The efficiency of Polytendon Complex (PC) and St. John’s Wort oil (Hypericum perforatum) on healthy Achilles tendon in rats

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Abstract

Aim: This experimental animal study aimed to investigate the effects of Tendoflex® (a polytendon complex) and St. John’s wort oil (Hypericum perforatum) on healthy Achilles tendons in rats.

Material and Method: Twenty Wistar albino rats weighing 250-350 g were randomly allocated into four groups. In Group A, Tendoflex® capsule per 2.5 kg/day was administered orally via gavage. In Group B, Hypericum perforatum 300mg/kg/day was given orally via gavage. In Group C, Tendoflex® and Hypericum perforatum were given orally via gavage at the doses mentioned above. In Group D, no intervention was done to the control group. After four weeks, all rats were sacrificed and biomechanical tests of the Achilles tendon and histological examinations were performed.

Results: Histological results revealed a significant difference between the groups regarding Type-1 and Type-3 immunopositivity in the Achilles tendon tissues (p <0.05). Immune-positivity values were high in group B, moderate in group C, mild in group A and insignificant in group D. The highest top tendon strengths in the biomechanical tests were recorded in the Hypericum perforatum and mix groups at the fourth week (83.75±16.1N and 81.875±9.7 N, respectively) followed by the Tendoflex® group (66.875±7.5 N). On the other hand, the lowest tendon strengths were obtained in the control group (54.375±7.1N).

Discussion: Tendoflex® and Hypericum perforatum increased the Achilles tendon tensile strength in rats. This result may be related to the fact that Type-1 and Type-3 collagen immunity was higher in all groups compared to the control group. Tendoflex® and Hypericum perforatum can be used to prevent tendon rupture or to avoid re-rupture in patients undergoing tendon repair.

Keywords
Achilles Tendon, Tendoflex, Hypericum perforatum, Prophylaxis
Introduction

Achilles tendon is the strongest and biggest tendon in the body. However, this tendon is commonly torn in middle-aged men who exercise. The incidence of tendon rupture is estimated to be 18/100 000 [1]. Medical aids for preventing the tear or reducing the risk of re-rupture, which is frequently encountered in patients with tendon repairs, can minimize labor loss, hospital stay, and hospitalization costs [2,3].

Many medicines and substances have been studied in the literature that investigates the tendon healing process and are thought to affect this process positively [4-10]. Studies are investigating the contribution of elements such as vitamin C and collagen contained in Tendoflex to tendon healing [4]. St. John's wort (centaury oil, Hypericum perforatum) is obtained from a medical plant and is used today to treat many different diseases, such as ulcer, burn, diabetic wounds, infections, and cancer [11-13]. Considering the mechanism of action of H. perforatum, its effects such as shortening the inflammatory process, increasing the collagen level, and decreasing fibroblast migration, which is thought to positively affect the tendon healing process, have been shown in publications [14].

As far as we know, there is no study in the literature on how Tendoflex and hypericum perforatum will affect the load capacity of intact tendons. This study aimed to investigate the effects of Tendoflex and St. John's wort on the pulling load capacity of tendons and its histopathological mechanisms of action.

Material and Methods

The experiment was initiated after obtaining approval from the Atatürk University Local Ethics Committee for Animal Experiments. This study was carried out per the principles of "Care and Use of the Laboratory Animals," and animal rights were protected [15]. Twenty Wistar-Albinorats weighing 250-350 grams were randomly allocated into 4 groups of 5 rats. In Group A, Tendoflex® (Mega-Farma, Turkey) capsule/2.5 mg/day was given orally via gavage. In Group B, Hypericum perforatum 300 mg/kg/day was given orally through gavage. In Group C, Tendoflex® and Hypericum perforatum were administered orally through gavage at the daily doses mentioned above. Group D was designated as the control group, and no intervention was given to this group. The study was conducted in accordance with the principles of the Declaration of Helsinki.

