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THE EFFECT OF INTRALESIONAL PROPOLIS ON TRANSFORMING GROWTH FACTOR-B (TGF-B) EXPRESSION IN TENDON HEALING: AN EXPERIMENTAL STUDY IN A WISTAR RAT MODEL OF PARTIAL ACHILLES TENDON RUPTURE

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ABSTRACT
Transforming growth factor-beta (TGF-β) regulates tendon repair, but dysregulated expression contributes to fibrosis. Propolis contains flavonoids and phenolics with immunomodulatory activity and may influence TGF-β signaling. This study evaluated intralesional propolis on TGF-β expression in a Wistar rat partial Achilles rupture model. A true experimental post-test only control group design was used. Male Wistar rats underwent standardized partial Achilles transection (30–40% thickness) and were randomized to: 50% propolis 0.1 mL intralesional (n=9), 0.9% NaCl 0.1 mL (n=9), or no injection (n=9). On day 35, tendons were harvested for immunohistochemistry using an IHC World ‑ based scoring (H-score 0 ‑ 300) assessed in ⩾5 high-power fields by two independent raters. Interobserver agreement was moderate (κ=0.444, p<0.001). TGF-β H-score categories differed significantly among groups (Kruskal–Wallis p<0.001). The propolis group shifted toward higher expression: 88.9% (8/9) were 101–200 and 11.1% (1/9) were 51–100, whereas both comparator groups clustered at 0 ‑ 50 (44.4%) and 51 ‑ 100 (55.6%), with none ⩾101. Intralesional 50% propolis increased TGF-β immunohistochemical expression at day 35 after partial Achilles rupture, suggesting enhanced regenerative signaling during tendon healing. Further studies should assess dose–response effects and biomechanical outcomes in vivo.

Keywords: achilles tendon; immunohistochemistry; propolis; transforming growth factor-beta

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INTRODUCTION

The Achilles tendon is the largest and strongest tendon in the human body, connecting the gastrocnemius and soleus muscles to the calcaneus (Miller & Thompson, 2023; Myhrvold et al., 2022; Xergia et al., 2023). Partial ruptures of the Achilles tendon, defined as a partial tear of tendinous fibers without complete discontinuity, are common injuries, particularly in active individuals aged 30-50. These injuries, often resulting from sudden, explosive movements or degenerative conditions, can lead to chronic pain and functional impairment, and may progress to a complete rupture if not managed appropriately (Miller & Thompson, 2023; Schulze-Tanzil et al., 2022).

Tendon healing is a complex biological process orchestrated in three overlapping phases: inflammation, proliferation, and remodeling (Schulze-Tanzil et al., 2022). The proliferation and remodeling phases are critically dependent on the activity of cytokines and growth factors. Transforming Growth Factor-beta (TGF-β) is a key multifunctional cytokine that regulates fibroblast proliferation, collagen deposition (primarily Type I), and extracellular matrix (ECM) remodeling (Deng et al., 2024; Randall et al., 2025). While essential for initiating and propagating the healing cascade, excessive or prolonged TGF-β expression is strongly linked to pathological fibrosis and scar tissue formation, which can permanently impair the tendon's biomechanical elasticity and function (Li et al., 2023).

Current management for partial Achilles ruptures ranges from conservative (immobilization, physical therapy) to surgical intervention, particularly for large tears (>50%) or in high-demand athletes (Aminlari et al., 2021; Mansfield et al., 2022; Yang et al., 2025). However, there remains a need for adjuvant therapies that can optimize the quality of tissue regeneration and accelerate functional recovery.

Propolis, a natural resin collected by bees (Trigona sp.), is rich in bioactive compounds such as flavonoids, phenolics (e.g., Caffeic Acid Phenethyl Ester - CAPE), and terpenoids (Balasubramaniam et al., 2025; Mansfield et al., 2022). It has demonstrated potent anti-inflammatory, antioxidant, and antimicrobial properties. Recent studies suggest propolis acts as a pro-healing agent by modulating immune responses and stimulating regenerative pathway. Specifically, propolis has been shown to modulate the TGF-β signaling pathway, potentially balancing the regenerative signals with anti-fibrotic effects (Zulhendri et al., 2022). However, the specific effect of propolis on TGF-β expression in the context of partial tendon rupture has not been fully elucidated. This study aims to investigate the effect of intralesional propolis administration on TGF-β expression in a Wistar rat model of partial Achilles tendon rupture. We hypothesize that propolis will modulate TGF-β expression, thereby supporting an enhanced regenerative environment.

