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Histopathological investigation of the effect of *Viola odorata* and *Pistacia atlantica* extract on acute tendonitis in rats

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ABSTRACT

Background & Aim: Tendon is the connective tissue between bone and muscle. Because of poor blood flow and the possibility of adhesions during repair, tendon injury management is particularlyimportant.Therefore, finding a quick way to repair tendon tissue is one of the targets that has been pursued over the years.
Experimental: In this study, 24 male rats were divided into 4 groups, including first group without any treatment, second group receiving dexamethasone (1mg/kg), third group receiving 250 mg/kg *Viola odorata* and *Pistacia atlantica* extract, fourth group receiving 500 mg/kg and *Pistacia atlantica* and *Viola odorata* extract. After anesthesia of rat acute tendonitis developed. Al the mentioned groups were injected subcutaneously on bigining day, 7 and 21 days after surgery and histopathological evaluation was performed on day 21.
Results: The results showed no statistical significance in the number of fibroblasts between different groups. However, dexamethasone groups and the one receiving 500 mg/kg of extract differed significantly from the control group in terms of inflammation and bleeding. Furthermore, the number of fibrocytes in the groups receiving 500 mg/kg and 250 mg/kg doses of *Viola odorata* and *Pistacia atlantica* extracts was significantly different from the control and dexamethasone groups ($P<0.05$).
Recommended applications/industries: According to the results, the amount of collagen and fibrocytes in the group treated with 500 mg/kg had a significant difference, compared to other groups, probably due to the anti-inflammatory, antibacterial and antioxidant properties of *Viola odorata* and *Pistacia atlantica*. They can be used in traditional medicine along with other medicines.

1. Introduction

Herbs have long been a valuable resource for finding new medicines. Herbal and natural medicines due to factors, such as economic value and low cost of their production, no destructive effects on the environment (organic), low side effects, exclusive treatment of some diseases with herbal medicines, and the existence of different clinical experiences about medicinal plants have led to the fact that these medicinal resources have a special value and place in the treatment of livestock and humans (Mortazavian *et al.*, 2012).

Today, the anti-tumor effects of many medicinal plants have been tested by cell culture or using animal models. It is believed that the anti-cancer effects of plants are created by inhibiting cancer-stimulating enzymes, helping to repair DNA, stimulating the production of anti-tumor enzymes in cells, increasing

the body's immunity, and inducing antioxidant effects (Mortazavian *et al*., 2012).

Violet flower is one of the most important medicinal plants as a genus of flowering plants in the violet flower family *Viola odorata* and has rootstock and aromatic flowers in violet flower, rarely white and red. According to traditional Iranian medicine, all parts of the plant, including leaves, seeds, roots, and flowering branches, are used medicinally. It is used for treatment of whooping cough. Its drug is also anti-inflammatory, diaphoretic, diuretic, emollient, expectorant, antipyretic and laxative. It contains salicylic acid which is used to make aspirin hence effective for the treatment of headaches, migraine and visominia. The roots of the plant yield an alkaloid violin which is used as an expectorant. There is a general feeling that the populations of *V. odorata* are decreasing at an alarming rate (Nikniaz *et al.*, 2014)

The medicinal effect of this plant is related to polyunsaturated proteins with about 30 amino acids. Histotoxic compounds have been identified in this plant that has anti-cancer effect (Ahmadi *et al.*, 2011)

Mohagheghi *et al.* (2018) conducted a study on the effect of aqueous extract of Violet flower on testicular damage in streptozotocin-induced diabetic male rats and the results indicated that the consumption of aqueous extract of Viole t flower caused more testicular damage (Mohagheghi *et al.*, 2018)

Modarresi *et al*. (2016) studied and compared the anticonvulsant effect of carbamazepine and *Viola Odorat* in the animal model. The results showed that the hydroalcoholic extract of *Viola odorata* at a dosage of 200 mg/kg can be introduced as a proposed and effective drug in preventing seizures in animal models (Modaressi *et al.*, 2017)

