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Original article

The effects of some natural products compared to synthetic products on the metabolic activity, proliferation, viability, migration, and wound healing in sheep tenocytes

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ABSTRACT

Background: Tendinopathy or tendon injuries can affect many people, causing a huge impact on their movements and maintaining standing posture. Treatment options include physiotherapy, anti-inflammatory drugs, and alternative medicine. The use of physiotherapy or anti-inflammatory drugs may cause some side effects like pain and liver failure, respectively, therefore, alternative medicine will be a better choice.

Method: Tenocytes were isolated from sheep Achilles tendon and used in Alamar blue assay to assess the metabolic activity, proliferation, and viability of tenocytes over 24 hrs. and 48 hrs., using natural and synthetic products [i.e., olive oil, oleic acid, corn oil, *Inula viscosa* oil, *Inula viscosa* extract, *Nigella sativa* oil, naproxen sodium, and paracetamol and LED photobiomodulation]. Furthermore, tenocytes viability was assessed by FDA/PI stain. For migration and healing of a wound, the scratch assay was used.

Results: Alamar blue assay over 24 hrs. showed that *Nigella sativa* oil increased the metabolic activity, proliferation, and viability of tenocytes significantly, while Alamar blue over 48 hrs. showed that oleic acid, LED, and their combination increased these parameters for tenocytes significantly. Olive oil increased the viability of tenocytes significantly using FDA/PI stains. Scratch assay revealed that *Inula viscosa* oil, *Inula viscosa* extract, and paracetamol increased tenocyte migration and healing significantly.

Conclusion: *Nigella sativa* oil, olive oil, oleic acid, *Inula viscosa* oil, and *Inula viscosa* extract may be used as an alternative therapy for tendinopathy with less side effects.

1. Introduction

Musculoskeletal disorders are health problems spread across the world, they include tendinopathy and tendinitis which affect millions of people worldwide. It affects one in three people aged over 75 years (Martin, 1988). In literature, it was one of the most frequent self-reported disorders, affecting 159 out of 1000 adult women and 143 out of 1000 adult men (Rowlands & Moser, 1997). In general women have more tendinopathy than men, for example women have six times paratendinopathy of the wrist and hand more than men and this could be due to the heavy use of hand and thumb and could be promoted by diabetes and

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rheumatoid arthritis, tendinopathy of pelvic region more in women than men, this may be due to morphology of pelvis in women is different than men (Kaux, Forthomme, Le Goff, Crielaard, & Croisier, 2011). Another important example is the tendinopathy due to sports, woman have more tendinopathy due to starting younger in sports and practicing for longer period of times (Maffulli, Wong, & Almekinders, 2003). Musculoskeletal injuries can affect both active people and sedentary people (Hootman et al., 2002). On the other hand, tendinopathy is not only a chronic disease of tendons, but it is also a major problem that may affect people in sports and the workplace causing high morbidity rates among them (Sharma & Maffulli, 2005). It was estimated that tendon injuries account for 30–50 % of all injuries in sports (Järvinen, Kannus, Maffulli, & Khan, 2005). Therefore, it was suggested that inflammation is playing a major role in the initiation of tendinopathy (Fredberg & Stengaard-Pedersen, 2008). Tendinitis is a tendon injury that results from the inflammatory response to the repetitive mechanical load of the tendon (Almekinders & Temple, 1998). Symptoms accompanying tendinopathy include pain, irritability, and reduced functionality of the tendon (Cook & Purdam, 2009).

Treatment of tendinopathy may include physiotherapy, anti-inflammatory drugs, exercise, stem cells, growth factors, sclerotherapy, shock wave therapy, surgery, nitric oxide patches, and using alternative medicine (Andres & Murrell, 2008; Wegener, 2000). Some of these treatments have limitations in curing tendinopathy, and some of them like anti-inflammatory drugs such as nonsteroidal anti-inflammatory drugs [NSAIDs] have bad side effects like intestinal ulcers, bleeding, intestinal perforations, colitis, and hepatic failure (Andres & Murrell, 2008; Bjarnason & Hayllar, 1993; Sgro et al., 2002). Therefore, there is a need for alternative anti-inflammatory treatment with naturally occurring substances that enhance healing and cause fewer side effects.