The active substance doses specified in the publications were administered in the intervention groups. Since Tendoflex® contains more than one active substance, the treatment given has been decided by looking at these active ingredients one by one [5,16-19]. In one study, bromelain was found beneficial at a dose of 30 mg/kg for tendon injury [5]. In another study, it was reported that L-arginine was used in rats at doses of 30-60 mg/kg [18]. In another study, methyl sulfonyl methane was used at a dose of 50 mg/kg [19]. In the light of all these studies, 1 capsule of Tendoflex® was weighed to correspond to a 2.5 kg rat weight. It was given daily with the help of gavage according to the weight of the animal. The Hypericum perforatum extract was applied orally via gavage at a dose of 300 mg/kg daily, as suggested in the literature [12-13].

Four weeks later, the rats were sacrificed, and tissue samples were examined histologically following biomechanical tests of the Achilles tendons.

Biomechanical investigations:

Achilles tendons of the animals sacrificed in the fourth week were measured regarding their durability in the mechanical test device (Figure 1). Load capacities measured by 0.1% accuracy of the load cell of the device were entered into the software, and force values (F) were obtained. The tests were terminated after force reduction and sample rupture. The highest force value obtained for each sample was used for data analysis.

Immunohistochemical studies:

Achilles tendon tissues were investigated after the necropsy of the rats in a 10% neutral formalin solution. Routine tissue follow-up was done by alcohol-xylool and transferred into paraffin blocks. Then, sections of 5 μm thickness were taken on poly-L-Lysine-coated slides, passed through xylol and alcohol series, washed with PBS, and kept for 10 minutes in 3% H₂O₂ inactivating endogenous peroxidase inactivation. To expose antigens in the tissues, treatment with antigen retrieval solution at 500 watts for 2x5 minutes was applied. The tissues which were washed with PBS were incubated for 20 minutes at room temperature with Type-1 Collagen (Abcam, Catalog No ab34710 1/200 dilution rate) and Type-3 Collagen (Abcam, Catalog No ab7778 1/200 dilution rate). Secondarily, "Large Volume Detection System: anti-Polyvalent, HRP" (Thermofischer, Catalog number: TP-125-HL) was applied as recommended by the manufacturer. DAB (3,3’-Diaminobenzidine) was used as a chromogen. The slides were covered with Entellan and examined under a light microscope after contrasting with Mayer’s Hematoxylin. As a result of the examination, grading in the Achilles tendon tissues were performed as no (-), mild (+), moderate (++), and strong (+++).

Statistical Analysis

All statistical analyzes were done using the IBM SPSS 20.0 statistical software (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean±standard deviation, frequency, and percentage. Differences between the groups were determined by the nonparametric Kruskal-Wallis test, and pairwise comparisons were made by the Mann-Whitney U test. The significance threshold was taken as p<0.05.

Results

None of the rats were lost during the study. In the examinations during the sacrifice of the rats, no macroscopic defect was detected in the integrity of the tendons.

Immunohistochemical Staining

In Achilles tendon tissues, there was a significant difference between the groups concerning Type-1 and Type-3 immunity (Table 1, p <0.05). Immunostaining of Type-1 and Type-3 in the Achilles tendon tissues of the control group rats were not significantly determined (Figures1 and 2). It was determined that the Tendoflex group had a mild level of Type-1 and Type-3 immunity to the Achilles tendon tissues (Figures1 and 2). While animals in the Tendoflex + St. John’s Wort group had moderate Type-1 and Type-3 immune positivity, Type-1 and Type-3 immune positivity in the St. John’s Wort group was strong (Figure 2 and 3).
Biomechanical measurements
When the strength of Achilles’ tendons at 4 weeks were examined, the best results were obtained in the Hypericum perforatum (Group B) and mixed (Group C) groups. The highest pulling load capacity was in the H. perforatum group (83.75 ± 16.1 N), followed closely by the mixed group (81.875 ± 9.7 N). The Tendoflex® (Group A) had the third rank in pulling load capacity (66.875 ± 7.5 N). The lowest tendon powers were seen in the Control Group (Group D) (54.375 ± 7.1 N) (Table 2).
Discussion

