DE GRUYTER
OPEN

herba polonica
From Botanical to Medical Research
Vol. 64 No. 1 2018

Received: 2017-03-12
Accepted: 2018-02-07

DOI: 10.2478/hepo-2018-0003

EXPERIMENTAL PAPER

Antioxidant properties of *Artemisia absinthium* accelerate healing of experimental Achilles tendon injury in rabbits

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Summary

Introduction: Delayed tendon healing is still found to be among the complications that occur most often after tendon repair.

Objective: The role of local injection of *Artemisia absinthium* was evaluated in healing of experimental Achilles tendon injury in rabbits.

Methods: . In nine adult New Zealand rabbits a partial thickness tenotomy was created on both hindlimbs. *A. absinthium* extract and normal saline were respectively injected daily to treatment and control groups for three days.

Results: On the day 7 after injury, the tendon sections showed that healing rate in *A. absinthium* treated group was higher than that in control group. Furthermore, at days 14 and 28, comparison between *A. absinthium* treated group and control group demonstrated that *A. absinthium* increased the healing rate but with no significance.

Conclusions: Results of this study have showed that application of *A. absinthium* extract can improve healing process of damaged Achilles tendon.

Key words: ***antioxidant, Artemisia absinthium, Achilles tendon, rabbits***

INTRODUCTION

Tendons are anatomic structures placed between muscles and bones and transmit force created in muscles to bones and make joint movements possible. Damage to these structures affects the natural balance between stability and mobility, thus altering joint kinematics and ultimately leading to destruction of the joint [1].

Delayed tendon healing and tendon adhesions are still found to be among the complications that occur most often after tendon repair. After tendon injury, the process of healing or tissue repair starts. The process can be largely divided into 3 overlapping phases: inflammatory, repairing and remodeling [2-4]. In all phases, antioxidants play a key role in healing process [5].

The process of inflammation normally leads to release of biologically active mediators to attract neutrophils, leucocytes and monocytes to the wound area and these attack foreign debris and microorganisms through phagocytosis. Then, this leads to the production of oxygen-free radicals such as hydrogen peroxide, superoxide anion, and hydroxyl anion and excess of these agents causes tissue damage in man or animal if they overwhelm the natural antioxidants of the host such as catalase, superoxide dismutase, and glutathione peroxidase. Therefore, antioxidants prevent the activity of free radicals and thereby prevent the damage of cells and tissues, providing protection to human and animal subjects, and also enhance healing of infected and non-infected wounds [5, 6].

Artemisia absinthium (*Asteraceae*), a species of wormwood, grows in temperate regions of Eurasia and Northern Africa. This species is an aromatic-bitter herb, used traditionally in Iran. Wormwood essential oil has been widely used mainly for its antimicrobial [7], antioxidant [8], antifungal [9], anthelmintic [10], antimalarial [11], antidepressant [12] and neuroprotective [13] properties.

This study investigates the effects of local injection of *A. absinthium* extract in healing of experimental Achilles tendon injury in rabbits.

MATERIAL AND METHODS

Animals

Nine adult New Zealand rabbits weighing 2±0.2 kg were used in this study. Before the beginning of the experiment, rabbits were housed for two weeks at the facility for acclimatization. The animals were supplied with standard pellet diet and tap water ad libitum throughout the experiment. All animals received sufficient care according to 'Guide for the Care and Use of Laboratory Animals' published by the National Institute of Health (NIH) – Approval No: B9/GVS/IAEC/0701 by the IAEC.

Plant materials and extract preparation

Aerial parts of the plant *A. absinthium* were purchased from official herbal drug center. Voucher specimens have been deposited at the Herbarium of the Faculty of Agricultural Science, Islamic Azad University, Garmsar, Iran. The aerial parts of the plant were dried and grounded into fine powder using an electric blender. The extract was prepared by cold maceration with distilled water for 24 h. 50 g of powder was suspended at 100 ml ethanol for 24 h at a room temperature. The mixture was then filtered using a fine muslin cloth followed by Whatman's No. 1 filter paper. The extract was concentrated using vacuum distillation.