Oleic acid is a major component in extra virgin olive oil, which has an anti-inflammatory effect on the body, including endothelial cells in the aorta (Harvey et al., 2010; Vassiliou et al., 2009). Oleic acid can reduce tumor necrosis factor-induced oxidative stress and increase the viability of cardiomyocytes *in vitro* (Al-Shudiefat, Sharma, Bagchi, Dhingra, & Singal, 2013). Extra virgin olive oil has an ibuprofen [anti-inflammatory drug] like effect as well as antioxidant properties and antimicrobial (Beauchamp et al., 2005; Bogani, Galli, Villa, & Visioli, 2007; Cicerale, Lucas, & Keast, 2012). Therefore, extra virgin olive oil and its major component oleic acid (Waterman & Lockwood, 2007) could serve as a natural alternative to anti-inflammatory drugs that have sometimes adverse side effects on health. In contrast, corn oil may cause inflammation in the intestine of mice fed alcohol (Kirpich et al., 2013). *Nigella sativa* oil has many beneficial properties, which include antioxidant, anticancer, and anti-inflammatory effects (Hadi, Mohammed, & Hameed, 2016). *Inula viscosa* also has antioxidant and anti-inflammatory properties (Lounis, Bergheim, Bouhaimi, Guigonis, & Belhamel, 2018), which make both *Nigella sativa*, *Inula viscosa* oils, and *Inula viscosa* extract probable and maybe a good alternative to look at instead of synthetic anti-inflammatory drugs in the healing of wounds by increasing metabolic activity, proliferation, and viability of tenocytes. Light-emitting diode [LED] is also reported to have a healing effect on tenocytes and rejuvenation of the skin (Opel et al., 2015; Seo, Park, Song, & Kwon, 2014). This study aimed to use natural products [olive oil, oleic acid, *Inula viscosa* oil, *Inula viscosa* extract, corn oil, and *Nigella sativa* oil] on injured sheep tenocytes and observe their effects on metabolic activity, proliferation, viability, and cell migration while comparing them to synthetic products such as paracetamol and naproxen sodium and LED.

2. Materials and methods

2.1. Preparation of sheep tenocyte cells

Tenocytes were isolated from hind limb Achilles tendon of sheep, obtained from a local slaughterhouse, aged 6–10 months as previously described (Alzyoud et al., 2019; Crockett, Centrella, McCarthy, & Grant Thomson, 2010). Briefly, tendon explants were sliced into small pieces with a size of about 1 mm^3 using a sterile scalpel and washed with phosphate buffer saline. Explants were cultured in Dulbecco's Modified Eagle's media [DMEM] (purchased from Thermofisher, UK) supplemented with penicillin and streptomycin and containing 5 % of fetal bovine serum [FBS] for one week using 75 cm^2 culture flasks, to allow cells to be released from the tissue into the culturing media and adhere to the flask at 37 °C and 5 % CO_2. After the release of cells [one week of culture], chopped pieces were removed. Cells were subcultured until they reached the third passage, then cells were harvested to be used in all experiments.

2.2. Preparation of treatments

Inula viscosa was collected from Zarqa city in Jordan in Al Hashimiyah municipality. *Inula viscosa* extraction was done according to the method published previously with some modifications (Merghoub, Amzazi, & Morjani, 2009). The plant was dried at room temperature for one week at the shadow, then it was grinded, and 20 g was added to 200 ml of absolute acetone for 72 hrs at room temperature with shaking, then the mixture was filtered using filter paper. After that, the solution was evaporated by a rotary evaporator at the boiling point of the solvent [acetone at 56 °C]. After evaporation, the obtained extract was diluted in dimethyl sulfoxide [DMSO] to get the concentration of 100 mg/ml stock solution. The solution was filtered by a 0.45 μm filter and then a 0.20 μm filter to get a sterile stock solution.

Oleic acid [50 μM] was prepared and used as described in previous work (Al-Shudiefat et al., 2013). Briefly, oleic acid [Sigma, USA], was diluted 1:1 with absolute filtered ethanol, then it was diluted one thousand times with sterile phosphate-buffered saline (PBS), a volume of 31.7 μL was taken for every ml of DMEM media. Olive oil [local store-Jordan], corn oil [local store-Jordan], *Nigella sativa* oil [Hemani Herbal/Pakistan], and *Inula viscosa* oil [Hemani Herbal/Pakistan], were prepared and used in the treatments the same way as oleic acid. 10 mM of naproxen sodium [Nopain commercial name, Hikma pharmaceutical Jordan] was used as described earlier (Aybar, Erkut, Yıldırım, & Bilir, 2004). Briefly, grinded 50 mg of naproxen sodium was dissolved in 5 mls DMSO and filtered using a 0.45 μm filter and then a 0.20 μm filter to get a sterile stock solution, from this stock solution 10 μL was added to each ml of media, so the final concentration of DMSO is 1 % (v/v). Paracetamol [commercial name Revanin, Hikma Pharmaceutical Jordan] was diluted in the same way as naproxen sodium and 10 μL also was added to each ml of media. Light-emitting diode [LED] was used as previously described (Alzyoud et al., 2019). A Photizo® device [Photon Therapy Systems LTD, 2012, South Africa] was used to generate LED with a single dose [4 J/cm2] that was applied to cells cultured on 24 well plates for 18 min after 24 hrs of seeding.

2.3. Alamar blue assay

Alamar blue [Invitrogen USA] was used to assess the metabolic activity, proliferation, and viability of tenocytes. It is based on the reduction of nonfluorescent Resazurin [blue] to the fluorescent dye resorufin [red] when it is entered viable cells, then the viability is

either measured by absorbance or fluorescent plate reader. Tenocytes were cultured in black 96 well plates with a black bottom and seeded with 10.000 cells per well. After 24 hrs. of seeding and attachment of tenocytes, 10 % v/v of Alamar blue was added to each well and incubated for 3 hrs. at 37 °C with 5 % CO_2 in dark. Two to three readings of Alamar blue were taken at zero time or beginning of treatment, 24 hrs, and 48 hrs. and these readings were recorded using a fluorescent plate reader [Bio-tekFLx800, USA] at an excitation /emission of 560 nm/590 nm filters. At the end of each Alamar blue assay reading, wells were washed with prewarmed media to remove any traces of Alamar blue and fresh media was added and cells were returned to the incubator.