Coriander tree is also considered as a source of gum production. The stem and trunk of the coriander tree carry a gum known as *pistacia atlantica* which is used in pharmacy, food industry, and painting (Samsam Shariat, 1995). *Pistacia atlantica* have traditionally been used to treat gastrointestinal, respiratory, cutaneous, and renal disorders. In addition, the antioxidant, anti-tumor, anti-asthma, and antimicrobial effect of *pistacia atlantica* have been proven in these modern medicines. Naderi *et al.* (2018) conducted a study on the analgesic and acute toxicity of *pistacia atlantica* fruit extract in mice. The results of the current investigation demonstrated the robust antinociceptive activity of *P. atlantica* fruit extract in mice. However, the exact mechanisms accountable for the pharmacologoical activities remain to be investigated (Naderi *et al.*, 2018)

The tendon is the second structure after the skin to be damaged by trauma. It also causes atrophy of muscles and bones and causes many functional problems (Oryan *et al.*, 2012).

Tendon's injuries are an important clinical argument for orthopedic surgeons. This unique texture plays an important role in the performance of the musculoskeletal system due to the fixation and steering of joints' motions (Sharma *et al.*, 2008).

Repairing tendons is a slow process that takes a lot of time. Achilles tendon is the strongest tendon in the body. The tendon in the leg ruptures when it is subjected to excessive stretching Slitting, rupture, or inflammation of tendon make a certain injury and decreased daily performance (James *et al.*, 2008). During stress activities such as the jump, a lot of pressure is applied to tendons (Isner-Horobet *et al.*, 2019). Despite the progress of surgical and rehabilitation techniques, early complications like rupture of the repaired area and post-surgery adhesions will still occur. A repaired tendon will need support for weeks to have enough strength. The tensile strength of the repaired tissue is never returned to normal.

Today, there are methods to treat tendon injuries. To date, the proper treatment techniques that have both physical improvements and return to normal biomechanical activity have not been found. One of the most common ones can be referred to as surgery. Surgery and tendon attachment also have problems, including constraints in autograft link preparation, lack of tissue structure, loss of adhesion, and the possibility of damage to the ligament, which eventually can be repaired with the expensive surgery (Oryan *et al.*, 2013). It has been mentioned that finding low-cost alternatives that lead to recovery, achieving the best results as well as reducing the side effects of repair methods. In this study, the effects of violet flower and *pistacia atlantica* extract on acute tendonitis in rats was studied from a histopathological point of view.

2. Materials and Methods

In present study, 24 male Wistar rats (220-250 g) were kept in the animal houses located at Islamic Azad

University, Shahrekord Branch, at a controlled temperature and light conditions was 12- hour darkness and 12- hour light, and had free access to water and food. In this study, all ethical cases were approved and observed by the Medical Ethics Committee on Laboratory Animals of Islamic Azad University, Shahrekord Branch. The animals were divided into 4 groups, including the experimental group without any treatment (A), the experimental group receiving dexamethasone (B), the experimental group receiving 250 mg/kg of extract of violet flower and *pistacia atlantica* (C), the experimental group receiving 500 mg/kg of extract of violet flower and *pistacia atlantica* (D), were categorized by simple random sampling. After collecting and identifying violet flower and *pistacia atlantica*, their extracts were made by a rotary apparatus and prepared at concentrations of 250 and 500 mg/kg. After anesthesia, intraperitoneal injection of an equal mixture of ketamine 50 mg/kg and xylazine 10 mg/kg was placed on the surgical table in the abdomen. The upper tarsus hair featured two cutaways, forearm and shoulder, and the lower bouts featured two cutaways, for easier access to the higher frets. The Achilles tendon area was exposed and crushed by cutting the skin on the lateral surface. The skin was then sutured with 0-2 nylon thread. After surgery, the rats received cefazolin at a dose of 15 mg/kg intramuscularly for 3 days and then kept individually in a restricted and controlled environment. After performing the above activities, all the mentioned groups were treated by subcutaneous injection on days of 0, 7 and 21 in the clinical evaluation of post-operation appetite, activity, infection, bleeding, and wound opening, they were examined and evaluated daily, and then hematoxylin-eosin staining was used for histological examinations. Samples of the mentioned tendon were removed at the end of the treatment phase and placed in 10% formalin after washing and sent to histopathology laboratory. They were stained by hematoxylin and eosin staining method.

Statistical analysis

Results were expressed as Mean ± SD. Data were analyzed with SPSS 25 software and non-parametric Kruskal-Wallis H and U-Whitney U-tests were performed to compare the means of the different groups. Significance was statistically acceptable at $P \leq 0.05$.

