A Comparative Study on the Efficacy of Hydro alcoholic Extract of Rose damascena, Dexamethasone, and Vitamin E in Promoting Healing of Acute Achilles Tendon Injuries in Rats

Abstract

Background: Tendinopathy, also called tendinitis, is a common issue in musculoskeletal consultations. Tendon healing is inferior to normal due to low metabolic rate and poor vascularity. Rose damascena anti-inflammatory, antioxidant, and healing effects. This study aims to investigate R. damascene's effects on Achilles tendon injury using histopathology and biochemical evaluations.

Methods: In a clinical interventional study, 56 male Wistar rats were subjected to mosquito crushing forceps to induce Achilles tendonitis and then randomly divided into seven groups, which included sham, control group and three tendinitis groups with different doses of RDHE (250, 500, 1000 mg/kg) were administered intraperitoneally to these groups, while the reference groups received Dexamethasone and Vitamin E. Histopathological assessment and antioxidant activity measured by analyzing serum malondialdehyde levels and conducting ferric-reducing antioxidant power assays on days 10 and 20.

Results: Different dosages of RDHE, particularly 1000 mg, improved inflammatory intensity, angiogenesis, fibroplasia, and complete tendon regeneration on the 10th and 20th days after the injury, while also reducing unsaturated fatty acid per oxidation and increasing the whole serum's antioxidant capacity on the 20th day.

Conclusion: After a thorough review, it can be concluded that R. damascena extract on inflammation and the reorganization of collagen bundles after tendon injury. The extract's safety and tolerability render it a prospective alternative for alleviating tendonitis symptoms.

Keywords: Tendon Injuries, Dexamethasone, vitamin E, Rose, Regeneration

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Introduction

Tendinopathy is a frequently occurring pathology that results in degenerative and inflammatory changes in tendons due to mechanical loading, leading to pain, reduced function, and tendon rupture, and is often misdiagnosed as "tendonitis" [(1,2)].Tendons are connective tissue that transmits force between muscle and bone. They are prone to injury due to mechanical stress. Tendon repair is challenging and slow due to limited vascularity and poor cellularity [(3)]. Despite advances in therapeutic approaches for tendon injuries, ranging from acute traumatic ruptures to chronic overuse and degenerative tendinopathy, function and motion continue to be negatively affected [(4)]. The management of tendinopathy typically involves non-surgical interventions, with limited supporting evidence for the various therapeutic options such as physiotherapy, splinting, taping, cryotherapy, extracorporeal shockwave therapy (ESWT), peritendinous injections with corticosteroids or Platelet Reach Plasma products (PRPs), non-steroidal anti-inflammatory drugs (NSAIDs) and dietary supplements [(5–9)].Certain biological compounds, such as anti-inflammatory and antioxidant agents, can aid in tendon healing by reducing inflammation and protecting cells against oxidative damage [(10,11)]. Glucocorticoids are often used to reduce inflammation and pain, but they can have negative effects on tendon healing and increase the risk of rupture when used for long periods of time [(12)]. Research has shown that this treatment can

inhibit tenocyte growth and collagen production, reduce tendon strength, and cause tendon stem/progenitor cells to differentiate into nontenocytes. While local injection of glucocorticoids can provide short-term pain relief and improve motor function, long-term use can lead to spontaneous tendon rupture [13–15].

