Histopathological study of *Capparis spinossa* on the healing of experimental Achilles tendon injury in rabbits

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Abstract
The critical role of tendons in body mechanics and injury and degeneration of this tissue can highly be debilitating, resulting in substantial pain, disability and costs. *Capparis spinossa* is one of the most common aromatic plants growing in Iran. The major objective of the study was to assess Achilles tendon healing in a rabbit model by local injection of ethanolic extract of *C. spinossa*. Nine adult white New Zealand male rabbits were anesthetized and partial thickness tenotomies were created on both hindlimbs. The *C. spinossa* extract and normal saline were respectively injected daily to the treatment and control groups for three days post-operatively. Histological analysis on days 7, 14 and 28 post-rupture demonstrated higher regenerating activity and capacity in treated groups than the control group. This was illustrated by fewer inflammatory cells, a larger number of blood vessels, further fibroblasts and increased structural organization with further longitudinally oriented collagen fibers in the treated group. In summary, these results suggest that use of *C. spinossa* extract can promote the healing process of damaged Achilles tendons in rabbits.

Key words: *Capparis spinossa*, Achilles tendon, Rabbit

Introduction
Tendon is a compositionally complex tissue with a predominantly mechanical function of translating muscular contractions into joint movement by transmitting forces from muscles to bones. Tendons are able to store elastic energy and withstand high tensile forces; on which, body locomotion is entirely dependent (Aslan et al. 2008). Damages to these structures affect the natural balance between stability and mobility; thus, altering joint kinematics and ultimately lead to destruction of the joints (Ferrara 2002). Owing to critical roles of this tissue in body mechanics, injury and degeneration of tendons can highly be debilitating, which can result in substantial pain, disability and health-care costs. Delayed tendon healing and adhesion are still complications that occur most often after tendon repairs. Following tendon injuries, process of healing or tissue repair starts that can largely be divided into three overlapping phases of inflammatory, repairing and...
remodeling (Bosch et al. 2011, Kuo et al. 2003, Tohidnezhad et al. 2011). Antioxidants play key roles in healing process of each phase (Martin 1996). The process of inflammation normally leads to release of biologically active mediators to attract neutrophils, leukocytes and monocytes to the wound area to attack foreign debris and microorganisms through phagocytosis. This process results in production of oxygen-free radicals such as hydrogen peroxide, superoxide anion and hydroxyl anion. Excesses in these free radicals cause tissue damages in humans or animals if they overwhelm natural antioxidant enzymes of the host such as catalase, superoxide dismutase and glutathione peroxidase. Therefore, antioxidants prevent activity of the free radicals and thereby prevent damages to cells and tissues, provide protection to humans and animals and enhance healing of infected and non-infected wounds (Houghton et al. 2005, Martin 1996).

*Capparis spinosa* or caper (Family Capparidaceae) is one of the most common aromatic plants growing wild in dry regions around the West and Central Asia and the Mediterranean basin. Caper has been known for centuries in traditional phytomedicine, which has described its properties for several purposes. Studies have revealed that *Capparis* includes antioxidative (Mousavi et al. 2016), anti-fungal (Shtayeh and Abu Ghdeib 1999), anti-inflammatory (Al-Said et al. 1999), anti-diabetic (Ziyyat et al. 1997) and anti-hyperlipidemic (Eddouks et al. 2005) activities and contains constituents of polyherbal formulations to treat liver ailments (Madrigal-Santillan et al. 2014), diuretic, antihypertensive and poultice (Calis et al. 1999). Various parts of the caper plant that can be used as drugs, cosmetics and foods are further used in various regions for landscaping, control of erosion and animal feeding (Ozcan and Akgul 1998). Based on the extensive anti-inflammatory effects of *C. spinosa* and effects of inflammatory control on tendon repair processes, the use of this plant is suggested to play effective roles in tendon repair processes. Therefore, the current study investigated the healing effects of local injections of *C. spinosa* extract on experimental Achilles tendon injuries in rabbits.