3. Results and discussion

Histopathological results after surgery in different groups

Control group on day 1 showed irregular collagen fibers and tissue necrosis (Figure 1).

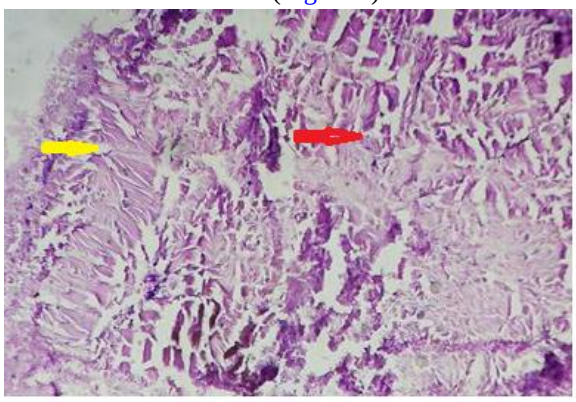

Figure 1. H&E stained microscopic sections (100X) tendon in rats after surgery (control group). Red arrow: the irregular collagen; and yellow arrow: tissue necrosis.

In control group (without treatment) on day 21: Delicate collagen tissue and lack of density of connective tissue categories and fibroblasts were observed (Figure 2).

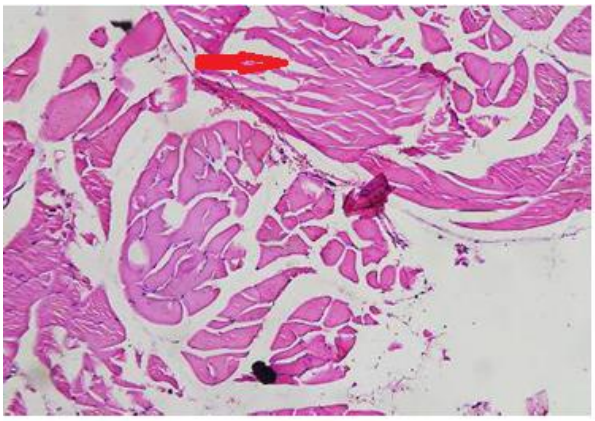

Figure 2. H&E-stained microscopic sections (100X). The tendon position on day 21 after post operation in the control group. View of fine collagen tissue (red arrow) and lack of density of connective tissue clusters and fibroblasts.

The groups receiving 250 mg/kg of violet flower and *pistacia atlantica* extracts on day 21 showed irregular and scattered collagen fibers and the presence of a small number of fibrocytes were visible (Figure 3).

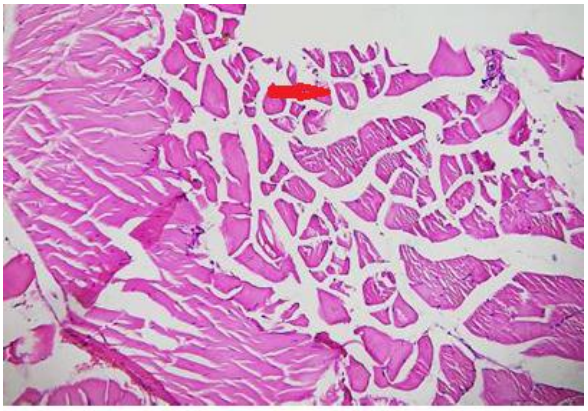

Figure 3. H&E stained microscopic sections (100X). The tendon position on day 21 post operation in the group receiving 250 mg/kg of extract of violet flower and *pistacia atlantica*. Irregular and scattered collagen fibers (red arrow) and low number of fibrocytes.

In group receiving 500 mg/kg of violet flower and *pistacia atlantica* extracts on day 21, dense collagen tissue, absence of inflammation and bleeding, and fibroblast cells were observed (Figure 4) and in group receiving dexamethasone on day 21 adult collagen fibers, fibroblasts, and no bleeding and inflammation were observed (Figure 5).