R. damascena, a member of the Rosaceae family, is a small plant that produces fragrant pink flowers in the spring. It is a popular ornamental plant and has various applications in the medical industry. R. damascena has been used in aromatherapy, skin care, wound healing, digestive health, cardiovascular health, respiratory health, and pain relief due to its anti-inflammatory, antioxidant, and analgesic properties [16–20]. The application of R. damascena topically on dermal wounds resulted in enhanced wound healing, including increased fibroblast proliferation, collagen deposition, wound maturation, and tensile strength, and when combined with retinoic acid, significant improvements were observed in wound closure rate, oxidative stress reduction, and antioxidant enzyme activity [20, 21]. Several studies have investigated the impact of reactive oxygen species (ROS) on tendon cells in vitro, which can lead to decreased cellular proliferation, migration, viability, and stemness [11,22]. Activation of mitochondrial aldehyde dehydrogenase 2 (ALDH2) has been found to mitigate oxidative stress and prevent H2O2-induced cell death and depolarization of mitochondrial membrane potential [23], while vitamin C treatment has been shown to decrease NO synthesis by tendon cells [24]. Vitamin E, a fat-soluble antioxidant, helps in protecting cells from damage caused by free radicals and has anti-inflammatory effects that may help alleviate pain and swelling due to tendon injuries [25–27]. This research endeavor was carried out with the intention of evaluating and contrasting the anti-inflammatory and antioxidant properties of hydroalcoholic rose extract, dexamethasone, and vitamin E with the objective of ascertaining the magnitude of their influence on the restoration of tendinitis.

Methods

The ethical committee of the university oversaw the treatment of animals in accordance with IR No. IAU. SHK. REC.1401.046 and the National Institute of Health Guide for the Care and Use of Laboratory Animals.

In May of 2022, flowers were gathered at the farm of Islamic Azad University, Shahrekord. Subsequently, the flowers were identified by the specialist from Shahrekord Medicinal Plants Research Center and assigned the code 0480001001. The herbarium of the University of Medical Sciences, Shahrekord, was the storage location for the flowers. After being relocated and dried in the shade, the extract was extracted. The ground petals were soaked in 300 ccs of 96% ethanol for 72 hours in the dark, and the resulting extract was filtered with Whatman 42 filter paper. The solvent, ethanol, was separated from the extract using a rotary device (IKA, Germany), and the extract was concentrated and sterilised using Millipore 0.22 microns. The final solution was stored in a dark glass container in the refrigerator for testing purposes [28]. Vitamin E and dexamethasone were purchased from Osve and Iran Hormone Pharmaceutical Co. (Tehran, Iran).

The research was carried out on a group of 56 healthy adult male Wistar rats with a weight range of 200-230 g, which were accommodated in steel cages for a week before the commencement of the study.

Seven different animal groups were assigned to various treatments, including sham, control, test, and reference groups. The sham group received a typical diet without any tendonitis induction or treatment, while the control group underwent the same surgical procedure without any treatment. The extract groups were treated with R. damascena hydroalcoholic extract (RDHE) intra-peritoneal at different doses of 250, 500, and 1000 mg/kg, and this treatment was provided for

20 days. Finally, the reference groups were treated with vitamin E (100 IU/kg) i.p. and dexamethasone (1 mg/kg) i.p., with treatment continuing for seven days. The animals were administered anesthesia through intraperitoneal injection of ketamine hydrochloride 80 mg/kg (Bremer Pharma Germany Company) and xylazine hydrochloride 10 mg/kg (Rotex Medical Company) via intraperitoneal injection. The Achilles tendon area was shaved and disinfected using a standard surgical aseptic technique. In rats, a longitudinal skin incision measuring 1.5 cm was created over the left hind leg to expose the Achilles tendon. The proximal area of the tendon, located roughly 4 mm from the heel tendon incision, was compressed with Halsted Mosquito forceps for one minute. The skin was then sutured with a nylon suture (0-5 SUPALON brand) and a needle (1.5 cm). Beginning from the first day after the onset of tendonitis, all rats were subjected to RDHE with varying doses of 250, 500, and 1000 mg/kg daily. The animals were granted the opportunity to engage in free-cage activities. At the termination of the treatment phase, on days 10 and 20, the creatures were administered with ether anesthesia. Their hearts were punctured to retrieve blood which was then transferred into sterilized test tubes and subjected to centrifugation using the Centurion Scientific Ltd Model Centrifuge 2041 at a speed of 4000 rpm for 10 minutes. The resultant blood serum was swiftly separated, transferred into Eppendorf micro tubes, and conveyed to the laboratory (n=4 for each group at each time point) for the purpose of biochemical analyses. Malondialdehyde (MDA) level, the index of lipid peroxidation, was measured by the method described by Buege and Aust [29].
The MDA level was estimated using the molar extinction coefficient of the product
(ε = 156 mM^{-1} cm^{-1}) and expressed as nM in the plasma. The antioxidant activity of Hydro alcoholic extraction of Rosa damascene was investigated using Ferric ion reducing antioxidant power (FRAP) assay of Oyaizu [30].
The results were expressed as µM Fe (II)/µg extract. Tendon tissue was harvested on days 10 and 20 after treatment (n=4 for each group at each time point). The tendon specimens were placed in 10% formalin solution for 24 h, embedded in paraffin, cut into 6-µm coronal sections, and stained with hematoxylin and eosin (HE) to assess the tendon morphology. Stained specimens were microscopically studied to assess the extent and severity of inflammation, angiogenesis, fibroplasia, and complete tendon healing, scaled from 0 to 3 by defined criteria [31] (Table 1).
Masson's trichrome is a three-color staining protocol for distinguishing cells from surrounding connective tissue. The trichrome was applied by immersion the fixated sample into Wiegert's iron Hematoxylin [32].

