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Research Article

Beneficial Effect of Herbal Oils on the Healing of Traumatic Tendons Injuries: In Vivo Model

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Abstract: Tendons exhibit a slow healing process in tendinopathies due to their relatively poor vascularization and low cellularity. Different treatment options have been developed to restore tendon function and structure; however, these treatments are inadequate for complete healing. Adjunctive novel treatments such as herbal oil ointments have been explored to enhance the healing process and the results are encouraging, with fewer side effects being observed in in vitro studies. In this study, histological, biochemical, hematological, and observational investigations were utilized to evaluate the effect of the topical treatment of different herbal oils or combinations (i.e., *Olive oil*, *Nigella sativa oil*, and *Inula viscosa oil*) on an in vivo model of a traumatized rat hind limb Achilles tendon. Forty-eight rats were divided into eight groups. The rats were treated daily after injury, and samples were collected on day six. An observational assessment of redness, swelling, and pain showed slightly improved symptoms among all the groups on day six. It was noted that groups treated with *inula viscosa* oil, *Olive* oil, and combined oils (*Olive + Nigella sativa*) achieved better scores. The histological findings showed similar changes of increased cellularity and extracellular matrix disorganization in the tendon matrix in the treated groups and the experimental control group, except for the rats treated with *Nigella sativa* oil, in which more changes were seen. The hematological findings showed a decreased neutrophil count and increased lymphocyte and monocyte counts in the blood of the model rats on day six. The general blood biochemical markers (AST, ALT) of inflammation and injury were slightly higher in the treated groups on day six than in the experimental control group, while the CPK values were notably elevated with high standard deviations. These variabilities in the data, especially with the small sample size (n = 6), make it difficult to determine any differences between the groups. In conclusion, the results support the traumatic in vivo model and show that, after one week of injury onset, the potential effect of treatment can be evaluated using observational assessments of pain, redness, and swelling. However, a longer experiment duration and the use of a larger sample size are needed to reflect the matrix changes.

Keywords: Tendon; Phytotherapy; *Nigella sativa*; *Inula viscosa*; *Olive oil*.

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1. Introduction

Tendons, viscoelastic connective tissue, have relatively poor vascularization and low cellularity, which results in a slow healing process in cases of tendinopathies (Schulze-Tanzil *et al.*, 2022). In football, traumatic injuries related to muscles and tendons of the knee and ankle joints comprise around two-thirds of cases (Gurau *et al.*, 2023). Different treatment options have been used for tendinopathies such as eccentric exercise, growth factors, phototherapy, and phytotherapy to boost the healing process by modulating collagen synthesis, cell division, oxidative stress, and matrix remodeling to restore tendon function and structure. However, none of these options are sufficient to cover the full treatment and rehabilitation plan (Cardoso *et al.*, 2019).

The use of phytotherapy (herbal medicine), as an adjunctive treatment, is growing worldwide, especially in sports musculoskeletal injuries. This approach works by modulating different biological effects against microbes, tumors, mutations, oxidative stress, aging, degeneration, and inflammation due to the rich source of bioactive compounds used in traditional and modern medicine (Sellami *et al.*, 2018; Forni *et al.*, 2019; Tran *et al.*, 2020). It has been shown that plant-based oils might help tendon repair during the three healing phases of a traumatic injury by modulating inflammation and oxidation levels (Cardoso *et al.*, 2019; Sellami *et al.*, 2018; Forni *et al.*, 2019; Tran *et al.*, 2020). Different herbal preparations such as essential oils, extracts, and ointments as adjunctive treatments have exhibited protective effects and their therapeutic outcomes are encouraging. In addition, these oil preparations in either oral or topical forms alleviate concerns about the adverse effects of pharmaceuticals and satisfy the desire for more personalized medical care (Aljaafari *et al.*, 2021; Cimino *et al.*, 2021).

