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Combined extract (*Curcuma longa-Glycyrrhiza glabra*) alleviates the inflammations of Achilles tendinopathy in Wistar rats

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Abstract. – OBJECTIVE: Achilles tendinopathy is a frequent pathological condition in adults with overused ankles, causing microtrauma, inducing tenocyte apoptosis and inflammatory response. Common treatment involves oral prescription or injection of anti-inflammatory agents, surgery, or shock-wave therapy. However, prolonged administration is not advisable due to adverse effects. Therefore, a novel and safe regimen is needed. *Curcuma longa* and *Glycyrrhiza glabra* extracts are known for their anti-inflammatory effects owing to their active compounds (curcumin and glycyrrhizin, respectively). This study aimed to determine the effect of combined extracts of *Curcuma longa* and *Glycyrrhiza glabra* on tendon healing in an animal model of Achilles tendinopathy (Wistar rats).

MATERIALS AND METHODS: This study took place from February to May 2022 and compared the regimens administered to 32 animal models of Wistar rats with 4 healthy rats as a control group to determine the most effective therapeutic regimen: immobilization, immobilization with ibuprofen, or immobilization with the combined extract. The outcomes were measured to find which intervention provided the lowest inflammatory markers [High Mobility Group Box-1 (HMGB-1), Tumor Necrosis Factor-α (TNF-α), Chemokin motif ligand 12 (CXCL-12)], and improved tissue morphology represented by the BONAR score, decreased cross-sectional area (CSA), and increased Macrophage 2 (M2) differentiation.

RESULTS: After Achilles tendinopathy was induced, total immobilization (I1) was proven to be the most effective with the lowest CSA, whereas immobilization+175 mg/kg *Curcuma longa*+110 mg/kg *Glycyrrhiza glabra* extract (I5) was the most effective with the lowest HMGB-1 levels and the lowest CXCL-12 levels. Immobilization+131 mg/kg *Curcuma longa*+82.5 mg/kg *Glycyrrhiza glabra* extract (I6) was the most effective with the lowest Bonar score, while immobilization+87.5 mg/kg *Curcuma longa*+55 mg/kg *Glycyrrhiza glabra* extract (I7) was proven to be the most effective with the highest M2 coverage area and the lowest TNF-α levels.

CONCLUSIONS: We found that combined extract therapy was the most effective intervention for treating Achilles tendinopathy due to its ability to provide the lowest inflammatory markers.

Key Words:
Bonar score, Chemokin motif ligand 12, *Curcuma longa*, *Glycyrrhiza glabra*, High Mobility Group Box-1, Macrophage 2, Tumor Necrosis Factor-α.

Introduction

Achilles tendinopathy is the most common Achilles tendon disorder among runners and people with high mobility. In Europe, 37 per 100,000 people suffer from Achilles tendinopathy. Approximately 30% of professional runners are having this condition, with an incidence varying between 7-9% in professional runners and 9% in recreational athlete[1,2].

Achilles tendinopathy occurs approximately 2-7 cm above the calcaneal insertion due to mechanical overuse of the Achilles tendon, which induces microtrauma and tenocyte apoptosis due to cellular stress[3]. Tenocyte apoptosis releases damage-associated molecular patterns,

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especially high-mobility group box 1 (HMGB-1)[4]. This HMGB-1 activates the nuclear factor kappa B (NF-κB) pathway by interacting with toll-like receptor 4 (TLR-4) and producing tumor necrosis factor-α (TNF-α) through its canonical pathway. Through its non-canonical pathway, NF-κB also secretes C-X-C ligand chemokine-12 (CXCL-12) that will enhance DAMP (Damage-Associated Molecular Pattern) release and recruits more inflammatory cell such as macrophage[5]. The prolonged inflammatory response degrades the collagen in the extracellular matrix of the Achilles tendon and replaces it with a stiffer matrix and alters its morphology. Hypercellularity, neovascularity, ovoid tenocytes, and collagen degradation are often found during the histopathological examination of Achilles tendinopathy. Together, these factors contribute to the tissue disruption that causes Achilles tendinopathy[6].

Unfortunately, universal therapy for severe Achilles tendinopathy has not been reported. Non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, have been frequently proposed as a general therapy to relieve the pain of Achilles tendinopathy with adverse effects due to prolonged use[7]. Tendon surgery and shock waves have also been proposed to manage Achilles tendinopathy. However, the results of this risky management have not always been satisfactory and consistent, causing patients to avoid the procedure[8].

Herbal medicines are known to have no significant side effects if consumed for prolonged period of time. *Curcuma longa* is an important herbal medicine owing to its curcumin component. Curcumin is known for its anti-inflammatory or immunomodulatory effects[9]. Curcumin has a positive effect on the inflammatory and regenerative response in tendinopathies. In addition, curcumin decreases and modulates the cell infiltration, activation, and maturation of leukocytes, as well as the production of pro-inflammatory mediators at the site of inflammation[10].

Glycyrrhiza glabra, also known as licorice, is a popular herbal medicine. *Glycyrrhiza glabra*, especially its roots and tubers, is used as a plant-based medicine for many diseases, owing to its anti-inflammatory properties due to its ability to inhibit the synthesis of pro-inflammatory cytokines such as interleukine-1β (IL-1β), IL-6, IL-8, and TNF-α[11].

Interestingly, glycyrrhizin is able to bind to HMGB-1, an important DAMP involved in Achilles tendinopathy pathophysiology[12]. In tendon mechanical overloading *in vivo*, HMGB-1 was released to the tendon matrix and initiated an inflammatory cascade, and this inflammation was inhibited by the administration of glycyrrhizin[13].