Surgical procedur

Animals were anesthetized through the intramuscular injection of 5% ketamine hydrochloride (35 mg/kg) and 2% xylazine (5 mg/kg). The anesthesia was maintained with inhalation isoflurane. Surgery was performed on both hindlimbs; with left one served as control. A longitudinal skin incision was made over the Achilles tendon, and the paratenon was identified and incised longitudinally as a separate layer. The three bundles of Achilles tendon were identified, and the central bundle was separated bluntly from the medial and lateral bundles (fig. 1A). A partial-thickness tenotomy (approximately 50% of tendon bundle width and 1 cm length) was created, beginning at the medial aspect of the bundle and 2 cm proximal to the calcaneus (fig. 1B). This partial tenotomy allowed the rest of the tendon to act as an internal splint for the non-immobilized repair. 1 ml of ethanolic extract of *A. absinthium* (5%) and normal saline were respectively injected daily to treatment and control groups for three days postoperatively. After the surgery, rabbits were recovered from the anesthesia in a heated recovery chamber under continuous observation. Following recovery, animals were returned to individual cages for the rest of experiment. Five percent enrofloxacin (5 mg/kg, IM) was administrated to rabbits one hour preoperatively and continued for three days.

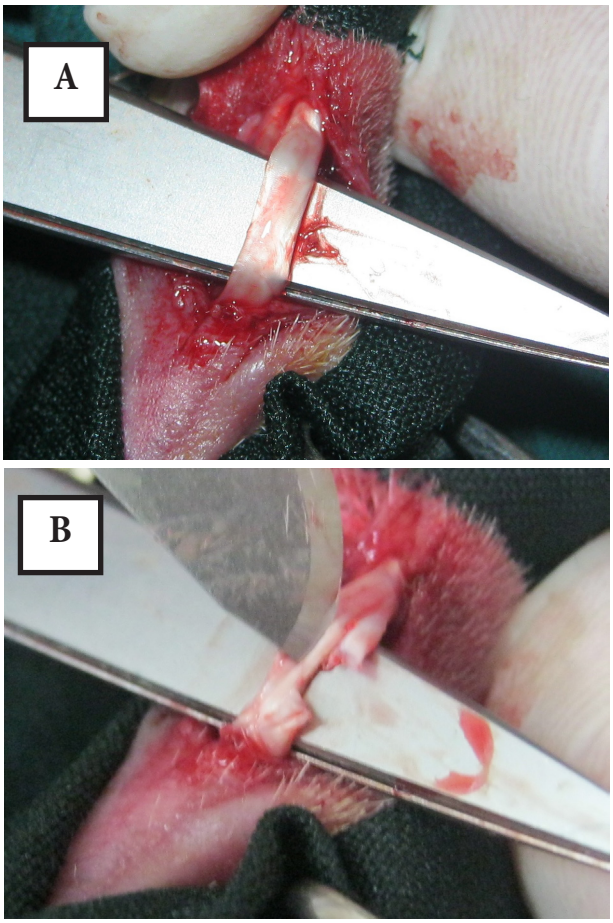

Figure 1.

A) Intact Achilles tendon, B) A partial-thickness tenotomy (approx. 50% of tendon bundle width and 1 cm length) was created, beginning at the medial aspect of the bundle and 2 cm proximal to the calcaneus

Histopathological studies

On days 7, 14 and 28 post surgery, three rabbits of each group were euthanized using sodium thiopental and Achilles tendon specimens were collected. Specimens were fixed in 10% buffered formalin and routinely processed using standard procedures and then stained with hematoxylin and eosin (H&E), and observed with light microscopy. Histopathological samples were scored qualitatively and semi-quantitatively based on Oryan *et al* scoring system [14].

Statistical analysis

Statistical analysis was performed using SPSS software v16.0 (SPSS Inc., USA) and Mann-Whitney U test. Data were expressed as mean ± standard deviation (SD). Differences were considered significant when $p<0.05$.