METHOD

A true experimental post-test only control group design was employed to evaluate the effect of intralesional propolis following induction of a partial Achilles tendon rupture model. Male Wistar rats (*Rattus norvegicus*). This study was prepared in accordance with the ARRIVE 2.0 guidelines for reporting animal research and adheres 3R Principles. This study was reviewed and approved by the Health Research Ethics Committee of Universitas Sumatera Utara (Approval No. 1257/KEPK/USU/2025). After model induction, the allocated intervention was administered according to group assignment, and outcomes were assessed at a predefined endpoint through macroscopic evaluation of tendon healing and histological assessment of TGF-β expression in Achilles tendon tissue.

The study was conducted from September to October 2025 across two settings: an animal laboratory for model induction and intralesional administration, and an Anatomical Pathology laboratory for tissue processing and evaluation of TGF-β expression. Animals were selected using simple random sampling, and the experimental unit was one animal. At the terminal procedure, Achilles tendon specimens were harvested for macroscopic documentation followed by histological examination of TGF-β expression. Male Wistar rats, aged 8–12 weeks with a body weight of 200–250 g were included if they were clinically healthy, active, free from visible physical abnormalities, and had no prior tendon wounds or injury. Animals were excluded if they died before or during the intervention period, sustained additional injuries other than the intended partial Achilles tendon rupture, developed systemic infection or other illness during the study, or if harvested tendon specimens were damaged and unsuitable for histological analysis.

Group allocation and experimental groups

Male Wistar rats were randomly allocated by simple randomization into three groups (n = 10/group): (1) propolis group, receiving a single intralesional injection of 50% propolis solution (0.1 mL) at the injury site; (2) placebo group, receiving a single intralesional injection of 0.9% NaCl (0.1 mL); and (3) negative control group, receiving no intralesional treatment following injury induction. The experimental unit was one animal.

Preparation of propolis solution

A stock propolis extract was obtained through a heating and filtration process to yield purified propolis. A 50% propolis solution was prepared by mixing 10 mL of 100% propolis with 10 mL of

sterile distilled water (aquadest/aquabidest) under aseptic conditions until homogeneous, and the solution was used for intralesional administration.

Partial Achilles tendon rupture model and surgical procedure

Animals were anesthetized using standard intraperitoneal ketamine–xylazine anesthesia. After aseptic preparation of the posterior lower limb, a partial Achilles tendon rupture was created via a small incision using a microscalpel, transecting approximately 30–40% of tendon thickness at approximately 3 mm proximal to the calcaneal insertion. The skin was closed with simple sutures. Post-procedure monitoring was performed to identify any adverse events or intercurrent illness in line with animal welfare standards.

Terminal procedure, necropsy, and macroscopic assessment

At the end of the experiment (day 35), animals were euthanized by anesthetic overdose using Euthal (150 mg/kg, intravenous). Death was confirmed by absence of respiration and heartbeat and pupillary changes. Following confirmation, the animals were disinfected with 70% alcohol, and Achilles tendon tissues were collected for macroscopic documentation and subsequent histological processing.

Immunohistochemistry for TGF-β and scoring

Tendon specimens were processed for immunohistochemistry (IHC) at the Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara. Sections were cut using a microtome, deparaffinized in xylene (two changes), and rehydrated through graded ethanol (96%, 90%, 80%, 70%). After washing in PBS (pH 7.4; three times, 5 min each), endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 20 minutes, followed by PBS washes. Non-specific binding was blocked using 1% BSA for 10–30 minutes at room temperature. Sections were incubated with primary antibody (anti–TGF-β1) for 1 hour at room temperature and then overnight incubation, followed by PBS washes and incubation with SA-HRP–labeled secondary antibody for 1 hour at room temperature. Visualization was performed using DAB chromogen for 10–20 minutes, followed by rinsing, hematoxylin counterstaining for 5 minutes, dehydration, and mounting with entellan. Slides were evaluated under light microscopy (100× and 400×). Quantitative assessment used the IHC World scoring approach, including the percentage of positively stained cells and staining intensity, with assessment in ≥5 high-power fields (400×) per sample by two independent raters; inter-rater agreement was planned using the Kappa statistic.