Olive oil, *Nigella sativa* oil, and *Inula viscosa* oil are among the most commonly used medicinal oils in potentiating the healing process. A recent *in vitro* study showed increased tendon cell viability over two days (Al-Shudiefat *et al.*, 2022). *Nigella sativa*'s antioxidant capability and anti-inflammatory effects have been reported by many researchers; for example, isolated compounds of *Nigella sativa* including thymoquinone, carvacrol, *t*-anethole, and 4-terpineol have appreciable free-radical-scavenging properties. In addition, *Nigella sativa* oil and thymoquinone, the main compound of *Nigella sativa* essential oil, inhibit non-enzymatic lipid peroxidation in liposomes (Dinakaran *et al.*, 2016; Bordoni *et al.*, 2019). Recently, an animal model of an injured tendon injected with thymoquinone extracts of *Nigella sativa* improved matrix organization and inflammatory cell infiltrates (Ouahchia *et al.*, 2020). *Olive oil* has antioxidant and anti-inflammatory effects through oleic acid and oleocanthal, respectively (Gavahian *et al.*, 2019; Pastor *et al.*, 2021; Al-Shudiefat *et al.*, 2022). An

in vitro study showed that oleic acid did not decrease the cell viability or gene expression of inflammatory or degenerative gene markers (Soltanfar *et al.*, 2023). The findings of an *in vivo* animal model of muscle injury support the use of *oleic acid* in *Olive oil*, in contrast to *linoleic acid*, which deteriorates the condition (Konar *et al.*, 2023). *Inula viscosa* plant-isolated bioactive components such as essential fatty acids and phenolic compounds have been reported for their appreciable antioxidant, anti-inflammatory, and analgesic properties in the wound healing process (Abreu *et al.*, 2016). A recent review reported evidence of the healing properties of *Inula viscosa*, and an *in vitro* study showed encouraging results from its use in treating sheep tendon cells (Al-Shudiefat *et al.*, 2022).

Evidence of herbal oils' potential modulation of healing at the cellular level has been reported. However, to the best of our knowledge, no previous studies have been conducted to explore the beneficial effects of different oil ointments on traumatically injured rat tendons. In this study, we hypothesize that reconditioning traumatically injured tendons topically with herbal oil ointments (i.e., *Olive oil*, *Nigella sativa* oil, *Inula viscosa* oil, or a combination) enhances the healing process by modulating the tendon matrix. If this is the case, these oils can reduce the duration of treatment and lessen side effects. Importantly, scientific evidence is needed for the safety and efficiency of such treatment (Mahmoudi *et al.*, 2016; Al-bahri *et al.*, 2023).

2. Materials and Methods

Forty-eight adult male Wistar rats weighing 275 to 350g (16-18 weeks of age, on average) were divided into eight groups. Each group had six rats (Table 1). The animals were housed in ventilated cages (two rats per cage) under an equal light/dark cycle, in an atmosphere with 55% humidity and a temperature range of 20–22°C (Brown, 2017), in the Animal House of the University of Jordan. The animals were acclimatized for one week, and were given filtered tap water and standardized food.

The skin on the posterior surface of the hind limb was shaved over the calcaneal tendon, which was traumatized bluntly and transversely by dropping a 200 gm weight from a fixed height (20 cm) perpendicular to the collagen fibers, 2 cm from tendon insertion, using a mini guillotine manufactured locally (Figure 1) (Joensen *et al.*, 2012; Salate *et al.*, 2005; Fillipin *et al.*, 2005; Oliveira *et al.*, 2009). After the injury, the skin over the traumatized area was treated daily for the next four days using moistened cotton with oil and dressed with a plaster around the injured area. In the experimental control groups, moistened cotton with water only was used as a placebo, and the skin was dressed with a plaster. No cast immobilization was used, and the rats were kept warm until they recovered consciousness. No analgesics were

administered during the trial, which was extended for five days.