RESULTS

The average score of the histopathological changes in the two groups are shown in table 1. Injured tendons in both treatment and control groups at 7 days post operation showed hypercellularity and formation of new blood vessels. However, compared to those of control group, swelling and cellularity of the lesions decreased and fewer blood vessels were seen in treatment group (fig. 2A, 2B). Average healing score in treatment group was 1.66; despite 1.33 in control group. The difference was not significant ($p\geq0.05$). An intense inflammatory response was seen at day 14 post operation in control group, while this response was absent in treatment group. Results of histopathological studies at day 14 showed collagen fiber deposition in parallel alignment in treatment group. Healing seemed to be delayed in control group while the newly regenerated fibrous connective tissue was hypercellular and hyperneovascular (fig. 3A, 3B).

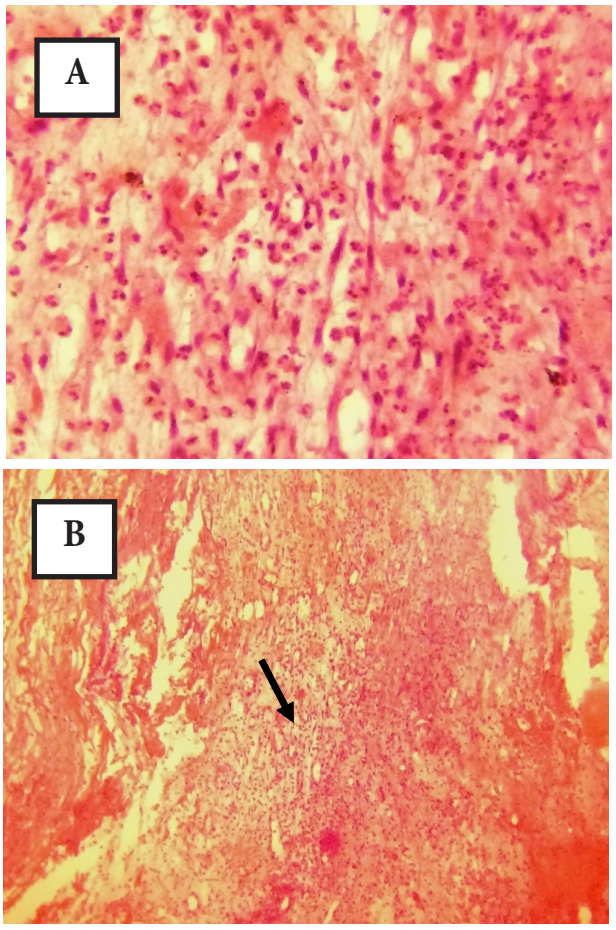

Figure 2.

Photomicrograph of the injured tendon in control group (A), and *A. absinthium*-treated group (B) at day 7 post operation. Note less hypercellularity, fewer new blood vessels and better healing process in treated group, (H&E×200)

Table 1.

Average score of the histopathological changes in the two groups

Day	*A. absinthium* extract	Control
7	1.66±0.57	1.33±0.57
14	2.33±0.57	2
28	3	2.33±0.57

Difference was not significant ($p \geq 0.05$)