As both herbal medicines have potent anti-inflammatory effects, in this study, we proposed combining their individual use to achieve a more potent effect. The effect of the combination on Achilles tendinopathy has never been observed before. Therefore, the primary aim of this research is to prove that oral administration of a combination of *Curcuma longa* and *Glycyrrhiza glabra* extract could be used as a therapy for inflammatory processes that induce Achilles tendinopathy, especially in rat models of Achilles tendinopathy.

Materials and Methods

Plant Material

It consisted of *Curcuma longa* and *Glycyrrhiza glabra* extract with plant specimens identified by Siti Mudaliana, a botanist searcher at the Herbal Laboratory Unit, Materia Medica Batu (Lahor Street No. 87, Batu City). Vouchers of *Curcuma longa* and *Glycyrrhiza glabra* were deposited at the Herbal Laboratory Unit, Materia Medica Batu, under number 201106.KNT.L.005 and 210401.KLG.H.001, respectively.

Extract Preparation

Dried preparations of *Curcuma longa* rhizome and *Glycyrrhiza glabra* root were obtained and confirmed by a plant taxonomist in UPTD Materia Medica, a medicinal plant cultivation center in Batu City, Indonesia. Each sample was ground into a coarsely dry powder using a blender, followed by ethanolic maceration, as previously described[14]. One gram of the powder was briefly macerated in 20 ml of 96% ethanol for 6 hours at room temperature. The filtrate was collected using filter paper Whatman 40 and washed thrice with sterile water. The solvent was evaporated in an oven at 100°C. The extract was stored at 4°C until further use.

Thin Layer Chromatography: Active Compound Identification

The active compounds of *Curcuma longa* and *Glycyrrhiza glabra* extract were determined using thin-layer chromatography as described. The Rf values of chromatograms were used to determine the active compounds based on the Rf values in the references[15].

Animal Model Preparation

Male Wistar rats (Rattus norvegicus), 2 months old and weighing 250 g, were chosen as the animal model. The rats were obtained from the Bioscience Laboratory, Universitas Brawijaya Indonesia, and maintained in a new environment for two weeks. Institutional and national standards for the care and use of laboratory animals were followed. Four healthy rats were used as negative controls. Hence, no intervention was performed. To induce tendinopathy in the Achilles tendon, 32 rats ran on a treadmill at 25 m/min for 15 minutes with 10° inclination on the first 7 days, then increased to 25 m/min for 60 minutes on weekdays (5 days) for 3 weeks and divided into eight experimental groups. Four weeks after the Achilles tendinopathy induction procedure, oral therapy was administered to Achilles tendinopathy rats every 8 hours daily6. There were 2 control groups [negative control (CN) or healthy rats and positive control (CP) or Achilles tendinopathy-induced rats without oral therapy] and seven oral therapy groups. Each oral therapy group was administered different therapies: immobilization only, immobilization+20 mg/kg of ibuprofen, immobilization+175 mg/kg of *Curcuma longa* extract, immobilization+110 mg/kg of *Glycyrrhiza glabra* extract, immobilization+175 mg/kg of *Curcuma longa*+110 mg/kg of *Glycyrrhiza glabra* extract, immobilization+131 mg/kg of *Curcuma longa*+82.5 mg/kg of *Glycyrrhiza glabra* extract, and immobilization+87.5 mg of *Curcuma longa*+55 mg/kg of *Glycyrrhiza glabra* extract. After four weeks of oral therapy, the rats were sacrificed.

Cross-Section Area (CSA) of Achilles Tendon Measurement

After the rats were sacrificed, the perpendicular axis lengths of the right Achilles tendon were measured in the proximal, middle, and distal regions. The product of each perpendicular length marks the area of each region. The cross-sectional area represented the mean of the three regions.

Evaluating Bonar Score

Hematoxylin-eosin staining was performed on the histopathological slides to facilitate tissue observation. Observations were performed at 200× magnification. Previous publications were used as a guide for the Bonar score[16]. If the score is >11.6, Achilles tendinopathy can be considered.

Extracellular HMGB-1, TNF-α, and CXCL-12 Levels Measurement

The Achilles tendon in PBS (Phosphate Buffer Saline) was cooled to 4°C for 24 hours. The tendon was then homogenized in a protein extractor solution (Cat No. 17081) as described in the protocol. ELISA and ELISA kits were used to measure extracellular HMGB-1, TNF-α, and CXCL-12 levels. ELISA kit (Cat. No. E0257Ra) was used to measure extracellular HMGB-1 levels. ELISA kit (Cat. No. E0764Ra) was used to measure TNF-α levels. ELISA kit (Cat. No. E1538Ra) was used to measure CXCL-12 levels.

Evaluating M2 Differentiation

Immunohistochemical staining was performed to detect the macrophage 2 differentiation. Antibody CD-163 (Cat. No. SC-58965) was chosen as the macrophage 2 marker[17]. An immunohistochemical staining system [N-Histofine Simple Stain max PO (M), code: 414131F] was used to stain antibody-marked tissue and cells. Observations were performed at 400× magnification.

Statistical Analysis

Bonar Score is a score that measures tendinopathy based on histological images. It consists of 5 components, namely: (1) changes in tenocyte morphology; (2) accumulation of ground substance; (3) architecture of collagen bundles; (4) vascularization; (5) cellularity. The combination of the five assessment components determines the Bonar score (each component ranging from 0 to 3).