On day six of the experiment, all rats were humanely euthanized, blood samples from rat orbits were collected using the retro-orbital bleeding procedure, and whole-tendon samples were excised and immediately fixed via

immersion in fresh 10% (v/v) neutral buffered formaldehyde solution for 48h at room temperature (Akamatsu *et al.*, 2014). The fixed tendon samples were processed for histological sections ready for hematoxylin and eosin staining at the Medical Laboratory Sciences Department, The Hashemite University.

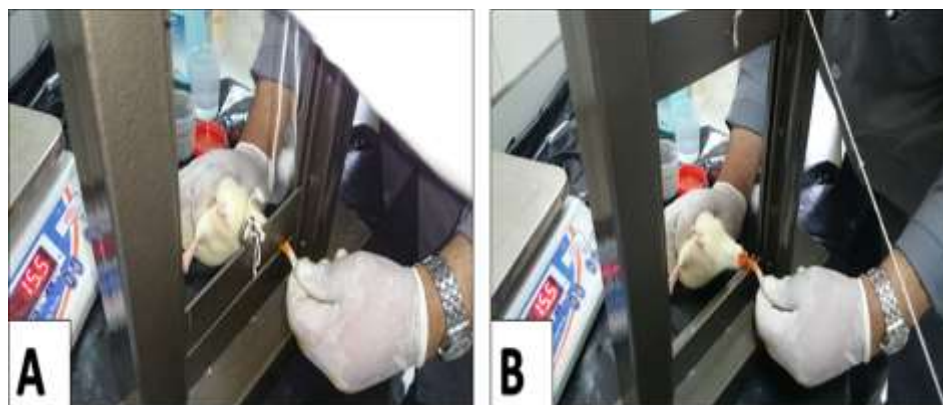


Figure 1. (A) Mini guillotine, manufactured by a local blacksmith, with rat positioned for Achilles injury. (B) Close-up of the tendon hit location.

Table 1. Features of animal groups.

Group	Group Name	Trauma	Treatment
I	Experimental control	Yes	Placebo
II	Olive oil	Yes	Olive oil
III	<i>Nigella sativa</i> oil	Yes	<i>Nigella sativa</i> oil
IV	<i>Inula viscosa</i> oil	Yes	<i>Inula viscosa</i> oil
V	Mixed oils (<i>Olive</i> + <i>Nigella</i>)	Yes	Mixed oils (<i>Nigella</i> and <i>Olive</i>)
VI	Mixed oils (<i>Olive</i> + <i>Inula</i>)	Yes	Mixed oils (<i>Inula</i> and <i>Olive</i>)
VII	Corn oil	Yes	Corn oil
VIII	Normal group	No	No

A light microscope system with a digital camera and laptop was used to capture images of H&E-stained slides. A quantitative, four-point scoring system was used to score and analyze the effects of the treatments on cellular and matrix features by three trained, blinded researchers (Table 2). The observational criteria of redness, swelling, and pain with walking ability were scored based on a four-point system during the experiment period by three blinded researchers (Table 2). Blood samples for hematology (collected in EDTA tubes) and blood chemistry (collected in plain tubes) were collected to test for the erythrocyte sedimentation rate (ESR), complete blood count (CBC), C-reactive protein (CRP),

alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and creatine phosphokinase (CPK) at the end of the experiment for each group. The results were expressed as means and standard deviations for the different rat groups (n = 48). Statistical analysis was performed using IBM Statistical Package for the Social Sciences software (SPSS, version 27) and Excel Microsoft Office software. The distribution of data was tested using the Shapiro–Wilk test. Parametric tests (i.e., ANOVA and T-test) were employed for normally distributed data, while non-parametric tests (i.e., Kruskal–Wallis tests) were applied to non-normally distributed data. Multiple comparisons were made for the different treatment groups accordingly. Statistical significance was determined at a p-value of 0.05 or below.

Table 2. Four-point scoring system for histological and observational criteria.