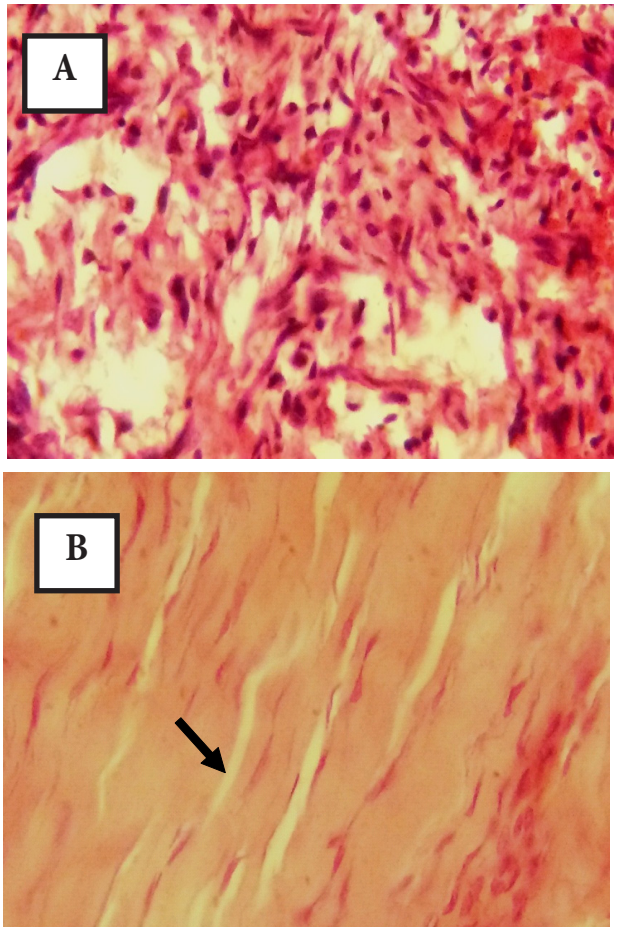

Figure 3.

Photomicrograph of the injured tendon in control group (A), and *A. absinthium* treated group (B) at day 14 post operation. Note less hypercellularity and more mature fibrous connective tissue and better healing process in *A. absinthium* treated group, (H&E×200)

At day 28, histhopatological results showed thick collagen fibers in parallel arrangement in treatment group. However, the control group showed deposition of thin collagen fibers, high blood vessels and hypercellularity characterized with increased fibroblasts (fig. 4A, 4B). Based on the findings at days 14 and 28, the mean healing rate in treated group was higher than that in control group. However, the difference was not statistically significant ($p \geq 0.05$).

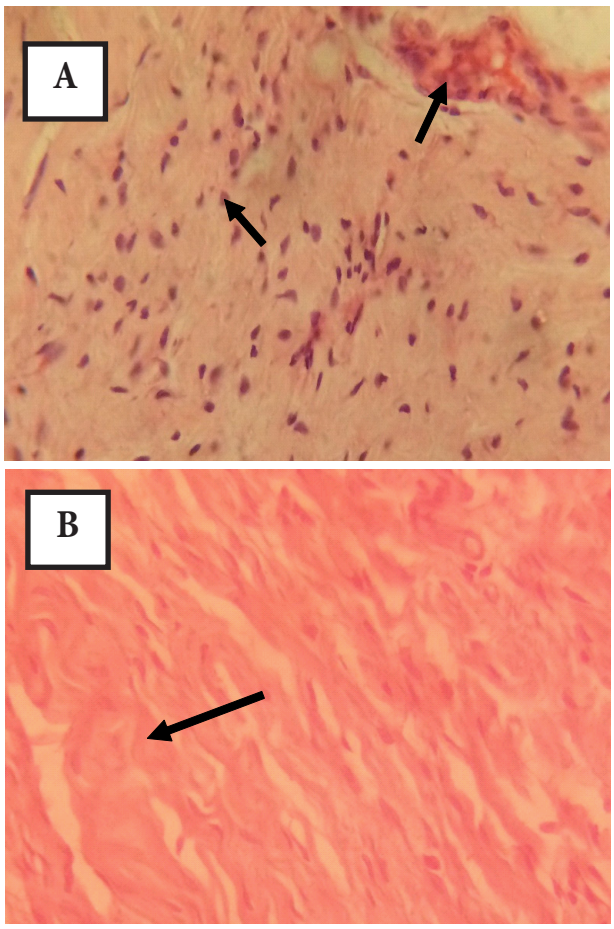

Figure 4.

Photomicrograph of the injured tendon in control group (A), and *A. absinthium*-treated group (B) at day 28 post operation. Note thick collagen fibers in parallel arrangement in treatment group. However, the control group showed deposition of thin collagen fibers with blood vessels and hypercellularity, (H&E×200)

DISSCUSION

Injuries and degenerative conditions of tendons represent almost 50% of the musculoskeletal injuries treated in orthopaedic clinics [15]. Like other connective tissue repair processes, tendon repair process has been an interesting subject of research for many years. It is well known that increased blood supply enhances the repair process in all kinds of connective tissues [16, 17].