HMGB-1 is a non-histone nuclear protein that functions as an endogenous danger signal molecule and triggers an inflammatory response when released into the extracellular matrix. HMGB-1 levels will be evaluated using the ELISA method, which targets the HMGB-1 protein (HMGB-1 Elisa Kit, catalog number E0257Ra, units ng/mL). TNF-α is a pro-inflammatory cytokine produced by macrophages and functions in inducing inflammation in tissues and migrating inflammatory cells. TNF-α levels will be measured using ELISA (TNF-α Elisa Kit, catalog number E0764Ra, unit ng/L). CXCL-12 is a chemokine that plays a role in inflammation (non-canonical NF-κB pathway) and cellular immune responses as a modulator of cell migration. CXCL-12 levels will be measured using ELISA (CXCL-12 Elisa Kit, catalog number E1538Ra, unit ng/L).

The data normality was evaluated using Shapiro-Wilk, while data homogeneity was by the Levene method. If the results of the Shapiro-Wilk

test are more than 0.05, the data distribution is normal. If the Levene test results are more than 0.05, the data is homogeneous. Statistical analysis was performed using the Statistical Package for Social Science (SPSS) 21.0 version (IBM Corp., Armonk, NY, USA). Homogenous and normally distributed data was compared using ANOVA comparative test, while Kruskal-Wallis was used when the data was not homogenous and normally distributed. The data was considered statistically significant if the *p*-value was lower than 0.05.

Results

Active Compound of Curcuma Longa and Glycyrrhiza Glabra Extract

3 active compounds are found in *Curcuma longa* extract through thin-layer chromatography, xanthorrhizol, thymol, and curcumin. Meanwhile, the active compounds found in the *Glycyrrhiza glabra* extract through thin-layer chromatography are glycyrrhizin and chalcone.

Effects of Combined Extract Toward Cross-Section Area of Achilles Tendon

Figure 1 shows the correlation between the intervention of the combined extract and CSA of the Achilles tendon after Achilles tendinopathy induction. From Figure 1, we can see that Achilles tendinopathy induction caused Achilles tendon edema and was proven by a larger CSA. Among the interventions provided to alleviate tendon edema, Intervention 1 (I1) or total immobilization was proven to be the most effective, providing the lowest CSA after Achilles tendinopathy was induced.

The *p*-value between the positive control (CP) and all interventions was <0.001. The *p*-value between the CP and I1 was <0.001.

Effects of Combined Extract towards Bonar Score

Figure 2 shows the correlation between the intervention of the combined extract and the Bonar Score of the Achilles tendon after Achilles tendinopathy induction. From Figure 2, we can see that the induction of Achilles tendinopathy changed the tissue morphology of the Achilles tendon and was proven by a higher Bonar Score. Among the interventions provided to alleviate tissue degeneration in the Achilles tendon, intervention 6 (I6) or immobilization+131 mg/kg *Curcuma longa*+82.5 mg/kg *Glycyrrhiza glabra* extract was the most effective, providing the lowest Bonar score after Achilles tendinopathy was induced.

The *p*-value between the positive control (CP) and all interventions was <0.001. The *p*-value between CP and I6 was 0.018. Figure 3 shows the differences

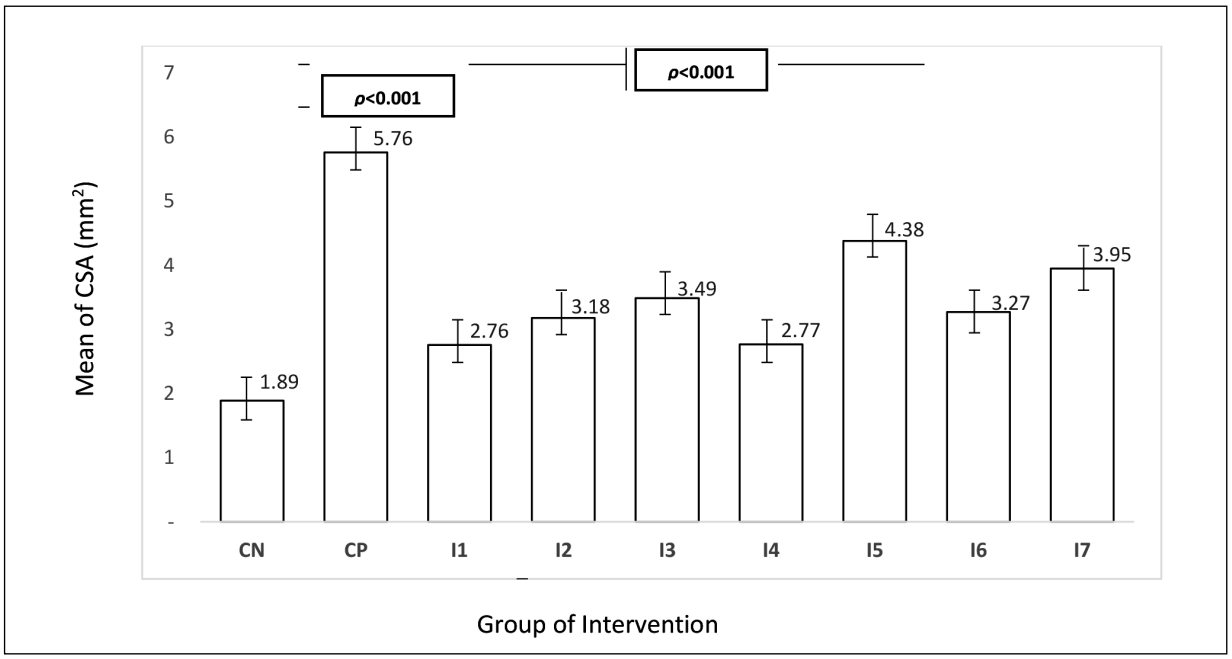

Figure 1. The cross-section area (CSA) of controlled groups and intervention groups.

