Rats

Safrizal Rahman[1]*, Eka Husnawaty Bahsoan[2], Sri Wahyuni[3]

[1]Division of Orthopedics and Traumatology, Department of Surgery, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia

[2]Residency Program in General Surgery, Department of Surgery, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia

[3]Laboratory of Anatomy, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia

*Corresponding author: safrizal.rahman@usk.ac.id

ABSTRACT

Achilles tendon rupture is a common injury with prolonged recovery and risk of poor remodeling. Hemp seed oil, rich in cannabidiol, essential fatty acids, and antioxidants, has anti-inflammatory and regenerative potential, yet evidence for its role in tendon healing remains limited. Therefore, the aim of this study was to evaluate the effect of topical hemp oil administration on the healing of surgically induced Achilles tendon rupture in white rats (Rattus norvegicus). Thirty-two adult male rats were randomly assigned to a control group or a treatment group receiving daily topical hemp seed oil following surgically induced partial Achilles tendon rupture. Functional recovery was evaluated on day 18 using footprint analysis, while histological parameters—including tenocyte morphology, vascularization, and collagen organization—were assessed by Bonar scoring. Footprint analysis demonstrated a higher proportion of normal gait in the treatment group compared with controls (75% vs. 62.5%). Histological evaluation revealed elongated tenocytes with more organized collagen fibres and reduced neovascularization in the treatment group, consistent with advanced tendon remodeling. Quantitative analysis showed significantly lower Bonar scores in the treatment group for tenocyte morphology ($p=0.002$), vascularization ($p=0.033$), and collagen disorganization ($p<0.001$). In conclusion, topical hemp seed oil accelerates both functional and structural recovery of the Achilles tendon, supporting its potential role as an adjuvant therapy in tendon injury management.

Keywords: Hemp oil, tendon rupture, tenocyte, collagen, Bonar score.

How to Cite: Safrizal Rahman, Eka Husnawaty Bahsoan, Sri Wahyuni, (2025) Hemp Oil as a Potential Agent to Promote Achilles Tendon Healing: An In Vivo Study in Rats, *Journal of Carcinogenesis, Vol.24, No.4*, 295-302.

1. INTRODUCTION

Achilles tendon rupture is one of the most frequent tendon injuries, with an annual incidence ranging from 7 to 40 cases per 100,000 individuals.1 The condition primarily affects adults between 30 and 50 years of age, sudden eccentric contraction (the muscle lengthens while contracting) particularly those who engage in recreational or competitive sports.2 Achilles tendon, despite being the body's largest and strongest tendon, is characterized by limited vascularity, which contributes to its vulnerability to degeneration and delayed healing after injury.3 The natural repair of a ruptured tendon follows a complex and overlapping sequence of biological phases: an initial inflammatory response characterized by infiltration of neutrophils and macrophages; a proliferative phase marked by fibroblast activation, tenocyte proliferation, and deposition of type III collagen; and a remodeling phase during which type III collagen is gradually replaced by type I collagen to restore tensile strength.4,5 Despite the orchestrated biological repair process, Achilles tendon healing is often slow and prone to complications that can compromise mechanical strength and functional recovery.6,7

To enhance tendon regeneration and improve clinical outcomes, various biological therapies have been explored. Platelet-rich plasma (PRP) and growth factor–enriched preparations have been investigated for their ability to stimulate tenocyte activity and accelerate collagen synthesis.8–11 PRP and growth factor–enriched preparations enhance tendon healing in animal models, but robust clinical trials in humans have not demonstrated consistent functional or structural benefits for Achilles tendon injuries.12,13 Therefore, there remains a need for more accessible, cost-effective, and non-invasive agents that can modulate inflammation, support collagen remodeling, and shorten the overall healing time.

Natural bioactive compounds—especially plant-derived antioxidants and polyunsaturated fatty acids (PUFAs)—demonstrate promising effects in preclinical tendon healing models by enhancing collagen deposition, reducing oxidative stress, and supporting tissue remodeling.14,15 Although not directly studied in tendon models within the literature, cannabidiol is recognized for its anti-inflammatory and antioxidant effects in other tissue injury models, suggesting potential benefits for tendon healing through attenuation of cytokine release and oxidative stress.16–19 Hemp oil extracted from Cannabis sativa made by cold pressing hemp seed, contains a unique combination of cannabidiol, omega-3 and omega-6 fatty acids, and antioxidant molecules such as tocopherols and polyphenols.19 Cannabidiol has been reported to attenuate inflammatory cytokine release and oxidative stress in various tissue injury models.16–18 Omega-3 and omega-6 fatty acids serve as precursors to anti-inflammatory lipid mediators, promoting neovascularization and fibroblast proliferation, while antioxidants help protect tenocytes and extracellular matrix components from reactive oxygen species.20,21