Histological Criteria	Four-point Scoring			
	0	1	2	3
Collagen fibers of wavy appearance	Normal	Slight change	Moderate change	Abnormal
Cellularity	Normal	Slight increase	Moderate increase	High increase
Nucleus shape	Flattened	Semi-flattened	Semi-rounded	Rounded
Observational Criteria				
Redness	Normal (no redness at all)	Light redness	Moderate redness	Strong redness
Swelling	Normal (no swelling at all)	Light swelling	Moderate swelling	Strong swelling
Pain with walking ability	Normal (no pain with good walking)	Slight pain with slight difficulty walking	Moderate pain with moderate difficulty walking	Strong pain with difficulty walking

3. Results

Weight changes and Observational notes

The results showed slight changes in the mean body weight of all the treated groups (ranging between 2 and 9 gm) when compared with the experimental control group (Group I) (Table 3); however, this change was not statistically significant when compared to the experimental control group (ANOVA test, p-value = 0.066).

The observational records showed increased redness, swelling, and pain with decreased walking ability in the first half of the experimental period and improvement in the second half. The observational assessment showed that the groups treated with *Inula viscosa* oil, *Olive* oil, and combined oils (*Olive* + *Nigella sativa*) had better scores on day six (Table 4).

Table 3. Weight change data.

Treatment Group	Initial Weight (gm)		Weight Change (gm)	
	Mean	SD	Mean	SD
Experimental control	326.33	32.57	0.83	9.06
<i>Olive</i> oil	324.83	40.07	-8.83	6.44
<i>Nigella sativa</i> oil	327.33	26.82	-8.50	4.12
<i>Inula viscosa</i> oil	320.67	38.57	-3.17	2.64
Mixed oils (<i>Olive</i> + <i>Nigella</i>)	328.33	12.36	-2.83	9.14
Mixed oils (<i>Olive</i> + <i>Inula</i>)	320.00	30.18	-2.50	7.59
Corn oil	326.67	12.40	-2.00	7.96

SD: Standard deviation.

Table 4. Changes in the observational criteria scores on day six.

Observational Criteria	Scoring Day	Recorded Scores for Each Group						
		Control	Olive Oil	Nigella Oil	Inula Oil	Mixed Olive and Nigella oil	Mixed Olive and Inula oil	Corn oil
Redness	1	1.5	1.5	1.5	1.5	1.67	2	1.83
	6	0.83	0.67	0.67	0.83	0.17	1.33	0.67
	1 – 6	0.67	0.83	0.83	0.67	1.5**	0.67	1.16
Swelling	1	2.33	2.33	1.5	2	2	2.17	2
	6	1.33	0.83	0.83	0.33	0.33	0.83	0.5
	1 – 6	1	1.5	0.67	1.67**	1.67**	1.34	1.5
Pain and walking ability	1	2	2	2	2	1.83	1.33	1.83
	6	0.83	0.5	0.5	0.33	0.33	0.67	0.33
	1 – 6	1.17	1.5	1.5	1.67**	1.5	0.66	1.5

**: Greatest change in score.

Hematology and Blood Biochemistry Analysis (Tables 5 and 6)

There was an increase in the white blood cell (WBC) count on day six in the treated groups (II-VII) when compared with the experimental control group. The statistical analysis data showed no significant difference on day six between all groups (I-VII) regarding WBC count (ANOVA test, p-value = 0.054) (Table 5). The ESR data on day six showed slight changes; however, these changes were not significant between the groups (I-VII) (ANOVA test, p-value = 0.267) (Table 5). The WBC differential analysis data showed changes regarding neutrophils (decrease), lymphocytes (decrease), and monocytes (increase). However, the lymphocyte difference was not significant when a comparison was made between all groups (I-VII) (ANOVA test, p-value = 0.326). The monocyte percentages on day six showed significant differences between all groups (I-VII) (ANOVA test, p-value = 0.016). Post hoc multiple comparison

tests showed that the *Nigella sativa* oil-treated group exhibited a significant increase compared to the experimental control group (post hoc multiple comparison tests, p-values = 0.025). The neutrophil percentages on day six showed significant differences between all groups (I-VII) (ANOVA test, p-value < 0.001). Post hoc multiple comparison tests showed that *Olive* oil, *Nigella* oil, *Inula* oil, and the two mixed oil (but not the corn oil group)-treated rats exhibited a significant decrease compared to those in the experimental control group (post hoc multiple comparison tests, p-values < 0.05) (Table 5).