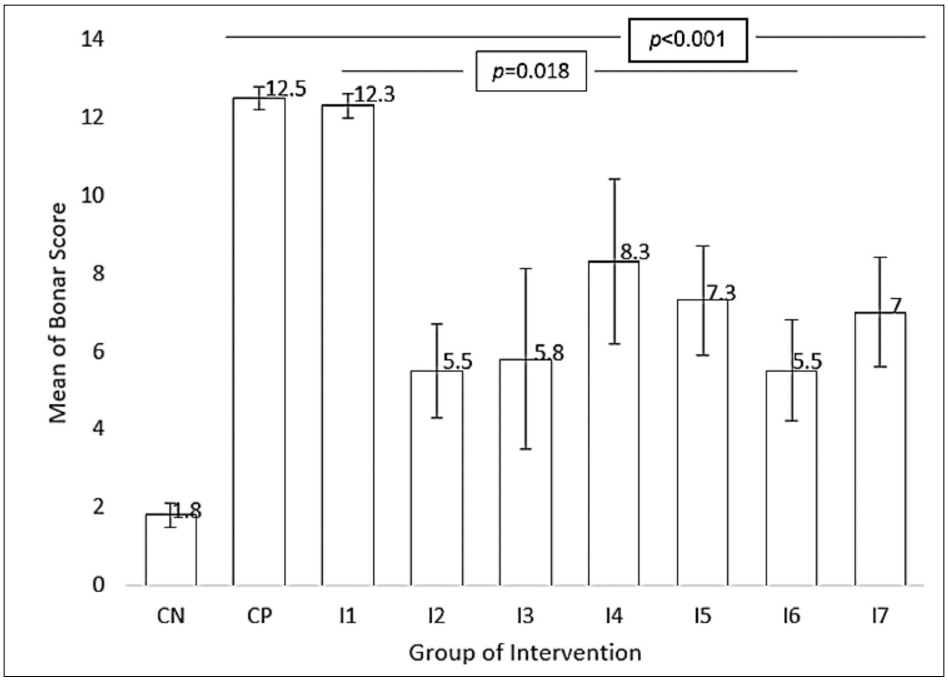

Figure 2. The Bonar Score of Controlled Groups and Intervention Groups.

in tissue morphology among the negative control, positive control, and intervention groups.

Effects of Combined Extract towards M2 Differentiation

Figure 4 shows the correlation between the intervention of the combined extract and the M2 coverage area of the Achilles tendon after Achilles tendinopathy induction. From Figure 4, we can see that Achilles tendinopathy induction increased the M2 coverage area as an attempt to heal the tendon. Among the interventions provided for tendon healing by increasing the M2 coverage area, intervention 7 (I7) or immobilization+87.5 mg/kg *Curcuma longa*+55 mg/kg *Glycyrrhiza glabra* extract was proven to be the most effective, providing the highest M2 coverage area after Achilles tendinopathy was induced.

The *p*-value between the positive control (CP) and all interventions was <0.001. The *p*-value between CP and I7 was <0.001. Figure 5 shows the differences in immunohistochemical staining among the negative control, positive control, and intervention groups.

Effects of Combined Extract on Extracellular HMGB-1 Level

Figure 6 shows the correlation between the intervention of the combined extract and HMGB-1 of the Achilles tendon after Achilles tendinopathy induction. Figure 6 shows that the induction of Achilles tendinopathy enhances HMGB-1 release from the Achilles tendon. Among the interventions to alleviate tissue degeneration in the Achilles tendon, intervention 5 (I5) or immobilization+175 mg/kg *Curcuma longa*+110 mg/kg *Glycyrrhiza glabra* extract was the most effective, providing the lowest HMGB-1 levels after Achilles tendinopathy was induced.

The *p*-value between the positive control (CP) and all interventions was <0.001. The *p*-value between CP and I5 was <0.001.

Effects of Combined Extract on TNF-α Level

Figure 7 shows the correlation between the intervention of the combined extract and TNF-α in the Achilles tendon after Achilles tendinopathy induction. From Figure 7, we can see that Achilles tendinopathy induction enhanced TNF-α release from the Achilles tendon. Among the interventions provided to alleviate tissue degeneration in the Achilles tendon, intervention 7 (I7) or immobilization+87.5 mg/kg *Curcuma longa*+55 mg/kg *Glycyrrhiza glabra* extract was the most effective, providing the lowest TNF-α levels after Achilles tendinopathy was induced.

The *p*-value between the positive control (CP) and all interventions was <0.001. The *p*-value between CP and I7 was 0.001.