In our study, on day 7 after injury, tendon micrographs showed that healing rate in *A. absinthium* treated group was higher than that in control group, however the difference was not significant ($p \geq 0.05$). Furthermore, at days 14 and 28 after injury, the comparison between the *A. absinthium* treated group and control group demonstrated that treatment with *A. absinthium* increased the healing rate with no significant difference. Based on the previous studies,

antioxidant and free-radical scavenging activity [18-20] and anti-inflammatory activity [18] have been reported for essential oil of *A. absinthium*. In recent years, oxidative stress has been implicated in a variety of degenerative processes and diseases; these include acute and chronic inflammatory conditions such as wound [21]. Tendon injuries have been shown to benefit from antioxidant therapy [22]. Antioxidants enhance the healing of infected and non-infected wounds by reducing the damage caused by oxygen radicals [6]. Positive correlation has been demonstrated between antioxidant activity and phenolic content of plant extracts [23]. It has been determined that the antioxidant effect of plant products is mainly attributed to phenolic compounds, such as flavonoids, phenolic acids, tannins and phenolic diterpenes [24]. Iranian wormwood essential oil was characterized by the predominance of β-pinene and β-thujone [25]. Aerial parts of *A. absinthium* have been reported to contain flavonoids [26-28], thymol, and carvacrol as well as other phenolic compounds [29]. These pharmacophores have been shown to possess potent antioxidant and free radical scavenging activity [30]. Many studies reported that phenolic compounds display antioxidant activity as a result of their capacity to scavenge free-radicals. Phenolic compounds can also act as antioxidants by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system [31, 32].

Various mechanisms, including reducing capacity, prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging have been claimed to explain the antioxidant activities [33]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Extract of *A. absinthium* exhibit effective reducing capacity at all concentration points. The reducing capacity of the extracts increased with increase of the concentration. The reducing properties are generally associated with the presence of reductones [34], which have been shown to exert antioxidant action by breaking the free radical chain, by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation [35].

However, no significant differences were observed between treatment and control groups in current study, but the higher average healing rate in treatment group compared to control group suggest that positive effects of *A. absinthium* extract in antioxidant and free-radical scavenging activity affect the tendon healing. In conclusion, results of this study have showed the tendency of healing process improve of damaged Achilles tendon after application of *A. absinthium* extract.

Conflict of interest: Authors declare no conflict of interest.

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Właściwości antyoksydacyjne *Artemisia absinthium* przyspieszające gojenie w doświadczalnym uszkodzeniu ścięgna Achillesa u królików

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Streszczenie

Wstęp: Powolne gojenie jest jedną z komplikacji pojawiających się najczęściej po operacji odbudowy ścięgna.

Cel: Badano wpływ miejscowego podania *Artemisia absinthium* w iniekcji na gojenie doświadczalnego uszkodzenia ścięgna Achillesa u królików.

Metody: U dziewięciu dorosłych królików nowozelandzkich przeprowadzono częściową tenotomię ścięgien obu kończyn tylnych. Następnie podawano w iniekcji wyciąg z *A. absinthium* lub sól fizjologiczną odpowiednio w grupie doświadczalnej i w grupie kontrolnej przez trzy dni.

Wyniki: Siódmego dnia po uszkodzeniu okazało się, że tempo gojenia w grupie leczonej wyciągiem z *A. absinthium* było wyższe niż w grupie kontrolnej. Co więcej, porównanie przeprowadzone 14. i 28. dnia wykazało, że podanie *A. absinthium* przyspieszyło gojenie, ale nieistotnie.

Wnioski: Wyniki tego badania pokazują, że podanie wyciągu z *A. absinthium* może przyspieszyć process gojenia uszkodzonego ścięgna Achillesa.

Słowa kluczowe: ***antyoksydant, Artemisia absinthium, ścięgno Achillesa, króliki***