**Materials and Methods**

**Animals**

This study was carried out on nine adult white New Zealand male rabbits with a mean bodyweight of 2–2.5 Kg. Before beginning of the experiment, rabbits were housed for two weeks to adapt to the environment. The animals were maintained under controlled conditions of 25 °C ±1 and 12 h light-dark cycles and had free access to standard chow diet and water throughout the study. The study was approved by the Institutional Animal Care and Use and Committee and carried out based on the guidelines from the National Institute of Health (NIH).

**Plant materials and extract preparation**

Roots of *C. spinosa* were collected from Varamin, Province of Tehran, Iran. Specimens were deposited at the herbarium of the institute of medicinal plants, Karaj, Iran. Roots of the plant were dried and grounded into fine powder using electric blender. The extract was prepared using cold maceration with distilled water for 24 h. The plant powder (50 gr) was suspended at 100 ml of ethanol for 24 h at room temperature. The mixture was filtered using fine muslin cloths followed by Whatman no. 1 filter papers. Extract was concentrated using vacuum distillation (Ezatpour et al. 2015).

**Experimental protocol**

Rabbits were anesthetized with ketamine hydrochloride (5%, 35 mg/kg) in combination with xylazine (2%, 5 mg/kg) intramuscularly (Fazel and Moslemi 2017). The anesthesia was maintained with
inhalation isoflurane (2.5%). Surgery was carried out on both hindlimbs; with left hindlimb served as control (Fazel and Moslemi 2017). The surgical area was prepared aseptically. A longitudinal skin incision was made over the Achilles tendon and the paratenon was exposed and incised longitudinally as a separate layer. The three bundles of Achilles tendon were identified and the central bundle was separated bluntly. A partial-thickness tenotomy (approximately 50% of the tendon bundle width with 1 cm length) was created at 2 cm to the proximal side of the Achilles tendon-calcaneus junction. The severed tendon was not sutured. The partial tenotomy allowed the rest of the tendon to act as an internal splint for the non-immobilized repair (Fazel and Moslemi 2017). One milliliter of the *C. spinosa* extract and normal saline were injected daily to the treatment and control groups respectively for three days post-operatively. After the surgery, rabbits were recovered from the anesthesia in a warm recovery chamber under continuous observation. Following recovery, animals were returned to the individual cages for the rest of the experiment. Flunixin (Razak Co. Iran) as analgesic (2.5 mg/kg IM) and enrofloxacin as antibiotic (5 mg/kg, IM) agents were administrated to rabbits one hour pre-operation and were continued for three days.

**Histopathological studies**

Animals were postoperatively euthanized on Days 7, 14 and 28 using an overdose of thiopental sodium. Then, Achilles tendons were removed by dissection. Number of specimens was three per day. Specimens were fixed in 10% buffered formalin, dehydrated and embedded in paraffin. After 10 days, longitudinal sections of the tendon were stained with hematoxylin and eosin (H&E). Stained specimens were microscopically studied to assess extent and severity of inflammation, angiogenesis, fibroplasia and complete tendon healing, which were scaled from 0 to 3 by defined criteria (Hadjipour et al. 2008). These criteria are shown in Table 1.

<table>
<thead>
<tr>
<th>Extent and severity of the inflammation</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory cells</td>
<td>Observation of inflammatory cells were not seen</td>
<td>Observation of inflammatory cells at two microscopic fields</td>
<td>Observation of inflammatory cells at 3–5 microscopic fields</td>
<td>Observation of inflammatory cells at more than five microscopic fields</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>Blood vessels were not seen</td>
<td>Existing of 0–2 blood vessels</td>
<td>Existing of 3–4 blood vessels</td>
<td>Existing of more than four blood vessels</td>
</tr>
<tr>
<td>Fibroplasia</td>
<td>Recording of few thin collagen fibers with numerous fibroblasts</td>
<td>Recording of thin collagen fibers with very numerous fibroblasts</td>
<td>Recording of thick collagen fibers with numerous fibroblasts</td>
<td>Recording of abundant thick collagen fibers with few fibroblasts</td>
</tr>
<tr>
<td>Complete tendon healing</td>
<td>Observation of inflammatory cells; no observation of blood vessels, fibroblasts or collagen fibers</td>
<td>Contemporary observation of inflammatory cells, blood vessels, fibroblasts and collagen fibers</td>
<td>Contemporary observation of blood vessels, fibroblasts and collagen fibers; no observation of inflammatory cells</td>
<td>Observation of fibroblasts, thick and compact collagen bundle; no observation of inflammatory cells or blood vessels</td>
</tr>
</tbody>
</table>

Table 1. Grading system for the histopathological study

**Tendon repair assessment score**
**Data analysis**

Results were expressed as mean±SD (standard deviation). Significant differences between groups were assessed using Mann-Whitney U-test and SPSS Software v.16.0 (SPSS, USA). Differences were considered significant when P<0.05.