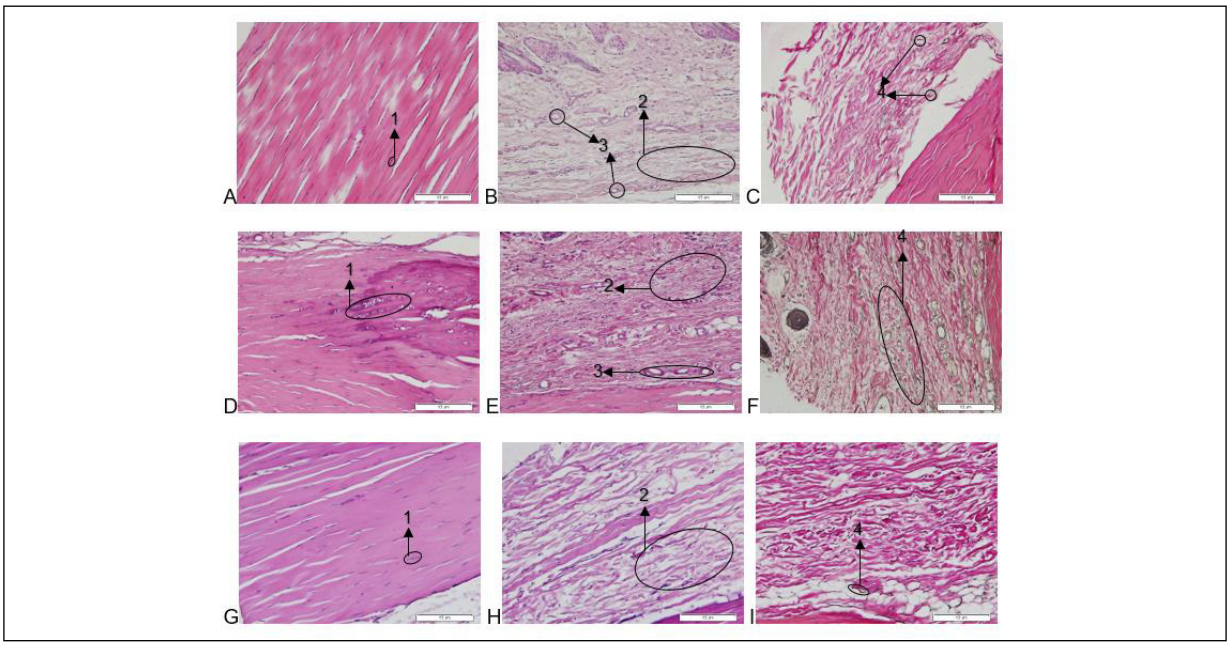

Figure 3. Histopathology observation of rats Achilles tendon; 1: nucleus, 2: collagen, 3: vascular, 4: ground substance (black); (**A-C**) come from cn group, (**D-F**) come from cp group, (**G-I**) come from I6 group. Magnification: 400x.

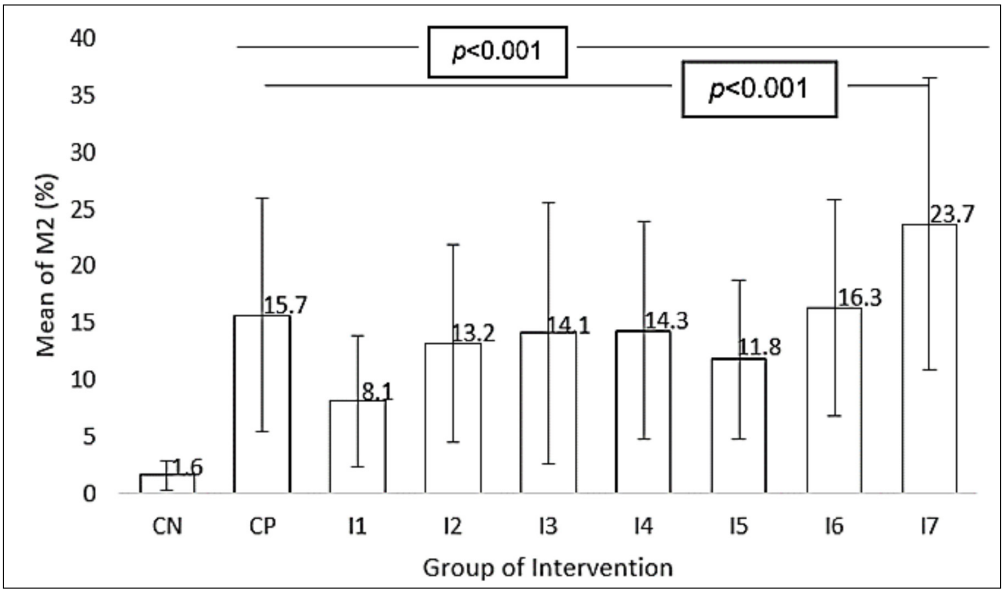

Figure 4. The M2 area of controlled groups and intervention groups.

Effects of Combined Extract on CXCL-12 Level

Figure 8 shows the correlation between the combined extract and CXCL-12 intervention in the Achilles tendon after Achilles tendinopathy induction. Figure 8 shows that the induction of Achilles tendinopathy enhanced CXCL-12 releases from the Achilles tendon. Among the interventions provided to alleviate tissue degeneration in the Achilles tendon, intervention 5 (I5) or immobilization+175 mg/kg *Curcuma longa*+110 mg/kg *Glycyrrhiza glabra* extract was the most effective, providing the lowest CXCL-12 levels after Achilles tendinopathy was induced.

The *p*-value between the positive control (CP) and all interventions was <0.001. The *p*-value between CP and I5 was <0.001.