Statistical analysis

The present investigation's outcomes were obtained by collecting, recording, and transference data to SPSS software version 26. Qualitative findings were also taken into account, including histopathological alterations that were evaluated based on standard deviation ± mean qualitative findings, as well as the rank of one-way analysis of variance (ANOVA "stands for analysis of variance, which is a statistical method used to analyze the differences among group means in a sample. It's often used to compare three or more group means simultaneously"). Differences between groups were examined using Tukey's polynomial and Kruskal Wallis (The Kruskal-Wallis test is a non-parametric method used to determine whether there are statistically significant differences between two or more groups) and Dunn tests (Dunn tests are a type of post hoc test used in statistics to compare multiple treatments after conducting an ANOVA. They help identify which treatments are significantly different from each other). A significance level of $P<0.05$ was adopted.

Results

Table 2 depicts the findings of the mass spectrometry analysis of the R. damascena extract collected from the agricultural land of Islamic Azad University, Shahrekord. The analysis revealed the presence of 17 compounds, accounting for 81.29% of the total compounds.

Table 1: The grading system for the histopathological study

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

The value 0 represents the absence of observation, while the value 3 represents the observation of the intended cell in the measurement. **Angiogenesis** is a vital biological process that facilitates the formation of new blood vessels from existing ones, playing a critical role in growth, development, wound healing, and tissue repair through the proliferation and migration of endothelial cells to generate new capillaries and larger blood vessels. The formation of fibrous tissue, known as **fibroplasia**, is a critical component of wound healing and is observed both in the normal healing process and in certain tissue abnormalities.

Beta-Citronellol was identified as the major constituent, comprising 41.78% of the extract. The phytochemical analysis further disclosed the presence of 19 compounds.

The alterations in the mean weight prior to and post the intervention within the cohorts indicated that as the quantity of intraperitoneal injection of the R. damascena hydroalcoholic extract heightened, the degree of weight reduction also amplified. The reduction of body mass in groups that underwent intraperitoneal treatment was notable ($P<0.05$) in a proportionate manner (Table 3). The assessment of total serum antioxidant capacity (FRAP) on days 10 and 20 after the onset of tendonitis in rats indicate that administering RDHE at a dosage of 1000 mg/kg led to a substantial increase in FRAP levels compared to the Sham, vitamin E groups, and RDHE 500 mg/kg, with no significant difference observed in other groups ($P<0.05$). The results also demonstrate that on the 20th day, the FRAP level was significantly higher in the RDHE 1000 mg/kg group compared to the control, Sham, dexamethasone groups, and RDHE 250 mg/kg, with no significant difference seen in other groups ($P<0.05$).