**Results**

The average scores of histopathological changes of the complete tendon healing in treatment and control groups are shown in Table 2. In the present study, number of fibroblasts and degree of neovascularization were higher in C. spinossa treated group than in control group on Days 7 and 14 of injury. In fibroplasia and angiogenesis on Day 7, mean±SD of C. spinossa treated group and control group were 1.66±0.57 and 1, respectively. These factors on day 14 were 2.33±0.57 and 1.66±0.57, respectively. Based on these findings, differences on day 7 were significant (P<0.05) while differences on day 14 were not significant statistically (P≥0.05). This result has suggested that C. spinossa promotes fibroblast migration and/or proliferation as well as neovascularization at early stages of tendon healing. At these times, injured tendons in treatment and control groups showed structural organizations of the new collagen fibers aligning with the functional loading axis in a further pronounced manner in C. spinossa treated group. This exhibited hypocellular areas of further disorganized collagens; for example, collagen fibers diverging from the functional axis (Fig. 1 and 2A, B). On day 7, the average score of histopathological changes of the complete tendon healing in C. spinossa treated group and control group were respectively 1.66±0.57 and 1 with significant differences (P<0.05). On day 14, the average score of histopathological changes of the complete tendon healing in C. spinossa treated group and control group were respectively 2.33±0.57 and 1.66±0.57 with no statistically significant differences (P>0.05). On day 28 for fibroplasia and angiogenesis, the mean±SD of C. spinossa treated group and control group were respectively 2.66±0.57 and 2 with statistically significant references (P<0.05). The number of fibroblast decreased in C. spinossa treated group at day 28. In consistency with these changes, thick collagen fiber bundles were aligned in one direction, parallel to the long axis of the tendon. However, the control group showed deposition of thin collagen fibers and hypercellularity characterized by increased fibroblasts (Fig. 3A, B). On day 28, the average score of histopathological changes of the complete tendon healing in C. spinossa treated group and control group were respectively 2.66±0.57 and 2 with statistically significant differences (P<0.05). Furthermore, results of various days from each group were compared to each other with differences not significant statistically (P>0.05).

<table>
<thead>
<tr>
<th>Day</th>
<th>C. spinossa extract</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1.66 ±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>14</td>
<td>2.33 ±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.66 ±0.57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>28</td>
<td>2.66 ±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

No significant differences were seen between similar capital letters in each column while significant differences were observed between the non-identical lowercases in each row. Data were represented as mean±SD with significant differences (P<0.05).
Fig. 1. Photomicrograph of the injured tendon in control group (A) and *C. spinossa* treated group (B) on day 7 post-operation. Note less hypercellularity, fewer new blood vessels and better healing process in treated group (H&E, ×200)

Fig. 2. Photomicrograph of the injured tendon in control group (A) and *C. spinossa* treated group (B) on day 14 post-operation. Note less hypercellularity and more mature fibrous connective tissue and better healing process in *C. spinossa* treated group (H&E, ×200)

Fig. 3. Photomicrograph of the injured tendon in control group (A) and *C. spinossa* treated group (B) on 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)

**Discussion**

Injuries and degenerative conditions of tendons represent almost 50% of the musculoskeletal injuries treated in orthopedic clinics (Schweitzer et al. 2010). Similar to other connective tissues, the tendon repair process has been an attractive...
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control groups in the current study suggest that *C. spinossa* is a good source of phenolic compounds, especially flavonoids. Despite high antioxidant activity of this plant and its effects on tendon healing, antioxidant assessment was not carried out in this study. Results of the study have demonstrated that *C. spinosa* includes potentials to improve the healing process of damaged Achilles tendons.

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**Conflict of interest**
The authors have no conflicts of interest to declare.

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**References**