The general blood biochemistry markers (AST, ALT) of inflammation and injury were slightly higher in the treated groups on day six than in the experimental control group. The CPK values were notably elevated, with a high standard deviation. These variabilities in the data, especially with the small sample size (n = 6), make it difficult to determine any differences between the groups (Table 6).

Table 5. Hematological findings for RBCs and WBCs.

Treatment Group	ESR (mm/hour)		WBC (103/mm3)		Neutrophils (%)		Lymphocytes (%)		Monocytes (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Experimental Control	1.00	0.00	2.98	1.15	56.38	17.65	48.77	27.20	13.88	2.00
<i>Olive</i> oil	1.17	0.41	4.67	2.20	26.12*	9.11	39.93	17.66	26.48	10.88
<i>Nigella sativa</i> oil	1.00	0.00	4.27	1.11	28.10*	8.07	32.82	9.01	30.55*	8.54
<i>Inula viscosa</i> oil	1.00	0.00	6.54	2.48	20.56*	5.24	49.95	16.35	25.25	7.38
Mixed oils (<i>Olive</i> + <i>Nigella</i>)	1.50	0.84	4.14	1.84	22.95*	3.84	44.37	9.42	25.75	5.22
Mixed oils (<i>Olive</i> + <i>Inula</i>)	1.00	0.00	4.09	1.16	28.93*	15.09	51.14	22.34	28.98	15.22
Corn oil	1.50	0.84	3.06	1.37	52.18	24.96	33.73	15.31	20.13	1.89

SD: Standard deviation; * Significant compared to the experimental control group: p-value < 0.05.

Table 6. Blood chemistry data.

Treatment Group	Creatinine (mg/dL)		AST (U/L)		ALT (U/L)		CPK (U/L)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Experimental control	0.23	0.02	85.00	12.29	31.67	10.26	357.00	124.11
Olive oil	0.34	0.06	133.00	9.54	54.00	7.55	1179.0	208.71
Nigella sativa oil	0.28	0.02	142.00	10.15	48.67	10.69	1129.3	32.56
Inula viscosa oil	0.26	0.01	123.00	13.00	46.33	4.16	1070.3	159.16
Mixed oils (Olive + Nigella)	0.31	0.07	131.33	25.15	53.67	4.93	934.00	421.56
Corn oil	0.27	0.01	101.00	14.00	44.67	4.62	540.67	136.43

SD: Standard deviation.

Histological Findings

Images of the traumatized tendon sections stained with H&E for the different groups (I-VII) and the data collected using the scoring system for matrix organization, cellular content, and cell morphology on day six are presented in Figure 2 and Table 7. The histological images show a traumatized area adjacent to normal non-traumatized tissue with an interface line between them. The traumatized regions are characterized by disorganized

collagen fibers and highly cellular infiltrates with round nuclei (Figure 2). At the end of the experiment, the findings showed that the increased cellularity and extracellular matrix disorganization of the tendons exhibited similar changes in the treated groups compared to the experimental control group (placebo), except for the rats treated with *Nigella sativa* oil, for which more changes were seen (Table 7).

Table 7. Tendon histological scoring for all groups at day six.