Despite these promising biological properties, evidence on the use of hemp oil for tendon healing remains scarce. This represents a critical gap in current research, as hemp oil is inexpensive, widely accessible, and easy to administer, making it a potential adjunctive therapy for tendon injuries. Therefore, the aim of this study was to evaluate the effect of topical hemp oil administration on the healing of surgically induced Achilles tendon rupture in white rats (Rattus norvegicus).

2. METHODS

Study design and setting

An experimental study was conducted following the ARRIVE guidelines and employed a post-test–only control group design.22 Thirty-two healthy male Wistar rats (3–4 months, 200–220 g) were randomly assigned to a control group or a treatment group receiving daily topical hemp seed oil (0.2 mL) for 18 days. A standardized partial rupture of the left Achilles tendon was surgically induced under ketamine–xylazine anesthesia. Functional recovery was evaluated on day 18 using footprint analysis, followed by histological assessment of harvested tendon tissue with hematoxylin and eosin staining and Bonar scoring.

Animals and housing

Sample size was calculated using the Federer formula for experimental research, which recommends a minimum of 16 animals per group when two groups are compared. To ensure adequate statistical power and allow for potential attrition, 16 rats were allocated

to each group, giving a total of 32 healthy male Wistar rats (Rattus novergicus). All animals were 3–4 months old, weighed 200–220 g, and were maintained under standard laboratory conditions with unrestricted access to food and water. Only male rats were selected to avoid the potential influence of hormonal fluctuations on tendon healing. Animals were housed in standard polypropylene cages (five to six rats per cage) under controlled environmental conditions (temperature 22 ± 2 °C; humidity 50–60%; 12-hour light/dark cycle). Rats had free access to a standard laboratory chow and water ad libitum. A one-week acclimatization period preceded the experimental procedures, during which rats were monitored daily to ensure normal activity and health status.

Randomization and group allocation

After acclimatization, rats were randomly allocated into two groups (n=16 per group) using a computer-generated random sequence. The control group received no topical intervention, whereas the treatment group received daily topical hemp oil application. Allocation was concealed until the start of the intervention.

Induction of Achilles tendon injury

Partial rupture of the left Achilles tendon was surgically induced in all rats. Anesthesia was achieved with intraperitoneal ketamine (80 mg/kg) combined with xylazine (10 mg/kg). Under aseptic conditions, a 1.5 cm skin incision was made over the Achilles tendon, and a standardized partial tendon rupture was created using a sterile size 11 scalpel blade. The paratenon and skin were closed with 4-0 absorbable sutures.

Postoperative care

All rats received a single subcutaneous dose of meloxicam (1 mg/kg) for analgesia immediately after surgery. No prophylactic antibiotics were administered. Animals were monitored daily for signs of pain, infection, or distress. Cages were cleaned daily and animals were observed for gait, feeding behavior, and wound healing.

Intervention

Hemp seed oil (Refreshing Hemp Oil, Green Angel, New York, USA) was used as the treatment agent. The treatment group received daily topical application of 0.2 mL cold- pressed hemp seed oil directly over the surgical site for 18 consecutive days, beginning 24 hours after surgery. The control group underwent the same surgical procedure but received no topical application.

Functional gait assessment

Functional recovery was evaluated on day 18 using a standardized footprint analysis protocol. Each rat was guided to traverse a 100 cm × 10 cm runway lined with white paper, with the distal hind paws lightly coated in non-toxic, water-based ink. Three consecutive runs were recorded for each animal to ensure reproducibility. Footprints were photographed and analyzed by an observer blinded to group allocation. Step length, stride width, paw placement symmetry, and pressure distribution were evaluated from inked footprints. Based on these measurements, gait was categorized as normal if footprints demonstrated consistent step length, balanced stride width, symmetrical paw placement, and even pressure distribution. Any deviation from these criteria was classified as abnormal gait.