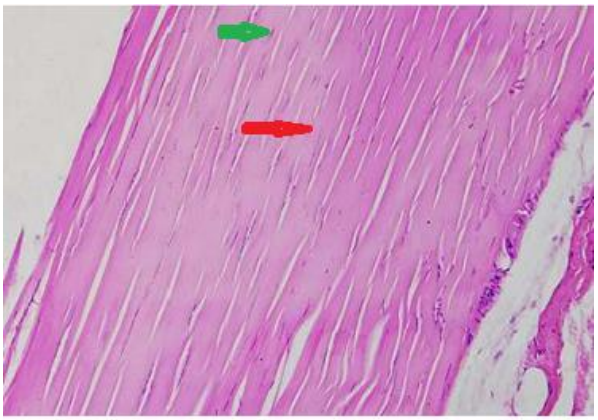

Figure 4. H&E-stained microscopic sections (100X). The tendon position on day 21 post operation in the group receiving 500 mg/kg of extract of violet flower and *pistacia atlantica*. Dense collagen tissue (red arrow), absence of inflammation and bleeding and observation of fibrocytes (green arrow).

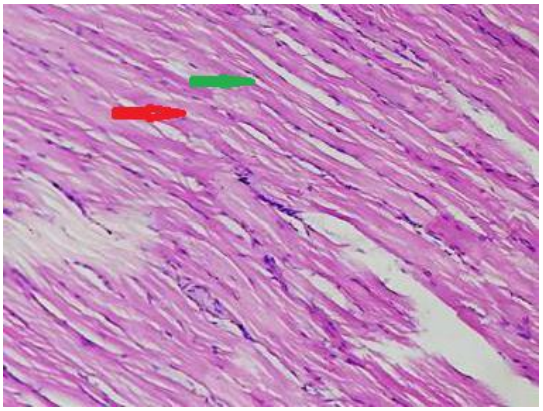

Figure 5. H&E-stained microscopic sections (100X). The tendon position on day 21 post operation in the dexamethasone group. Adult collagen fibers (red arrow), fibrocyte cells (green arrow), absence of bleeding and inflammation.

Tendon injuries are an important clinical issue for orthopedic surgeons. There are obstacles in the way of repairing tendon injuries, which are mentioned below. Despite advances in surgical and rehabilitation techniques, early complications such as ruptured repair and postoperative adhesions may occur. A repaired tendon needs support for weeks to become strong enough. Adhesion after an intra-synovial tendon injury is a major clinical problem. Amputation of the synovial sheath during injury or surgery allows granulation tissue and tenocytes to invade the repair site from surrounding tissues. Exogenous cells dominate the tenocytes. It allows surrounding tissues to attach to the site of repair, resulting in adhesion (Snedeker *et al.*, 2017). The most common complications after tendon repair are flexion, rupture, tendon adhesion, and joint contraction. In bending tendon repair. Adhesion of soft tissues to the surrounding sheath is one of its problems (Chen *et al.*, 2019). Despite the rearrangement of biochemical and mechanical properties, restorative tendon tissue is never as healthy as tendon tissue. After the injury, the healing process in the tendon is followed by the formation of fibrotic scar tissue. The mechanical, organizational, and structural properties of restorative tissue are poorer than those of a healthy tendon.

Although properties improve over time, they will never return to their original state (Woo *et al.*, 2019). Also, the tensile strength of the restored tissue never returns to normal. Tendons have poor blood flow and low repair capacity. Therefore, tendon injury management is necessary. Regardless of the nature of the acute or chronic tendon injury, reconstruction and surgery of large defects are complex (Saether *et al.*, 2016; Yang *et al.*, 2013). Therefore, knowing the function of the tendon, along with knowledge of biology and the ability to heal is essential to achieve a new advanced solution in improving the tendon repair process, and in recent years, much research has been done to improve the tendon repair process. Hogel *et al.* (2020) conducted a study on the therapeutic effects of a pulsed electromagnetic field on the healing of the rat's Achilles tendon. The results showed that the distinctive effects of the Pulsed Electromagnetic Field (PEMF) pulse electromagnetic field emphasize tendon-to-bone healing and support the testing of possible clinical treatment strategies before use (Huegel, 2020). Yadegari *et al.* (2020) conducted a study on the effects of general injection of tendon on the repair process in rats by ultrasonography and histopathology. The results of this study showed that tendon was effective in tendon repair and reducing inflammation. Although today different methods are used to treat and repair such tears (Yadegari *et al.*, 2020), so far no effective treatment has been provided that has no side effects. Medicinal plants can be of particular importance in this regard because medicinal plants have rich sources of natural antioxidants that are used in traditional medicine to control and treat many diseases. Serious use of medicinal plants due to the lack of various side effects and effective compounds in plants has emerged (Huegel, 2020).