Group Name	Collagen fibers' wavy appearance score	Cellularity score	Nucleus shape score
Control	3	3	3
Olive oil	2	3	2
Nigella sativa oil	3	3	3
Inula viscosa oil	2	3	2
Mixed oils: Olive and Nigella sativa	2	3	2
Mixed oils: Olive and Inula viscosa	2	3	1
Corn oil	2	3	2

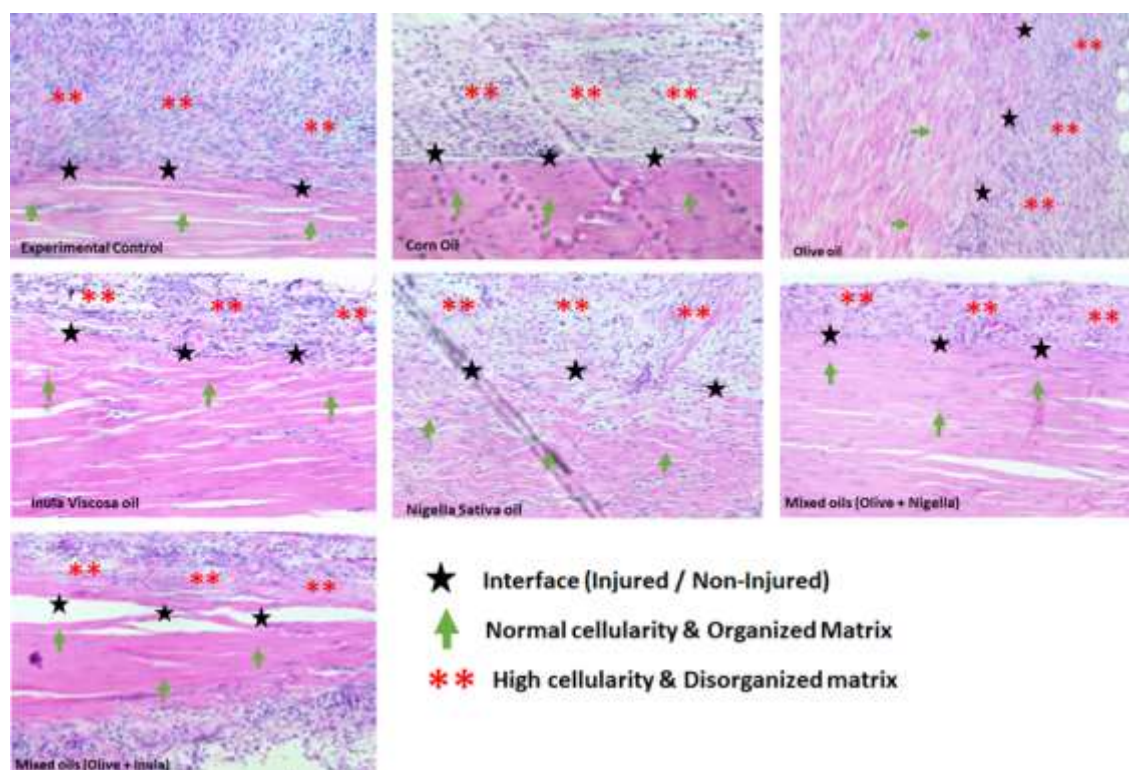


Figure 2. Histological H&E images showing changes in matrix organization and cellular content on day six for all groups; magnification power: 10X.

4. Discussion

The response of the tendons to traumatic injury was similar for all groups in terms of redness, swelling, pain, and walking ability, and the results reported by the evaluators showed improved symptoms among all groups on day six to varying degrees. The findings showed that the groups treated with *Inula viscosa* oil, *Olive* oil, and combined oils (*Olive* + *Nigella sativa*) had better scores. Topical applications of these oils have been reported to improve pain symptoms and oxidative stress status in animal models of skin wound healing (Mark *et al.*, 2007). In our model, both the tendon and the overlying skin were traumatized, so it is expected that topical application will help to ameliorate the signs and symptoms of both.

The histological findings showed similar changes, namely, increased cellularity and extracellular matrix disorganization in the tendon matrix in the treated groups compared to the experimental control group, except for the rats treated with *Nigella sativa* oil, for which more changes were seen. These findings could be explained by the fact that the short experiment period of six days spans the hemostasis, inflammatory phase, and the onset of the proliferative phase of the healing process of the traumatized tendon, which explains the continued presence of high inflammatory cell infiltrates at the end of the experiment (day six). In addition, the process of synthesizing collagen fibers and angiogenesis is still at the onset stage (Schulze-Tanzil *et al.*, 2022; Mark *et al.*, 2007).