Microscopic evaluation

Following macroscopic assessment, all animals were euthanized with an overdose of intraperitoneal pentobarbital (200 mg/kg). The injured Achilles tendons were carefully excised, fixed in 10% neutral-buffered formalin, dehydrated, and embedded in paraffin. Longitudinal sections (4–5 µm) were stained with hematoxylin and eosin. Histological healing was evaluated by a blinded pathologist using the Bonar scoring system, which assesses four parameters: tenocyte morphology, collagen fiber organization, ground substance, and vascularization.23 Each parameter was scored from 0 (normal) to 3 (marked abnormality), with lower scores reflecting better tendon healing.23 Collagen content in Achilles tendon sections stained with picrosirius red (PSR) was quantified using ImageJ software. Digital images of the stained preparations were captured under polarized light, and the collagen area fraction was calculated to determine relative collagen content.

Statistical Analysis

Data were analyzed using SPSS version 25 (IBM SPSS, New York, USA). Normality of continuous variables was tested with the Shapiro–Wilk test. For normally distributed data, intergroup comparisons were performed using the independent t-test; for non- normally distributed data, the Mann–Whitney U test was applied. Results were expressed as mean ± standard deviation (SD) or median (min–max) as appropriate. A p-value <0.05 was considered statistically significant.

3. RESULTS

Functional gait assessment

Footprint analysis performed on day 18 revealed a clear improvement in locomotor function in rats treated with hemp seed oil. As illustrated in Figure 1, the treatment group demonstrated a more symmetrical gait pattern with even pressure distribution across the hind limbs compared with the control group. Quantitatively, 12 of 16 rats (75%) in the treatment group showed a normal gait, while only 10 of 16 rats (62.5%) in the control group achieved a comparable outcome (Table 1). The higher proportion of normal gait in the treatment group indicates superior functional recovery of the Achilles tendon following hemp seed oil application.

Table 1. Comparison of gait examination outcomes between control and treatment groups on day 18 based on footprint analysis.

Gait examination	Control group, n (%)	Treatment group, n (%)
Normal	10 (62.5)	12 (75)
Abnormal	6 (37.5)	4 (25)

Histological characteristics of tenocytes and collagen matrix during tendon healing

Microscopic evaluation highlighted distinct differences in tendon healing characteristics between the two groups. In the control group (Figure 2), numerous tenocytes with round nuclei were observed, accompanied by irregular and loosely arranged collagen fibres, reflecting immature tendon repair. In contrast, the treatment group (Figure 2) displayed tenocytes with elongated spindle-shaped nuclei and a more organized collagen matrix, indicating more advanced tendon remodeling.

Histological characteristics of vascularization changes during tendon healing

Assessment of vascularization showed that neovascular proliferation was more pronounced in the control group, while the treatment group exhibited a reduction in vascular density (Figure 3). This pattern is consistent with the later stages of tendon healing, during which excessive vascularization typically regresses as tissue maturation occurs.

Histological characteristics of collagen organization during tendon healing Collagen

architecture analysis using hematoxylin–eosin and picrosirius red staining (Figure 4) further supported these observations.

The treatment group exhibited a denser and more regularly aligned collagen network, whereas the control group retained irregular collagen fibre arrangement and lower collagen content.

Histological comparison and Bonar score analysis

Independent t-test analysis confirmed significant differences between the groups. The treatment group demonstrated significantly lower Bonar scores across key histological parameters, including tenocyte count (mean±SD: 1.44±0.73 vs. 2.38±0.81; p=0.002), vascularization (1.63±0.81 vs. 2.25±0.77; p=0.033), and collagen disorganization (1.19±0.40 vs. 2.31±0.48; p<0.001) (Table 2).

Table 2. Comparison of histological parameters of the Achilles tendon between control and treatment groups.

Variable	Control group, mean±SD	Treament group, mean±SD	*p*-value[a]
Tenocyte	2.38±0.81	1.44±0.73	0.002*
Vascularisation	2.25±0.77	1.63±0.81	0.033*
Collagen	2.31±0.48	1.19±0.40	<0.001*

aAnalyzed using independent t-test
*Statistically significant at p<0.05

4. DISCUSSION

This experimental study demonstrated that topical hemp seed oil enhances both functional recovery and histological tendon remodeling after surgically induced Achilles tendon rupture in rats. On macroscopic assessment, footprint analysis revealed improved gait symmetry, more normal step length, and better paw placement in the treatment group compared with controls, reflecting superior restoration of tendon function. Microscopic evaluation further showed elongated spindle-shaped tenocytes, a well- aligned and denser collagen matrix, and significantly lower Bonar scores across all key histological parameters. Together, these findings indicate accelerated progression from the proliferative to the remodeling phase of tendon healing in the hemp oil group.