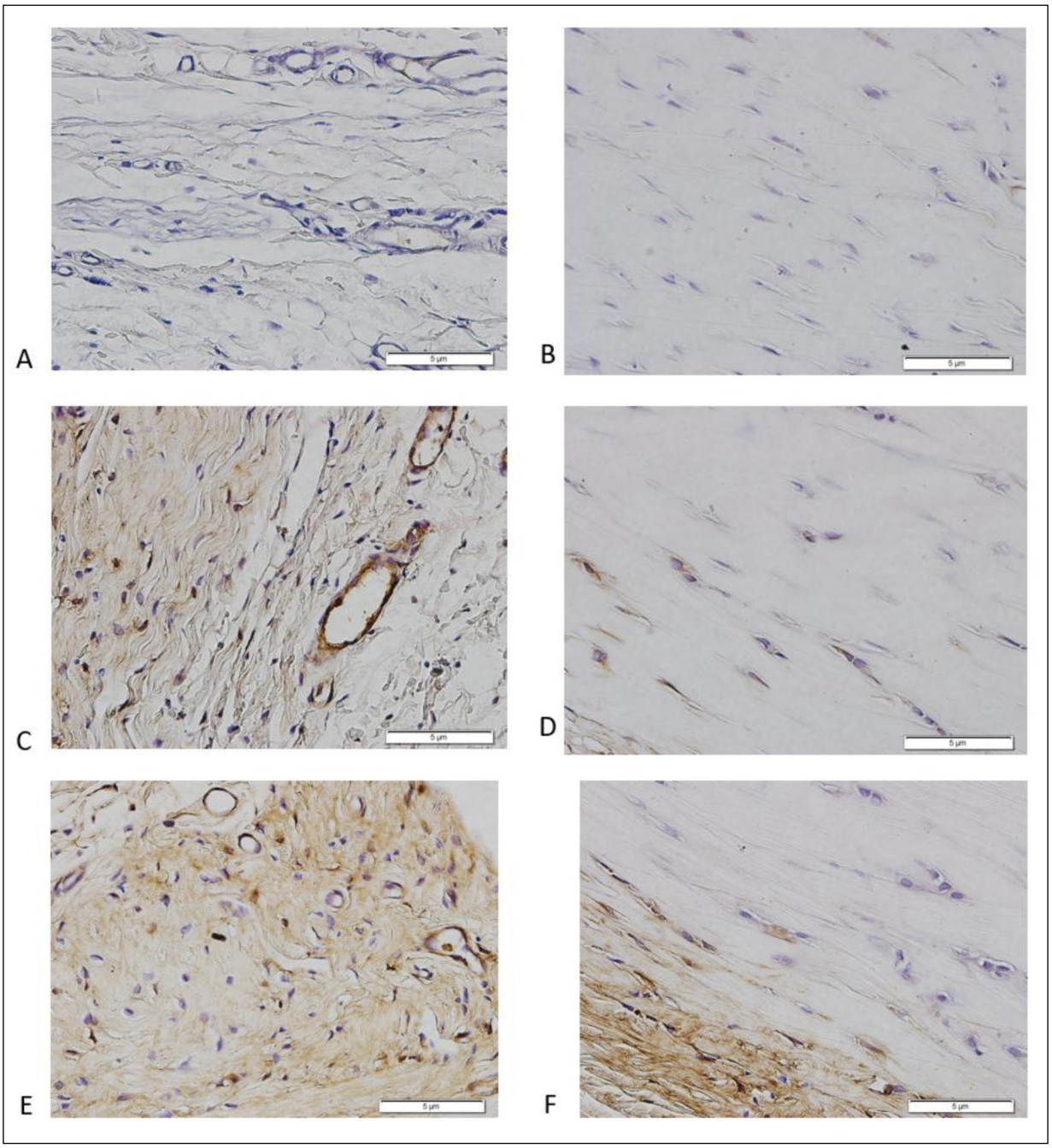

Figure 5. Immunohistochemical staining of rats Achilles tendon, brown color shows M2 coverage area; (**A, B**) come from CN group; (**C, D**) come from CP group; and (**E, F**) come from I7 group. Magnification: 400x.

Discussion

Before we discuss the effects of the combined extract on Achilles tendinopathy in rat models, we must realize that this study succeeded in proving that the extracts used in this study had the most potent active compounds as herbal medicines, namely curcumin in *Curcuma longa* extract and glycyrrhizin in *Glycyrrhiza glabra* extract. These results were confirmed by the book "Plant Drug Analysis A Thin Layer Chromatography Atlas" which describes each Rf of each active ingredient[15]. In addition, using molecular docking, this study succeeded in predicting the interaction of curcumin with TLR-4 and glycyrrhizin with HMGB-1 which plays a role in the inflammatory

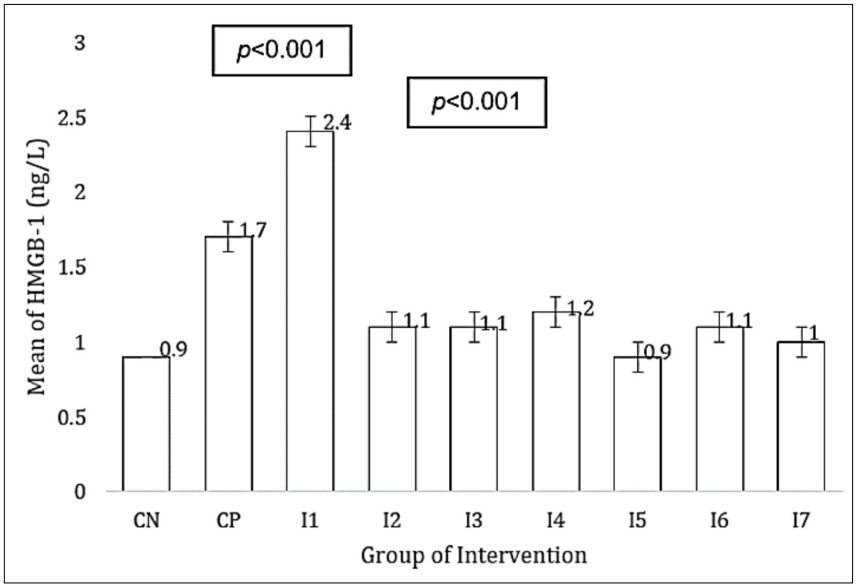

Figure 6. The HMGB-1 Levels of controlled groups and intervention groups.