Our model represents the inflammatory and proliferative phases, which extend for a few days after the trauma.

This is reflected by the increase in the WBC count, especially the precursors of macrophages, which predominate on day six (Sunwoo *et al.*, 2020). Moreover, the hematological findings showed a decrease in the neutrophil count and an increase in lymphocyte and monocyte counts in the blood of the model rats on day six. In addition, these initial phases result in the release of many vasoactive and chemotactic factors that stimulate inflammatory cells, tenocyte proliferation, and the migration and synthesis of the extracellular matrix, especially collagen fibers. However, these changes in matrix organization typically commence in the second week. Moreover, the changes in cell morphology are supported by the decreased activity of the rats as a result of trauma (Chen *et al.*, 2021; Soltanfar *et al.*, 2023).

Moreover, WBC's role in healing follows a time frame where neutrophils are the first cells to appear at the injured site, followed by monocytes and lymphocytes (Marsolais *et al.*, 2001). Considering the life span of each cell, it is expected that the lymphocyte and monocyte count is still high, while the neutrophil count is low. Neutrophils have been reported to accumulate in a tendon tissue inflammatory model during the inflammatory phase (1-3 days), while the increase in the macrophage count lasts for one week (Kaya *et al.*, 2022). Moreover, a study similar to ours reported an increase in WBC counts in patients with active tendinopathy relative to normal controls, and the neutrophil percentage was decreased (Warden, 2007), so our findings support the role of WBCs in the pathophysiology of tendinopathy. This decrease in neutrophils can be explained by the fact that measures were taken on day six when the inflammatory phase subsided, especially neutrophils, which normally

increase in the first three days. Moreover, increased cellularity, as depicted from the histological sections of traumatized tissues, is a common feature reported in different lesions of tendinopathy. In our model, the increased cellularity is mostly attributed to inflammatory cell infiltrates, as stated previously (Alzyoud *et al.*, 2019; Alzyoud *et al.*, 2022; Jomaa *et al.*, 2020). A review reported that macrophages were the most common cells present in inflammation in tendinopathy lesions; macrophages are the precursors of monocytes, which increased in our study on day six (Leong *et al.*, 2020; Sallehuddin *et al.*, 2020).

Herbal oil treatments have been reported to ameliorate tendinopathy with satisfactory outcomes (Muzy *et al.*, 2023), and the approach is considered an adjunct treatment to improve signs and symptoms, tendon histology, and hematological and biochemistry tests (Sallehuddin *et al.*, 2020; Kmail *et al.*, 2023). Published research summarized the potential healing effect of *Nigella sativa* due to its thymoquinone content, which helps modulate inflammation, oxidative stress, and bactericidal effects, as reported in an animal model of an injured tendon injected with thymoquinone extracts from *Nigella sativa*, which improved matrix organization and inflammatory cell infiltrates (Jimenez-Lopez *et al.*, 2020; Hohen-schurz-Schmidt *et al.*, 2023). Our previous work demonstrated the safety and potential effects of several medicinal herb oils, i.e., *Nigella sativa*, *Olive*, oleic acid, and *Inula viscosa*, upon cells extracted from sheep tendons (Alzyoud *et al.*, 2019). *Inula viscosa*'s active ingredients, terpenoids, flavonoids, and lignins, have been reported to modulate inflammation, oxidative stress, and antiseptic processes (Mahmoudi *et al.*, 2016). The findings of our study regarding the effects of *Inula viscosa* oil could be explained by the isolated bioactive components such as essential fatty acids and phenolic compounds that have been reported to have appreciable antioxidant, anti-inflammatory, and analgesic properties in the wound healing process (Abreu *et al.*, 2016). *Olive* oil's active ingredients, such as oleic acid and phenolic compounds, can modulate inflammation and oxidative stress and possess antimicrobial effects (Bucciantini *et al.*, 2021). These effects explain the changes in the rat tendons in this experiment. Similar effects were reported in an in vivo animal model of muscle injury and the findings support the use of *oleic acid* in *Olive* oil, in contrast to *linoleic acid*, which deteriorates the condition (Abreu *et al.*, 2016). *Olive* oil has antioxidant and anti-inflammatory effects through oleic acid and oleocanthal, respectively (Ouahchia *et al.*, 2020; Gavahian *et al.*, 2019). An in vitro study showed that oleic acid did not decrease cell viability or the gene expression of inflammatory or degenerative gene markers (Soltanfar *et al.*, 2023). In this study, non-injured rats treated with normal saline as a placebo were used as an experimental control group to control for confounding biases during treatment and to satisfy internal validity. The general blood biochemical markers (AST, ALT) of inflammation and injury were slightly higher in the treated groups on day six than in the experimental control group. The CPK values were notably elevated, with high standard deviation values. These variabilities in the data, especially with the