The biological plausibility of these effects is supported by previous evidence on the anti-inflammatory and pro-regenerative properties of hemp-derived bioactive compounds.24–28 Cannabidiol, one of the principal constituents of hemp oil, has been reported to suppress the expression of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), while promoting polarization of macrophages toward the M2 reparative phenotype.18,24,25,29,30 This macrophage shift plays a critical role in resolving inflammation and initiating matrix remodeling.24,25,31 Moreover, hemp seed oil is naturally rich in omega-3 and omega-6 fatty acids, which are known to reduce oxidative stress, enhance fibroblast activity, and stimulate collagen synthesis—processes essential for tendon regeneration.20,21,24,27 Antioxidants present in hemp oil may further limit tissue damage from reactive oxygen species during the early inflammatory phase.20,21,24,27

When compared with other biological interventions, such as PRP, hemp seed oil presents several notable practical advantages. PRP therapy necessitates venipuncture to collect autologous blood, followed by centrifugation to concentrate platelets, and subsequently an injection into the injured tendon under sterile conditions.32,33 This multistep process requires specialized equipment, trained personnel, and strict procedural sterility, which increases both cost and complexity. Furthermore, PRP injections may be associated with local discomfort, potential infection risk, and the need for repeated applications to achieve optimal efficacy.34–36

In contrast, hemp seed oil can be administered as a simple topical agent, eliminating the need for invasive procedures or sophisticated laboratory preparation. Its application does not require specialized training, making it feasible for use not only in tertiary care centers but also in primary care facilities and outpatient rehabilitation settings. The cost of hemp oil is substantially lower than that of PRP preparation and delivery, which is particularly advantageous in resource-limited healthcare systems. Moreover, the non-invasive nature of hemp oil application minimizes patient discomfort and procedural complications, thereby improving treatment acceptability and adherence. These attributes highlight hemp seed oil as a practical and accessible adjunct to standard surgical repair and post-operative rehabilitation protocols for tendon injuries. Its ease of use and cost-effectiveness make it especially appealing in low- and middle- income countries where advanced biologic therapies such as PRP or stem cell–based treatments may be financially or logistically unattainable. By providing a readily available option to enhance tendon healing, hemp oil has the potential to bridge the gap in regenerative therapy accessibility across diverse clinical settings.

Despite these promising results, some important limitations should be acknowledged. The study did not evaluate a dose–

response relationship or determine the optimal concentration and frequency of hemp oil application. In addition, no direct comparison was made with established biological agents such as PRP or mesenchymal stem cell–based therapies, which limits the ability to position hemp oil within the hierarchy of regenerative treatments. The experimental follow-up was limited to 18 days and did not assess the long-term mechanical strength or durability of the healed tendon. Furthermore, although the rat Achilles tendon model is widely accepted for preclinical research, species-specific differences mean that the findings cannot be directly extrapolated to humans without further validation. Future research should focus on determining the optimal dose and application schedule, assessing the mechanical properties of the regenerated tendon, and evaluating long-term outcomes. Well-designed comparative studies against PRP and other regenerative modalities, as well as carefully controlled clinical trials, are warranted to establish the translational relevance and safety of CBD-based topical preparations in human tendon injuries.

5. CONCLUSION

Topical hemp seed oil significantly enhanced functional recovery and histological remodeling of Achilles tendon rupture in rats, evidenced by improved gait and lower Bonar scores. Its anti-inflammatory and regenerative properties, combined with non- invasive and low-cost application, highlight hemp seed oil as a promising adjunct for tendon healing. Further studies are needed to define optimal dosing, long-term outcomes, and clinical safety.

Competing interests
All the authors declare that there are no conflicts of interest.

Funding
This study received no external funding.

Underlying data
Derived data supporting the findings of this study are available from the corresponding author on request.