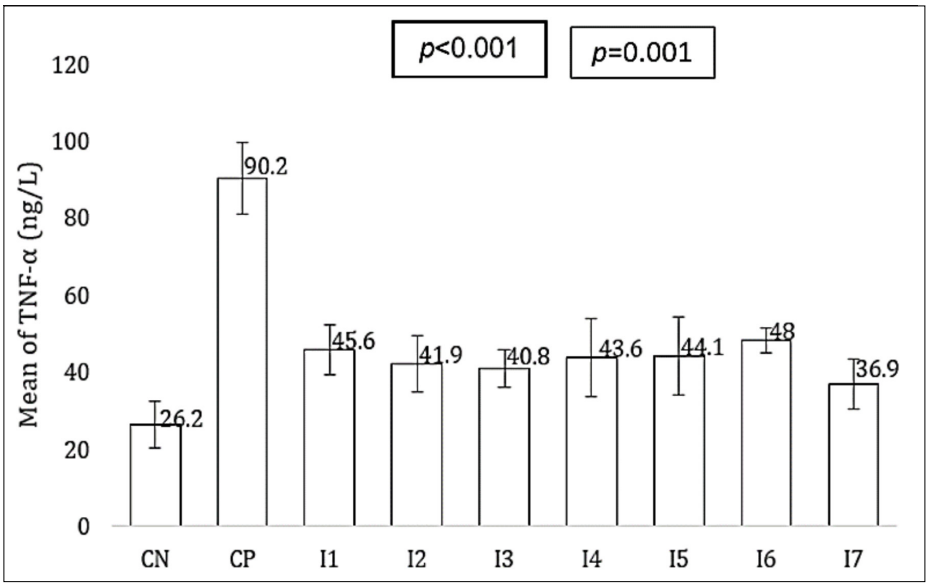

Figure 7. The TNF-α levels of controlled groups and intervention groups.

process. Since prolonged inflammation is the main pathophysiology of Achilles tendinopathy, the success of molecular docking in predicting the interaction between curcumin and TLR-4 and glycyrrhizin with HMGB-1 supports the hypothesis of the role of *Curcuma longa* and *Glycyrrhiza glabra* extracts are anti-inflammatory agents.

Based on the findings of this study, we can see that combined extract therapy provided the most satisfactory results, as it not only improved the quality of tissue morphology, but also increased healing through M2 differentiation. The data on inflammatory biomarkers also supported this result by showing that the lowest inflammatory biomarker level can be obtained under the effect of the combined extract. The ability of the combined extract to repair tissue, which was represented by a lower Bonar score,

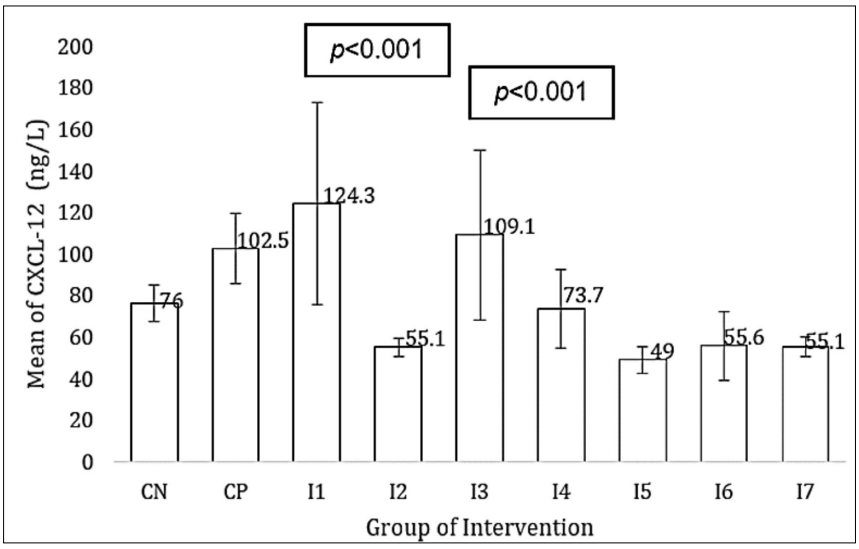

Figure 8. The CXCL-12 levels of controlled groups and intervention groups.

was due to the content of curcumin and glycyrrhizin in the combined extract.

Tissue degeneration in the Achilles tendinopathy rat model was caused by recurrent exposure to the inflammatory response due to tendon overuse[6]. But combined extract therapy can inhibit the inflammatory response, thereby preventing further degeneration and inducing tendon healing. Curcumin in *Curcuma longa* extract has potent anti-inflammatory, anti-oxidant, and anti-apoptotic properties and is capable of alleviating the damage to Achilles tendon tissue[18]. Glycyrrhizin in *Glycyrrhiza glabra* extract also plays a role in dampening the activity of DAMP, especially HMHB-1, which plays a central role in the pathogenesis of Achilles tendinopathy[12]. Together, they improve tendon healing and repair tissue damage in the Achilles tendon.

Regarding the effect of the combined extract toward HMGB-1 secretion in Achilles tendinopathy rat models, a previous study performed at the Konkuk University of Seoul, in which rats were injected with lipopolysaccharide to induce systemic inflammation, and another study in Jianzhu, China, in which rats were induced to suffer hepatitis, showed that *Curcuma longa* extract inhibited the secretion of HMGB-1 into the extracellular compartment[19,20] This occurred because curcumin in *Curcuma longa* extract acted as a TLRs inhibitor and prevented the activation of the NF-κB pathway[19].