small sample size ($n = 6$), make it difficult to determine any differences between the groups.

5. Conclusions and Recommendations

Our findings validate the in vivo model of traumatic tendon injury, and the results from the topical application of herbal oil treatments show the potential of this approach to improve the general symptoms and enhance the healing process of traumatized tendons. An important limitation of this study is that the treatment period of five days spans the hemostasis, inflammatory phase, and the onset of the proliferative phase of the healing process of the traumatized tendon, which explains the continued presence of high inflammatory cell infiltrates at the end of the experiment (day six). In addition, the process of synthesizing collagen fibers and angiogenesis is still at the onset stage. Moreover, WBCs' role in healing follows a time frame where neutrophils are the first cells to appear at the injured site, followed by monocytes and lymphocytes (Russo *et al.*, 2022); considering the life span of each cell, it is expected that the lymphocyte and monocyte count is still high, while the neutrophil count is low. Another limitation of this study is that the findings only represent the topical application of these herbal oils as no other treatment modalities were applied.

Finally, taking these considerations into account, it is recommended that the treatment be extended for a longer period to cover the proliferative phase so that matrix changes can be revealed histologically. In conclusion, the results support this traumatic in vivo model and show that, after one week of injury onset, the potential effect of treatment can be evaluated using observational assessments of pain, redness, and swelling. However, a longer experiment using a larger sample size is needed to reflect the matrix changes.

Supplementary Materials: The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Author Contributions: Conceptualization, Jihad A.M. Alzyoud, Abd Al-Rahman Salem Al-Shudiefat, and Adham N.M. Al-Rousan; data curation, Adham N.M. Al-Rousan, Samya A. Omoush, and Dalal A.O. Shuqair; formal analysis, Jihad A.M. Alzyoud; methodology, Jihad A.M. Alzyoud, Abd Al-Rahman Salem Al-Shudiefat, and Adham N.M. Al-Rousan; project administration, Jihad A.M. Alzyoud, and Abd Al-Rahman Salem Al-Shudiefat; supervision, Jihad A.M. Alzyoud and Abd Al-Rahman Salem Al-Shudiefat; validation, Jihad A.M. Alzyoud, Abd Al-Rahman Salem Al-Shudiefat, Adham N.M. Al-Rousan, Samya A. Omoush, and Dalal A.O. Shuqair; visualization, Jihad A.M. Alzyoud and Abd Al-Rahman Salem Al-Shudiefat; writing—original draft, Jihad A.M. Alzyoud, Abd Al-Rahman Salem Al-Shudiefat, Adham N.M. Al-Rousan, Samya A. Omoush, and Dalal A.O. Shuqair; writing—review and editing, Jihad A.M. Alzyoud, Abd Al-Rahman Salem Al-Shudiefat, Adham N.M. Al-Rousan, Samya A. Omoush, and Dalal A.O. Shuqair. All authors have read and agreed to the published version of the manuscript.

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