The assessment of peroxidation of unsaturated fatty acids (MDA) at days 10 and 20 post-induction of tendonitis in rats indicate that on day 10, there was a significant decrease in MDA levels among rats treated with RDHE 1000 mg/kg, RDHE 250 mg/kg, and the Sham group as compared to RDHE 500 mg/kg. However, no significant difference was observed in the other groups ($P<0.05$). On the 20th day post-tendonitis induction, rats treated with RDHE 1000 mg/kg, RDHE 500 mg/kg, and vitamin E group demonstrated a significant increase in MDA levels compared to the Sham and control groups ($P<0.05$).

Table 2: shows the chromatogram results of Rosa Damascene, the agricultural land of Islamic Azad University, Shahrekord

Range	Combinations	RI* (Min)	IAU SHk (Percent)
1	Cyclohexanone	11/41	-**
2	Menthone	11/72	2/71
3	Menthol	11/98	1/04
4	Beta-Citronellol	13/77	41/78
5	Z-Citral	14/2	6/11
6	Octadien-3-Ol	14/6	6/11
7	Geranial	15/17	9/04
8	6-Octen-1-Ol	15/27	4/8
9	Thymol	15/85	21/3
10	Camphane	15/9	0/1
11	Menthyl Acetate	15/94	0/1
12	Spathulenol	24/66	2/22
13	Caryophyllene Oxide	24/84	5/24
14	Isobutyl Phthalate	32/41	0/1
15	Heptadecene	32/5	0/1
16	Nonadecane	33/14	0/1
17	Eicosane	35/55	-
18	Heneicosane	37/86	0/1
19	Tricosane	42/2	0/1
			81/29

*RI:Retention indices

**-: not detected

The present histopathological investigation examined the severity of inflammation, angiogenesis, fibroplasia, and the degree of complete tendon repair. A significant difference in the investigated factors was observed between the 10th and 20th day after tendonitis induction (P<0.05) (Table 4). On the 10th day after surgery, the intensity of inflammation was significantly higher in the control group (Figure 1-A) than in the other groups (P<0.05). The intensity of inflammation was higher in the groups receiving a hydroalcoholic extract of rose, vitamin E, and dexamethasone (Figure 1-B), except for the hydroalcoholic extract with a dose of 250 mg of rose. The level of angiogenesis in the groups receiving the hydroalcoholic extract of rose with doses of 500 (Figure 1-C) and 1000 mg/kg was significantly lower than the control group (Figure 1-A) (P>0.05). The rank of fibroplasia in the groups receiving vitamin E and the groups receiving RDHE with doses of 250, 500 (Figure 1-C), and 1000 mg/kg was significantly higher than the control group (Figure 1-A) but lower than the Sham group (P<0.05). The groups receiving vitamin E and RDHE with doses of 250 and 1000 mg/kg improved complete tendon repair (P<0.05), while the groups receiving dexamethasone (Figure 1-B) and RDHE with a dose of 500 mg/kg (Figure 1-C) did not differ from the control group (Figure 1-A) in terms of complete tendon repair. The intensity of inflammation and angiogenesis in the groups receiving the RDHE with doses of 250 (Figure 2-E), 500, and 1000 mg/kg on the 20th-day tendonitis induction was significantly lower than the Sham group and the same as the control group (P>0.05) (Table 4). The rate of fibroplasia and complete tendon repair in the group receiving vitamin E (Figure 2-F) and the rate of complete tendon repair in the group receiving dexamethasone were lower than the Sham group, and a significant difference was observed. Moreover, the amount of fibroplasia and complete tendon repair in the groups receiving the RDHE with doses of 250 (Figure 2-E), 500, and 1000 mg/kg was significantly higher than the control group, with complete tendon repair observed in these groups on the 20th day after tendonitis induction (P<0.05).