Statistical Analysis

Data were analyzed using SPSS version 30 (IBM Corp.), and a two-sided p value < 0.05 was considered statistically significant. Descriptive statistics were reported as mean ± SD for approximately normally distributed continuous variables, median (IQR) for non-normally distributed or ordinal variables, and frequency (%) for categorical variables. The primary outcome, TGF-β immunohistochemistry, was analyzed both as a continuous H-score (0–300) and, where applicable, as an ordinal category (low, low–moderate, high–moderate, high). Between-group comparisons of H-scores across the three groups were performed using one-way ANOVA when normality and homogeneity assumptions were satisfied; otherwise, the Kruskal–Wallis test was used, followed by post-hoc pairwise comparisons with appropriate adjustment for multiple testing (e.g., Dunn–Bonferroni). Comparisons of categorical/ordinal classifications were conducted using the Chi-square test, with Fisher's exact test (or likelihood-ratio alternatives) applied when expected cell counts were small. Inter-rater agreement for IHC scoring between the two independent assessors was evaluated using the Kappa statistic.

RESULT

This study evaluated the effect of intralesional propolis on TGF-β expression during healing of a partial Achilles tendon rupture model in Wistar rats. TGF-β expression was assessed on day 35

using immunohistochemistry (IHC) and summarized using the histoscore (H-score), which integrates the percentage of positively stained cells and staining intensity.

Interobserver Reability

Interobserver reliability for IHC scoring was examined using the Cohen's kappa (κ) coefficient. The analysis demonstrated $\kappa = 0.444$ with $p < 0.001$, indicating moderate agreement between observers. These findings suggest acceptable consistency of TGF-β IHC assessment across raters. TGF-β expression was compared across groups using categorized H-score strata (0–50, 51–100, 101–200, 201–300). The Kruskal–Wallis test indicated a significant overall difference in TGF-β expression among the propolis, NaCl, and negative control groups ($p < 0.001$) (Table 4.2). The distribution of H-score categories showed a clear shift toward higher expression in the propolis group: 8/9 specimens were classified in the 101–200 category and 1/9 in the 51–100 category, with none in the lowest (0–50) or highest (201–300) strata. In contrast, both comparator groups clustered in lower categories, with NaCl and negative controls showing 4/9 specimens in 0–50 and 5/9 in 51–100, and no specimens reaching ≥101. Collectively, these findings suggest that intralesional propolis is associated with higher TGF-β IHC expression at day 35 compared with placebo and untreated controls (Table 1).

Tabel 1.
Overall differences in TGF-β immunohistochemical H-score categories among groups

H-score category	Propolis (n=9)	NaCl / placebo (n=9)	Negative control (n=9)	P-Value
0–50	0 (0.0)	4 (44.4)	4 (44.4)	
51–100	1 (11.1)	5 (55.6)	5 (55.6)	
101–200	8 (88.9)	0 (0.0)	0 (0.0)	
201–300	0 (0.0)	0 (0.0)	0 (0.0)	

P-value<0.01

DISCUSSION

This study demonstrated a significant difference in TGF-β expression as reflected by the H-score ($p < 0.001$). This finding indicates increased TGF-β protein expression in tendon tissue following intralesional propolis intervention. The propolis group showed the highest proportion of elevated TGF-β expression. Overall, these results position propolis as a promising bioactive agent with potential to modulate tendon healing. The increased TGF-β expression observed in the propolis group suggests an active stimulation of tissue regenerative processes. These findings are consistent with previous work by El-Sakhawy et al. (El-Sakhawy et al., 2023). Another study by Oršolić et al. (2022) showed that active compounds in propolis, such as CAPE and flavonoids, can stimulate fibroblast proliferation and enhance type I and type III collagen synthesis; in the present study, propolis derived from Trigona sp. bees contained flavonoids at 55.2 mcg/L (Irsyam et al., 2025; Oršolić & Jazvinšćak Jembrek, 2022). Taken together, these consistent results strengthen the theory that propolis exerts immunomodulatory and regenerative effects, potentially through activation of the TGF-β/Smad signaling pathway, thereby contributing to improved healing of connective tissues, including tendon. A study by Zhu et. Al. (2023) using bone marrow stem cells also reported increased TGF-β expression, stimulation of fibroblast proliferation, and accelerated as well as enhanced tendon healing (Zhu et al., 2023).