REFERENCES

[1] Park SH, Lee HS, Young KW, Seo SG. Treatment of Acute Achilles Tendon Rupture. Clin Orthop Surg. 2020;12(1):1.
[2] Čretnik A, Košir R. Incidence of Achilles tendon rupture: 25-year regional analysis with a focus on bilateral ruptures. Journal of International Medical Research. 2023 Nov 17;51(11).
[3] Zhang J, Li F, Williamson KM, Tan S, Scott D, Onishi K, et al. Characterization of the structure, vascularity, and stem/progenitor cell populations in porcine Achilles tendon (PAT). Cell Tissue Res. 2021 May 26;384(2):367–87.
[4] Frankewycz B, Henssler L, Weber J, Silva NPB da, Koch M, Jung EM, et al. Changes of Material Elastic Properties during Healing of Ruptured Achilles Tendons Measured with Shear Wave Elastography: A Pilot Study. Int J Mol Sci. 2020 May 12;21(10):3427.
[5] Saarensilta A, Chen J, Reitzner SM, Hart DA, Ahmed AS, Ackermann PW. Novel tissue biomarker candidates to predict both deep venous thrombosis and healing outcome after Achilles tendon rupture. Sci Rep. 2025 Mar 1;15(1):7318.
[6] Gatz M, Driessen A, Eschweiler J, Tingart M, Migliorini F. Open versus minimally- invasive surgery for Achilles tendon rupture: a meta-analysis study. Arch Orthop Trauma Surg. 2021 Mar 7;141(3):383–401.
[7] Attia AK, Mahmoud K, d'Hooghe P, Bariteau J, Labib SA, Myerson MS. Outcomes and Complications of Open Versus Minimally Invasive Repair of Acute Achilles Tendon Ruptures: A Systematic Review and Meta-analysis of Randomized Controlled Trials. Am J Sports Med. 2023 Mar 15;51(3):825–36.
[8] Yu TY, Pang JHS, Lin LP, Cheng JW, Liu SJ, Tsai WC. Platelet-Rich Plasma Releasate Promotes Early Healing in Tendon After Acute Injury. Orthop J Sports Med. 2021 Apr 1;9(4).
[9] Padilla S, Sánchez M, Vaquerizo V, Malanga GA, Fiz N, Azofra J, et al. Platelet-Rich Plasma Applications for Achilles Tendon Repair: A Bridge between Biology and Surgery. Int J Mol Sci. 2021 Jan 15;22(2):824.
[10] Wong CC, Huang YM, Chen CH, Lin FH, Yeh YY, Bai MY. Cytokine and Growth Factor Delivery from Implanted Platelet-Rich Fibrin Enhances Rabbit Achilles Tendon Healing. Int J Mol Sci. 2020 May 2;21(9):3221.
[11] Park SH, Kim DY, Lee WJ, Jang M, Jeong SM, Ku SK, et al. Effect of platelet-rich plasma in Achilles tendon allograft in rabbits. J Vet Sci. 2024;25(2).
[12] Huang D, Vithran DTA, Gong HL, Zeng M, Tang ZW, Rao ZZ, et al. Effectiveness of platelet-rich plasma in the treatment of Achilles tendon disease. World J Orthop. 2023 Jun 18;14(6):485–501.
[13] Ali Elsiddig Ahmed E, Muharib R Alruwaili K, Alruwaili AH, Talal M Alruwaili A, Ahmed S Aljudia H, Mohammed N Alhadi N. Efficacy of Platelet-Rich Plasma in Treatment of Achilles Tendinopathy: Systematic Review and Meta-Analysis. Cureus. 2025 Feb 26;