Table 3: Effects of R. damascena hydro alcoholic extract (RDHE) on mean animal weight in rats before and after treatment, on day 20

Groups	Route	Weight changes (g)		p-value
		Before treatment	After treatment	
Sham	P.O.	221.00 ± 4.71	224.00 ± 4.65	<0.001
Control	P.O.	228.17 ± 4.95	216.50 ± 3.57	0.004
RDHE 250	I.P.	226.17 ± 2.31	211.83 ± 2.27	0.001
RDHE 500	I.P.	229.75 ± 7.92	197.75 ± 2.29	0.018
RDHE 1000	I.P.	230.83 ± 3.75	206.83 ± 2.36	0.007
Vit E	I.P.	222.00 ± 2.62	222.17 ± 2.26	0.822
Dex	I.P.	229.17 ± 4.09	227.67 ± 4.28	0.076

Data are expressed as the mean ± SEM (n=4). P-values in the last column show the comparison of the two groups before and after the treatment. P.O.; Oral, I.P.; intraperitoneal, RDHE; R. damascena hydroalcoholic extract (RDHE) at 250, 500, and 1000 mg/kg doses, Vit E (100 IU/kg), Dex; dexamethasone (1 mg/kg).

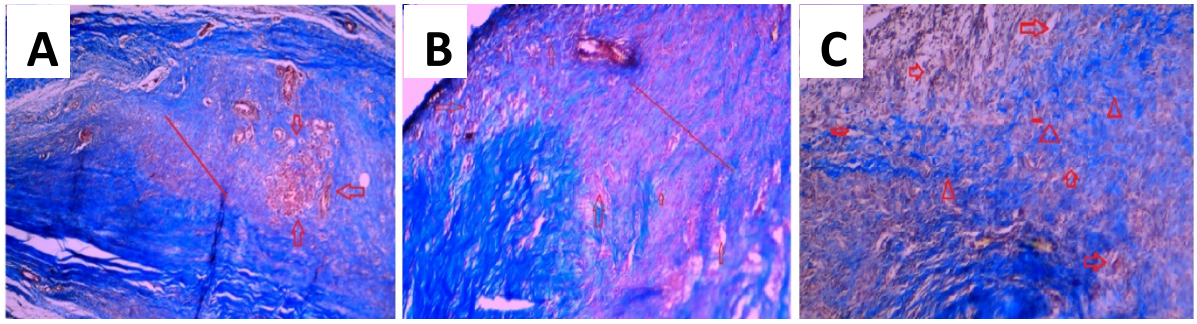

Figure 1: Tendonitis-induced tendon degeneration on day 10; Trichrome staining 100.
(A); Control group: development of thin collagen fibers (continuous line) and vascularized, cell-filled germinal tissue (red arrow); Dex: Formation of thin collagen fibers (straight line) and vascularized, cell-filled germinal tissue (red arrow); RDHE 500: Formation of thin disordered collagen fibers (arrowhead) and vascular, cell-filled budding tissue (red arrow).