The fact that Achilles tendon rupture is a common issue, and there are potentially severe complications after surgery, increases the interest in the prevention and treatment strategies [6]. In this study, the contribution of various active substances to the Achilles tendon endurance in healthy rats was investigated mechanically and histologically. If the Achilles tendon endurance can be increased, the risk of rupture will naturally decrease. The use of Hypericum perforatum in rats increases the stamina of Achilles’ tendons in the best way. It was also found that the use of Tendoflex increased tendon strengths to a lesser extent than Hypericum perforatum. When using these two substances, higher values were obtained in the tendon strengths than the group using only Tendoflex. As a result, better pulling load capacities were obtained in all groups compared to the non-intervention group. Histological examinations supported these tensile test results too. Type 1 and Type 3 immunopositivity rates were in line with the load capacity test results.

Although Hypericum perforatum is used in a wide variety of diseases, studies show that it increases tensile strength in wounds and contributes to wound healing [20]. In vivo and in vitro studies have shown that the H. Perforatum shortens inflammation time, stops the migration of fibroblasts, and increases collagen accumulation during wound healing [21]. Tensile strength is an objective criterion of wound healing and is known as the clinical entity that best reflects this process. Kahyaoglu et al. attributed to H. perforatum increasing the tensile strength of the wound, having antibacterial effects, increasing the migration of fibroblasts, and increasing collagen [12]. In this study, it was determined that H. perforatum increased the strength of the healthy Achilles tendons and did this by increasing the amount of Type 1 and Type 3 collagen in the tissue.

One of the products containing Polytenon complex, which is recommended by health care professionals to accelerate healing and increase the collagen amount of the repair-tissue after Achilles tendon injuries, is a commercial product called Tendoflex®. Studies investigate the effects of vitamin C and collagen, one of the factors included in this product, on tendon healing. In their research, Ömeroğlu et al. investigated the effects of parenteral high dose vitamin C on tendon healing. It increased angiogenesis and type 1 collagen synthesis in the early period [4]. In this study, the effect of Tendoflex® on the strong Achilles tendon of the rats was examined. This active substance increased the strength of the intact Achilles tendon by raising its collagen content.

Other studies in the literature examine the increase of tensile strength and healing of the Achilles tendon using various active substances. Majevski et al. applied an autologous serum to the Achilles’ tendons after repair and found that this application increased type 1 collagen and decreased type 3 collagen [7]. Liang J et al. showed a positive effect on tendon healing of hyaluronic acid/tenocyte locally after tendon repair in rat Achilles tendon rupture models [8]. Özer et al. showed that oral glucosamine chondroitin sulfate positively affected tendon healing in the rat Achilles tendon model [9]. Eren et al. showed a positive effect of low molecular weight heparin and rivotronxab administered subcutaneously in the rat Achilles model on tendon healing, but could not find any difference biomechanically and histologically [10]. In a study on rats, Kajikava et al. showed that collagen synthesis is higher in patients who received platelet-rich plasma [22]. A study by Tsai et al. showed that transforming growth factor-beta release was stimulated by pulse ultrasound in repaired tendon cells [23].

Some limitations of this study are the small number of subjects and the lack of clinical and functional results due to the experimental nature of the study.

Conclusion

The single and combined use of Tendoflex® and Hypericum perforatum increased the Achilles tendon stretching power in rats. This finding may be related to the fact that Type-1 and Type-3 immunopositivity was higher in all groups compared to the control group. These two factors can prevent tendon rupture in patients who are predicted to have tendon injuries. It can also be used to avoid re-rupture in patients whose tendons have been repaired surgically.

Scientific Responsibility Statement

The authors declare that they are responsible for the article’s scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: None

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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Tendoflex® and Hypericum perforatum on healthy Achilles tendon in rats

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How to cite this article:
Kutsi Tuncer, Mehmet Demir, Eyüp Şenocak, Ali Safa Mendil, Arzu Gezer, Basri Pür, Recap Oztürk. The efficiency of Polytendon Complex (PC) and St. John’s Wort oil (Hypericum perforatum) on healthy Achilles tendon in rats. Ann Clin Anal Med 2021;12(2):139-143.

143 | Annals of Clinical and Analytical Medicine