[14] Li Y, Wang W, Xu W. Mechanisms and new advances in the efficacy of plant active ingredients in tendon-bone healing. J Orthop Surg Res. 2025 Jan 29;20(1):106.
[15] Yurteri A, Mercan N, Çelik ZE, Yaykaşlı H, Yıldırım A. Does quercetin affect tendon healing? An experimental study in a rat model of Achilles tendon injury. Front Med (Lausanne). 2025 Feb 5;12.
[16] Niyangoda D, Muayad M, Tesfaye W, Bushell M, Ahmad D, Samarawickrema I, et al. Cannabinoids in Integumentary Wound Care: A Systematic Review of Emerging Preclinical and Clinical Evidence. Pharmaceutics. 2024 Aug 17;16(8):1081.
[17] Abbasifard M, Moosavi Z, Azimi M, Kamiab Z, Bazmandegan G, Madahian A, et al. Effect of Topical Hemp (Cannabis sativa L.) Seed Oil on Knee Osteoarthritis: A Randomized Double-Blind Controlled Trial. Pain Management Nursing. 2025 Feb;26(1):e74–81.
[18] Stauch CM, Ammerman B, Sepulveda D, Aynardi MC, Garner MR, Lewis G, et al. Biomechanical Effects of Δ9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD), the Major Constituents of Cannabis, in a Sprague Dawley Rat Achilles Tendon Surgical Repair Model: A Pilot Study. Am J Sports Med. 2021 Jul 7;49(9):2522–7.
[19] Rahman S, Rivai MA, Dasrul D, Ayu O. Effect of hemp seed oil on accelerating wound healing: Evaluation of wound size reduction, epithelialization, granulation tissue formation, and vascularization in murine models. Narra J. 2025 Apr;5(1):e1878.
[20] McGlory C, Calder PC, Nunes EA. The Influence of Omega-3 Fatty Acids on Skeletal Muscle Protein Turnover in Health, Disuse, and Disease. Front Nutr. 2019 Sep 6;6.
[21] Fernández-Lázaro D, Arribalzaga S, Gutiérrez-Abejón E, Azarbayjani MA, Mielgo- Ayuso J, Roche E. Omega-3 Fatty Acid Supplementation on Post-Exercise Inflammation, Muscle Damage, Oxidative Response, and Sports Performance in Physically Healthy Adults—A Systematic Review of Randomized Controlled Trials. Nutrients. 2024 Jun 27;16(13):2044.
[22] Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. BMC Vet Res. 2020 Dec 14;16(1):242.
[23] Zabrzyńska M, Grzanka D, Zielińska W, Jaworski Ł, Pękala P, Gagat M. The Bonar Score in the Histopathological Assessment of Tendinopathy and Its Clinical Relevance—A Systematic Review. Medicina (B Aires). 2021 Apr 9;57(4):367.
[24] VanDolah HJ, Bauer BA, Mauck KF. Clinicians' Guide to Cannabidiol and Hemp Oils. Mayo Clin Proc. 2019 Sep;94(9):1840–51.
[25] Atalay S, Jarocka-Karpowicz I, Skrzydlewska E. Antioxidative and Anti- Inflammatory Properties of Cannabidiol. Antioxidants. 2019 Dec 25;9(1):21.
[26] Citti C, Linciano P, Panseri S, Vezzalini F, Forni F, Vandelli MA, et al. Cannabinoid Profiling of Hemp Seed Oil by Liquid Chromatography Coupled to High-Resolution Mass Spectrometry. Front Plant Sci. 2019 Feb 13;10.
[27] Mikulcová V, Kašpárková V, Humpolíček P, Buňková L. Formulation, Characterization and Properties of Hemp Seed Oil and Its Emulsions. Molecules. 2017 Apr 27;22(5):700.
[28] Vitorović J, Joković N, Radulović N, Mihajilov-Krstev T, Cvetković VJ, Jovanović N, et al. Antioxidant Activity of Hemp (Cannabis sativa L.) Seed Oil in Drosophila melanogaster Larvae under Non-Stress and H2O2-Induced Oxidative Stress Conditions. Antioxidants. 2021 May 22;10(6):830.
[29] Stauch C, Ammerman B, Aynardi M, Garner M, Lewis G, Morgan D, et al. Biomechanical Effects of Δ9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD), the Major Constituents of Cannabis, on Tendon to Tendon Healing in a Sprague Dawley Rat Achilles Tendon Surgical Repair Model. Orthop J Sports Med. 2020 Jul 1;8(7_suppl6).
[30] Verrico CD, Wesson S, Konduri V, Hofferek CJ, Vazquez-Perez J, Blair E, et al. A randomized, double-blind, placebo-controlled study of daily cannabidiol for the treatment of canine osteoarthritis pain. Pain. 2020 Sep;161(9):2191–202.
[31] Claro-Cala CM, Grao-Cruces E, Toscano R, Millan-Linares MC, Montserrat-de la Paz S, Martin ME. Acyclic Diterpene Phytol from Hemp Seed Oil (Cannabis sativa L.) Exerts Anti-Inflammatory Activity on Primary Human Monocytes- Macrophages. Foods. 2022 Aug 7;11(15):2366.
[32] Chalidis B, Givissis P, Papadopoulos P, Pitsilos C. Molecular and Biologic Effects of Platelet-Rich Plasma (PRP) in Ligament and Tendon Healing and Regeneration: A Systematic Review. Int J Mol Sci. 2023 Feb 1;24(3):2744.
[33] Lu J, Li H, Zhang Z, Xu R, Wang J, Jin H. Platelet-rich plasma in the pathologic processes of tendinopathy: a review of basic science studies. Front Bioeng Biotechnol. 2023 Jul 21;11.
[34] Chen X, Jones IA, Park C, Vangsness CT. The Efficacy of Platelet-Rich Plasma on Tendon and Ligament Healing: A Systematic Review and Meta-analysis With Bias Assessment. Am J Sports Med. 2018 Jul 21;46(8):2020–32.
[35] Carmona JU, López C. Efficacy of Platelet-Rich Plasma in the Treatment of Equine Tendon and Ligament Injuries: A Systematic Review of Clinical and Experimental Studies. Vet Sci. 2025 Apr 18;12(4):382.
[36] Nauwelaers AK, Van Oost L, Peers K. Evidence for the use of PRP in chronic midsubstance Achilles tendinopathy: A systematic review with meta-analysis. Foot and Ankle Surgery. 2021 Jul;27(5):486–95.