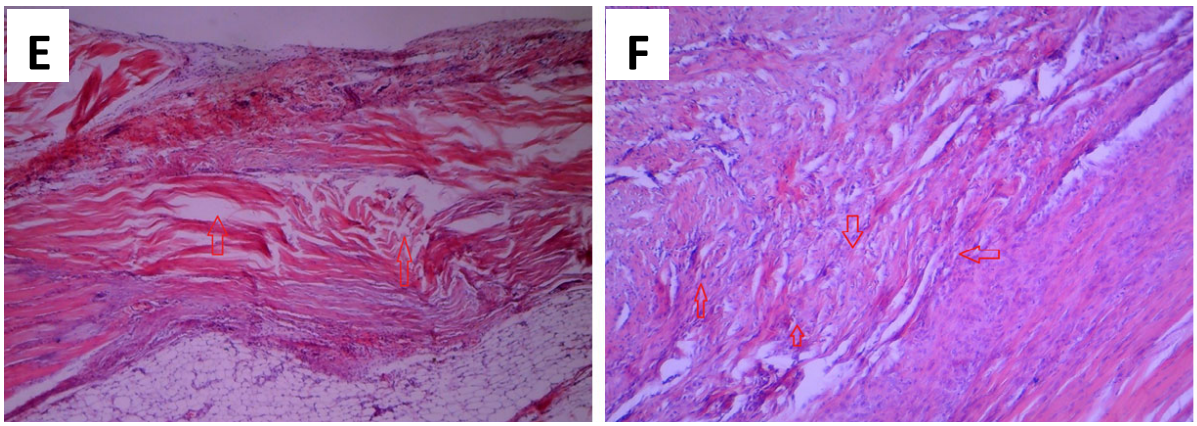

Figure 2: Tendonitis-induced tendon degeneration on day 20.
(E): In the group receiving the hydroalcoholic extract of rose with a dose of 250 mg, irregular formation of collagen strands at the injury site, hematoxylin and eosin staining × 40.
(F): In the group receiving vitamin E, irregular formation of collagen fibers at the injury site, hematoxylin and eosin staining ×100

Discussion

Tendinopathy is a common pathology present in up to 30% of musculoskeletal consultations [2]. The process of repairing tendons has been a focus for researchers due to the importance of increased blood supply, specifically angiogenesis, in the formation of vascular channels and cytokine delivery for tendon healing and remodelling [33]. The angiogenesis rate decreased significantly in groups receiving hydro alcoholic extract of R. damascena on the 10th and 20th day after surgery, especially in the groups receiving doses of 500 and 1000 mg/kg. Meanwhile, complete tendon repair was observed in groups receiving vitamin E and hydroalcoholic extract of R. damascena with doses of 250 and 1000 mg on the 10th day after surgery, and hydroalcoholic extract of R. damascena with all three doses on the 20th day after surgery ($P<0.05$).

The hydroalcoholic extract of R. damascena has vasoactive properties and antiangiogenic/proangiogenic effects. A study on mice showed that injecting rose placenta from R. damascena into wounds resulted in significantly smaller wounds on days 4, 7, and 10. The experimental group also showed increased expression of VEGF and EGF on day 2, decreased expression of TGF-β1 on days 7 and 10, and increased vessel density on day 10. The study suggests that rose placenta may be a potential drug for enhancing wound healing [20]. The results of the study align with our experiment; however, it is important to acknowledge that the level of angiogenesis may be linked to the amount and duration of the extract's usage. Further investigation is necessary.

Although inflammation is necessary for the initial stages of wound healing, previous research suggests that it can have negative effects on the repair process by reducing collagen deposition and vascularization, ultimately leading to a decrease in mechanical properties of the repairs [34, 35]. Consequently, it is important to reduce inflammation during the early stages of wound healing to improve the quality of the repairs [36]. Dexamethasone is a common treatment for tendon injury due to its anti-inflammatory capabilities, but it can cause negative effects such as impaired tendon healing and rupture. This study examined the effects of dexamethason treatment on human tendon stem cells and found that low concentrations stimulated cell proliferation while high concentrations decreased it. Dexamethason treatment also induced non-tenocyte differentiation of hTSCs at all concentrations used, leading to the formation of non-tendinous tissues and making tendon susceptible to rupture [37]. Our study demonstrated that dexamethasone was inadequate in fully restoring tendon tissue, only ameliorating movement in rats through inflammation and pain reduction.

Various studies have been conducted to reveal the anti-inflammatory properties of R. damascena. It has been observed that R. damascena can reduce the release of certain interleukins from inflammatory cells and exhibits anti-inflammatory effects on leg swelling similar to diclofenac [38, 39]. The study discovered that using a hydroalcoholic extract of rose at 500 and 1000 mg/kg decreased inflammation intensity compared to the sham group. Additionally, the research conducted by Latifi et al. has demonstrated that the hydroalcoholic extract of R. damascena, administered orally or through intraperitoneal injection, produces anti-inflammatory effects in animal models of colitis caused by acetic acid, akin to the effects of dexamethasone and prednisolone [18]. The anti-inflammatory effect of this extract has also been confirmed in mice models where edema was induced by carrageenan [40]. The occurrence of oxidative stress reactions and damage indices is augmented during tendonitis [24]. Beta-Citronellol, Thymol, and Geranial have been identified as the primary compounds in the hydroalcoholic extract from Rosa damascene through GC-MS analysis. Numerous studies have confirmed the antioxidant properties of Rosa damascene [41]. Tendonitis causes damage to tendon tissue in rats by increasing lipid peroxidation and