Findings of recent scientific studies approved some of these properties including antioxidant, antimicrobial, anti-inflammatory, analgesic, hypoglycemic, anti-cancer, and lipid-lowering, cardioprotective, and hepatic protection. They have also been shown to be beneficial for gastrointestinal disorders. Of course, most of the mentioned researches have evaluated the properties of coriander fruit. Studies have been done on other components of this plant sparingly. Studies on the skin, leaves, and green fruits of this plant have shown that its skin has antioxidant and antimicrobial properties, its leaves and fruits have anti-cancer effects. Currently, the most common uses of this plant in traditional medicine are the use of the fruit of this plant to strengthen the nerves, relieve anemia, soften bones and the gum of this plant is used as a laxative in the treatment of gastrointestinal disorders (Muqtada'I *et al.*, 2020).

Darugari *et al.* (2018) evaluated the analgesic effects of rubbing *pistacia atlantica* oil using formalin test in male rats. The conclusion showed that rubbing *pistacia atlantica* oil has an analgesic effect (Darugari *et al.*, 2018).

Muslimi *et al.* (2020) studied the histopathological study of *Capparis Spinoza* on the healing process of Achilles tendon injury in rats and found that the use of *Capparis Spinoza* extract can improve the healing process in Achilles tendon injury in rats (Muslimi *et al.*, 2020).

In a comparative evaluation of histopathological variables between different groups on the day 21, results showed that in the group treated with 500 mg/kg of extract, the highest amount of mature collagen was observed compared to all (Table 1). We also observed this increase in the groups treated with dexamethasone with a slight difference compared to the group treated with 500 mg/kg of extract of violet flower and *pistacia atlantica.*

Table 1. Comparison of various tissue parameters between different groups.

	Control	***Pistacia atlantica* extract and violet flower (250 mg/kg)**	***Pistacia atlantica* extract and violet flower (500 mg/kg)**	**Dexamethasone**
Adult collagen	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}	1.83 ± 0.41^{a}	1.33 ± 0.52^{ab}
Inflammation and bleeding	0.67 ± 0.52^{ab}	0.33 ± 0.52^{ab}	0.00 ± 0.00^{a}	$0.00 \pm {}^{a}0.00$
Fibroblasts	0.83 ± 0.41^{a}	0.50 ± 0.54^{a}	0.17 ± 0.41^{a}	0.17 ± 0.41^{a}
Fibrocytes	0.50 ± 0.55^{a}	1.17 ± 0.41^{b}	1.50 ± 0.55^{b}	1.00 ± 0.00^{ab}

[a, b]: Groups with different letters have statistically significant differences (P< 0.05). According to Table 1 and the Kruskal-Wallis test, there was no statistically significant difference only between the "fibroblast" variable between groups (P>0.05).

In the variable of inflammation and bleeding, the highest value belonged to the control group and then this variable was observed less than the control group in the group treated with 250 mg/kg of extract, but in the group treated with 500 mg/kg of extracts and dexamethasone, no inflammation and bleeding were observed.

In the fibrocyte variable, the highest number of fibrocytes was observed in the group treated with 500 mg/kg and 250 mg/kg of the extracts of violet flower and *pistacia atlantica*, respectively (Table 1).

It should be noted that there was no statistically significant difference between the fibroblast variable between the different groups (P>0.05). The mature collagen variable was highest in the dose of 500 extracts and dexamethasone. There was also a significant difference between the control group and the groups receiving the dose of 500 mg/kg of the extracts of violet flower and *pistacia atlantica* and dexamethasone in the variables of inflammation, bleeding, and fibrocytes (P<0.05). Confirmation of these findings can also be found in histopathological results.

4. Conclusion

In general, the results obtained from this study showed the favorable process of collagen formation and proper repair of tendon tissue in the groups treated with violet flowers and *Pistachia atlantica*, which is probably due to antimicrobial, antioxidant, anti-inflammatory properties of these two plants.

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