Figures

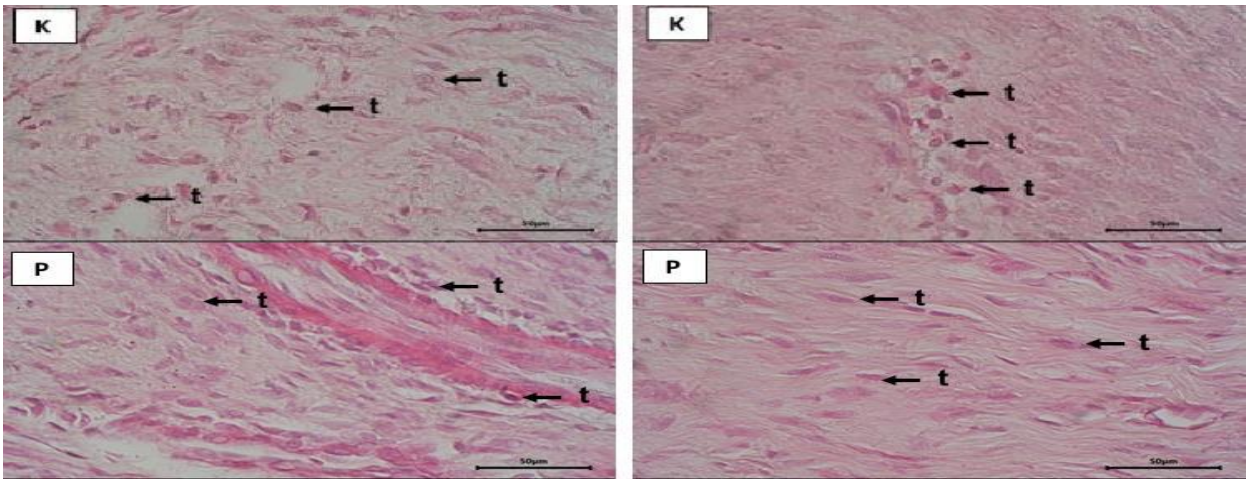

Figure 1. Histological appearance of the Achilles tendon. (A) Control group showing numerous tenocytes (t) with round nuclei and irregular collagen fibre arrangement. (B) Hemp oil treatment group demonstrating tenocytes (t) with elongated nuclei and more organized collagen fibre structure.