myeloperoxidase, which leads to severe tissue destruction[23]. A reduction in malondialdehyde levels in the blood serum of rats treated with rose hydroalcoholic extract suggests its protective effect in preventing lipid peroxidation, which is initiated by free radicals and causes damage to cell membrane compounds, cell necrosis, and inflammation, and promotes the formation of active eicosanoids in inflammation [42]. The study found that rats with tendonitis treated with 1000mg/kg of hydroalcoholic rose extract had a significant increase in FRAP compared to other groups. This increased the total antioxidant capacity of plasma to normal levels.

Conclusion

The findings presented in this research validate the use of R. damascene in traditional medicine to facilitate the healing process. The hydro alcoholic extract of Rose damascene, specifically at a dosage of 1000 mg, was observed to enhance the intensity of inflammation, angiogenesis, fibroplasia, and the rate of complete tendon repair on the 10th and 20th days after the induction of tendonitis, indicating a faster healing process. This is evident through increased angiogenesis and fibroplasia, which play a crucial role in providing sufficient blood flow and tissue repair at the injured tendon site, while also enhancing the antioxidant capacity of the total serum and reducing the peroxidation level of unsaturated fatty acids on the 20th day following tendonitis induction. In addition to its anti-inflammatory effect, this extract also improved the gait recovery of the injured animals. Further investigations are required to explore the impact of the R. damascene extract on inflammation and the reorganization of collagen bundles after tendon injury. The extract's safety and tolerability render it a prospective alternative for alleviating tendonitis symptoms.

Table 4: Evaluation of average rank (Q1-Q3) m histopathological parameters of tendon repair process on days 10 and 20 after tendonitis induction in male rats

Parameter / Groups	Sampling day	Inflammation	Angiogenesis	Fibroplasia	Complete tendon repair
Sham	Day 10	^{c}5/00	^{c}3/00	^{a}33/00	^{a}33/00
	Day 20	^{b}10/50	^{b}10/50	^{a}23/00	^{a}25/50
Control	Day 10	^{a}33/50	^{B}26/50	^{c}5/50	^{c}8/00
	Day 20	^{a}26/50	^{a}23/00	^{b}5/50	^{C}3/00
RDHE 250	Day 10	^{c}6/10	^{a}26/50	^{b}20/50	^{b}23/00
	Day 20	^{b}10/50	^{b}10/50	^{a}23/00	^{a}25/50
RDHE 500	Day 10	^{b}23/50	^{b}11/50	^{b}20/50	^{c}8/00
	Day 20	^{b}10/50	^{b}10/50	^{a}23/00	^{a}25/50
RDHE 1000	Day 10	^{b}23/50	^{b}11/50	^{b}20/50	^{b}23/00
	Day 20	^{b}10/50	^{b}10/50	^{a}23/00	^{a}25/50
Vit E	Day 10	^{b}20/10	^{a}20/50	^{b}20/50	^{b}23/00
	Day 20	^{a}31/00	^{a}20/50	^{b}5/50	^{b}10/50
Dex	Day 10	^{b}23/50	^{a}26/50	^{c}5/50	^{c}8/00
	Day 20	^{a}26/50	^{a}20/50	^{a}23/00	^{b}10/50
P value		0/001	0/001	0/001	0/001

Data are based on mean±0/001S.D, Number of rats (n=4) for each group at each time point, R. damascena hydroalcoholic extract (RDHE), Vitamin E, Dex; dexamethasone.
Different letters (a, b, c) in each column indicate significant differences (P<0.01).

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