Zulhendri et al. 2022 similarly reported that propolis increases TGF-β production and accelerates healing. TGF-β is a multifunctional cytokine with a central role in connective tissue repair (Zulhendri et al., 2022). In tendon tissue, TGF-β stimulates expression of type I collagen, fibronectin, and proteoglycans, which are essential for new matrix formation (Firmansyah et al., 2024). Activation of the Smad2/3 signaling pathway by TGF-β promotes fibroblast activity and collagen deposition, while negative regulation of MMP-1 and MMP-9 helps prevent excessive matrix degradation. In addition, TGF-β contributes to regulation of cell proliferation, tissue contraction, and the transition from the inflammatory phase to the remodeling phase (Zhou et al.,

2023). Importantly, TGF-β may exert both beneficial and adverse effects; excessive or deficient TGF-β can disrupt balanced healing, leading to scar formation or incomplete repair (Xiaojie et al., 2022).

Collectively, these findings support the hypothesis that propolis may accelerate tissue regeneration by enhancing growth factor activity—particularly TGF-β—which plays a key role in the proliferative and remodeling phases of tendon healing. However, some studies have reported divergent results. Bahari et al. (2025) reported that high-dose propolis does not necessarily increase TGF-β expression significantly and may even reduce fibroblast activity due to overly potent antioxidant effects, which can suppress the early inflammatory response required to initiate healing (Bahari et al., 2025). These observations suggest that the effectiveness of propolis in enhancing TGF-β expression is dose- and context-dependent and underscores the need for formulation standardization in future studies.

Interobserver reliability testing showed moderate agreement for H-score assessment, indicating some degree of interpretive variability between observers. This may be influenced by the relatively small sample size and the inherently subjective nature of semi-quantitative scoring. Nevertheless, the statistically significant agreement suggests that the observed concordance was meaningful and unlikely to occur by chance.

The present findings further suggest that propolis can stimulate TGF-β expression without inducing excessive overexpression, which is clinically important because appropriate TGF-β regulation may help prevent fibrosis, adhesion, and scar tissue formation. Fibrosis is a common complication in tendon healing that can permanently impair tissue function and elasticity, potentially resulting in functional limitation. The dose used in animal experiments may be estimated for humans using the human equivalent dose conversion formula as a preliminary reference for safe dosing in clinical studies. In this study, a 50% propolis concentration of 0.1 cc (equivalent to 50 mg) was administered to a 250 g rat; using human equivalent dose conversion, this corresponds to an estimated 32.4 mg/kg in humans.52

Several limitations should be considered when interpreting these results. First, the sample size was relatively small, limiting the generalizability of the findings to broader populations. Second, observation was limited to the earlier stages of healing, and therefore structural changes during late remodeling may not have been fully captured. Third, the assessment relied on descriptive histological evaluation without accompanying quantitative molecular analyses (e.g., gene or protein expression of TGF-β and collagen), which could provide deeper mechanistic insight into the biological pathways involved.

CONCLUSION

Intralesional propolis significantly increased TGF-β immunohistochemical expression at day 35 in a Wistar rat partial Achilles tendon rupture model compared with placebo (NaCl) and negative control groups ($p < 0.001$). These findings suggest that propolis may enhance tendon healing by modulating growth factor–related pathways involved in tissue repair. Further studies should assess additional molecular markers (e.g., VEGF and collagen I/III) using IHC and/or RT-PCR, explore dose–response relationships, and compare propolis with other regenerative therapies such as PRP or stem cells.

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