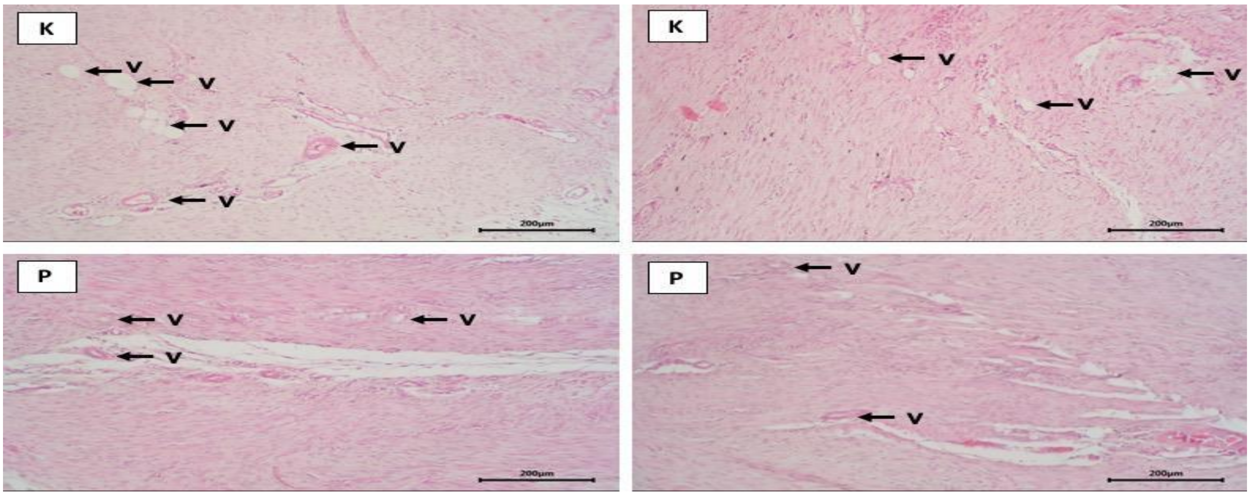

Figure 2. Vascularization in Achilles tendon tissue. (A) Control group (K) showing increased vascularization (v). (B) Hemp oil treatment group (P) showing reduced vascularization (v).

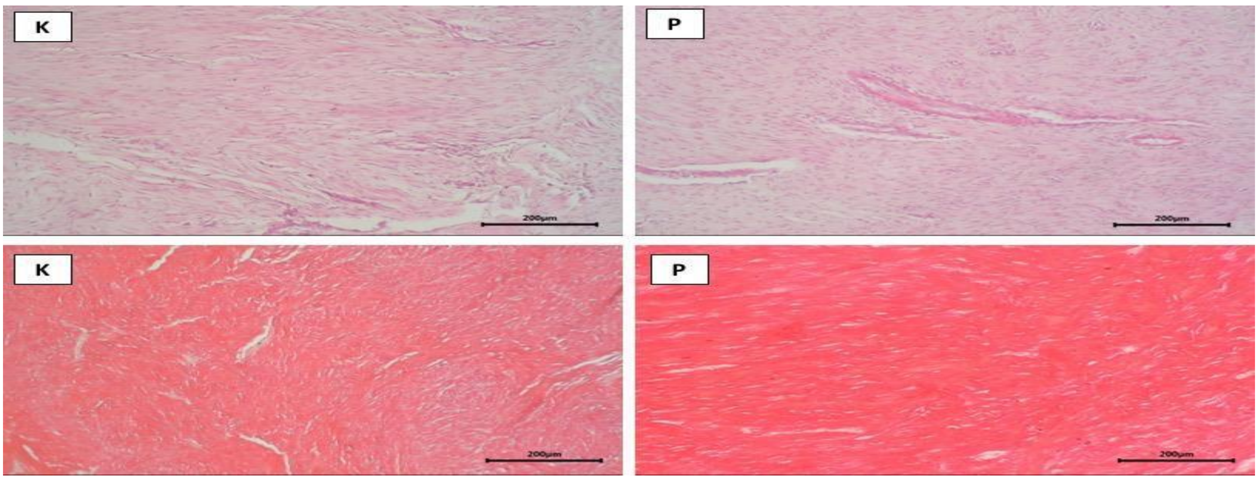

Figure 3. Collagen structure in Achilles tendon tissue. (A, top) Hematoxylin–eosin (HE) staining showing irregular collagen fibres in control rats (K) compared with more organized fibres in the treatment group. (B, bottom) Picrosirius red (PSR) staining demonstrating higher collagen content in the treatment group (P) than in the control group (K).

VETERINARY ETHICS COMMITTEE
FACULTY OF VETERINARY MEDICINE
SYIAH KUALA UNIVERSITY
BANDA ACEH, INDONESIA

Ethical Clearance Approval for Using Animals
(Certificate of Ethics)

Ref: 356/KEPH/X/2024

The Committee Veterinary Ethics for animals using in Faculty of Veterinary Medicine of Syiah Kuala University has studied carefully the design proposed research activity:

Researcher Lead	:	Eka Husnawaty Bahsoan
Research title	:	Pengaruh Pemberian Hemp Oil Terhadap Percepatan Penyembuhan Ruptur Tendon Achiles Pada Model Hewan Coba Tikus Putih (*Rattus norvegicus*).
Review material	:	1. Application form for animal use 2. Research proposal
Prosper Institution	:	Program Studi Dokter Spesialis Ilmu Bedah, Fakultas Kedokteran, Universitas Syiah Kuala

That research activity with animal subject as mentioned in the submission file by the applicant are stated to **fulfill the ethical feasibility requirements** to be implemented

Banda Aceh, October 16th 2024
Chief Committee,

Dr. Drh. Hamny, M.Si

This ethical approval can be revoked/removed if it is not in accordance with the approved research procedures.