On the other side, *Glycyrrhiza glabra* also plays its role in preventing the release of HMGB-1. In a study[21] performed in Guizhou, China, in 2022, mice induced with acute respiratory distress syndrome (ARDS) showed high levels of HMGB-1 in their lung. However, certain groups that received glycyrrhizin showed lower levels of HMGB-1. Just like curcumin, glycyrrhizin can also bind to TLR, thereby preventing NF-κB activation and p38. It also binds HMGB-1 to prevent its activity as a ligand of many receptors[22].

The effects of *Curcuma longa* and *Glycyrrhiza glabra* on TNF-α have been studied extensively. In a study performed in Bangalore, India, in 2013 using sepsis model rats, it was found that all cytokines formed from the p50 transcription factor (the result of the NF-κB canonical pathway), such as IL-6, IL-1, and TNF-α, were decreased after *Curcuma longa* extract was administered orally[23]. It also occurred because curcumin acted as a mitogen-activated protein kinase (MAPK) an inhibitor that prevented p53 (apoptosis inducer) activities[24].

Glycyrrhiza glabra affects TNF-α synthesis owing to its ability to disrupt IκBα activity, a mediator of canonical NF-κB. Glycyrrhizin can inhibit the c-Jun-N-terminal kinase (JNK) pathway, preventing apoptosis and secretion of TNF-α[25]. Glycyrrhizin also inhibits transcription factors that target the coding gene for TNF-α by manipulating three proteins in the MAPK pathway: MKP1, MKP3, and PP2A[26]. Together with *Curcuma longa*, *Glycyrrhiza glabra*, as a combined extract, can inhibit the NF-κB pathway more efficiently. The combined extract also targets the MAPK pathway, thus preventing TNF-α secretion.

Glycyrrhiza glabra affects the transcription of the coding gene for the CXCL-12 or Cxcl12 gene. Hence, the levels of CXCL-12 that recruits pro-inflammatory cells decreased[27]. A study[28] conducted in Seoul in 2019 using lung-injured mice found that CXCL-12 levels were not sufficient to activate its receptor if 50 mg/kg of *Glycyrrhiza glabra* was administered orally to the mice. This phenomenon decreased pro-inflammatory cell recruitment. *Curcuma longa* also affects CXCL-12 synthesis by inhibiting the non-canonical NF-κB pathway based on a previous study performed in Texas in 2012 using cancer cells. All receptors that use CXCL-12 as their ligand cannot be activated after the administration of *Curcuma longa* extract[29,30].

This study showed that the ideal dose of the extract combination to increase M2 differentiation was the combined dose of *Curcuma longa* extract (87.5 mg) and *Glycyrrhiza glabra* (55 mg/kg). Using this combination dose, the coverage area of M2 was obtained, which reached 23.7% of the entire Achilles tendon tissue in the Achilles tendinopathy rat model. The high-coverage area of M2 can reduce the symptoms of Achilles tendinopathy such as pain and edema.

Interestingly, *Glycyrrhiza glabra* increased the differentiation of M1 macrophages (pro-inflammatory macrophages) when administered to macrophage cultures isolated from mouse monocytes. In a study[31] conducted in Zhejiang, China, it was found that monocyte cells isolated from the bone marrow of mice could differentiate into M1 when administered with 100 µg/mL of glycyrrhizin isolated from *Glycyrrhiza glabra* extract. Although the exact mechanism is unknown, it is suspected that small doses of glycyrrhizin will induce the proliferation and differentiation of monocytes into macrophages and finally into M1, but the higher dose will increase the differentiation of M2.

In contrast, *Curcuma longa* increased the differentiation of macrophages into M2 macrophages. This is due to the ability of curcumin to increase the cytokines IL-4 and IL-13, which are produced by macrophages in an autocrine or paracrine manner to differentiate into M2. In a study conducted in 2015 in Shanxi, China, macrophages only synthesized the cytokines IL-4 and IL-13 after being exposed to 25 µmol/mL curcumin isolated from *Curcuma longa* extract[32]. Another study[33] conducted in 2016 in Niigata, Japan, showed an increase in the synthesis of IL-10 produced by M2 in nephrotoxic model rats after curcumin therapy was administered.

Strengths and Limitations

The limitations of this study included difficulties in finding the right age and size animal model. Furthermore, the immobilization of the tendon must be checked daily to avoid loosening due to the rats' activity. Another factor is the limited laboratory hours during the COVID-19 pandemic. It might also be preferable to work with a group of taxonomists instead of just one taxonomist. What also must be put into attention is the short availability of curcumin.

Meanwhile, the strength of this study was in its easy access to the plants, which are abundant in Indonesia, the safety of the extracts tested, and also its novelty.

In order to proceed with developing a commercial herbal medicine for patients, further research on a larger scale of experiments, including human trials, is required.

Conclusions

The combined extract of *Curcuma longa* and *Glycyrrhiza glabra* is very effective in reducing the inflammatory response in Achilles tendinopathy. This was confirmed by lower levels of HMGB-1, TNF-α, and CXCL-12. Combined extract therapy with *Curcuma longa* and *Glycyrrhiza glabra* also improved the morphology of Achilles tendon tissue, as demonstrated by the lower Bonar score. *Curcuma longa* and *Glycyrrhiza glabra* also increase the differentiation M2 hence enhancing tendon healing.

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Informed Consent

Not applicable.

Availability of Data and Materials

Not applicable.

Ethics Approval

This study was approved by the Health Research Ethics Committee of the Faculty of Medicine, University Brawijaya (23/EC/KEPK-S3/02/2022).

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Authors' Contributions
All authors were involved in the conception and design of the study, acquisition of data, or analysis and interpretation of data; drafting the article or making critical revisions related to relevant intellectual content of the manuscript; supervision; validation and final approval of the version of the article to be published.

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Conflict of Interest
The authors declare that they have no conflicts of interest.

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