2.4. Fluorescein diacetate [FDA]/propidium iodide PI [live/dead] stain

In FDA/PI stain, fluorescein diacetate is permeable in living cells and hydrolyzed in viable cells to fluorescent fluorescein with green color, while propidium iodide is not permeable in living cells and react with DNA fragments from dead cells to form a red fluorescent dye. Therefore, the cells colored green under the fluorescent microscope are living cells, while the ones colored red are dead cells. 4′, 6-Diamidino-2-Phenylindole [DAPI] purchased from Invitrogen was used as a counterstain, it forms a fluorescent dye with blue color. Both FDA/PI stains were purchased from Sigma-Aldrich. Cells were stained with FDA/PI according to the manufacturer's instructions and according to a previously published work (Zhang et al., 2014). Cells were cultured on round sterile cover slides inside a 6 well plate with seeding of 50,000 cells/3ml media/well. Media was aspirated off from cells and washed with PBS, then 1 ml of prewarmed [37° C] serum-free media was added to each well. 10 μL of FDA stock solution [20.8 μg/ml in acetone] and 10 μL of PI stock solution [2 mg/ml in distilled water] were added to each well. Cells were incubated for 30 min at 37°C and wrapped with aluminum foil, then media with FDA/PI were aspirated and a 500 μL of 10 % neutral buffered formalin [NPF] was added and incubated for 10 min at room temperature. Cells were washed three times with PBS and coverslips were removed from wells and a drop of DAPI stain was applied to each sample [SlowFade Diamond Antifade mountant with DAPI], and then the coverslips were inverted and placed on slides and left in a dark room for 2 hrs. at 4°C, cells were then analyzed using a fluorescent microscope. To assure that FDA/PI is working correctly, a negative control using 50 % DMSO that is toxic for tenocytes and kills them, making them appear in red is used and compared to the control without any treatment which should appear in green in color.

2.5. Scratch assay

The third passage of primary cell lines harvested from sheep Achilles tendon was used to prepare a cellular density of 200,000 cells per 2 ml/well in a 24 well plate and incubated them at 37 °C for 24 hr. to allow cell adherence. The procedure of scratch assay is adapted from (Alzyoud et al., 2019). All wells were scratched after checking confluency by a 100 μL sterile pipette tip simulating tissue injury as described previously (Liang, Park, & Guan, 2007). The scratched surface area was assessed at the scratch line at zero time, 24 hrs., and 48 hrs. using ImageJ software by two independent readers, and an average was calculated of their readings to measure the mean of the closed area after 48 hrs. for nine treatments [control, olive oil, oleic acid, corn oil, *Nigella sativa* oil, *Inula viscosa* oil, *Inula viscosa* extract, naproxen sodium, and paracetamol]. The higher the mean closed area is the better cell migration and healing of injured cells.

2.6. Experimental design

Sheep tenocytes were isolated from five different animals [n = 5 animals, two samples were taken from each animal, and each sample was repeated in triplicate]. The obtained *Inula viscosa* extract in DMSO [20 mg/ml] was optimized by taking five diluted concentrations in DMEM media from this stock [0.1 %, 0.5 %, 1 %, 5 %, and 10 %] to determine which concentration is the best to use in comparison to control. This was achieved by using Alamar blue assay on a 24 well plate with a cell density of 200,000 cells/well for 48 hrs., then Alamar blue readings were taken at zero time, and after 48 hrs. Each reading was taken after adding 10 % v/v Alamar blue to the 24 well plate and incubating for 3 hrs. in dark at 37 °C with 5 % CO_2, after that, the media from the 24 well plate was transferred to the black 96 well plate with black bottom for fluorescent plate reader at 560 nm and 590 nm excitation/ emission filters. Cells were assessed for their metabolic activity, proliferation, and viability in a black 96 well plate with black bottom over 24 hrs. using nine treatments [control, olive oil, oleic acid, corn oil, *Nigella sativa* oil, *Inula viscosa* oil, *Inula viscosa* extract, naproxen sodium, and paracetamol], to compare the effects of natural products and synthetic anti-inflammatory drugs on tenocytes using cell density of 10,000 cells/well in Alamar blue assay. Readings were taken at zero time, 24 hrs, and 48 hrs, but only the difference between zero and 24 hrs. readings were used in the analysis since the readings of 48 hrs. were revealed that cells were not in the exponential phase of growth. To test if oleic acid over 48 hrs. will give better than 24 hrs. for metabolic activity, proliferation, and viability of cells, and if LED treatment alone or in combination will affect cell viability, a 24 well plate was used with Alamar blue for 48 hrs. as described for *Inula viscosa* extract optimization.

The nine treatments were also tested for viability using FDA/PI staining and taking pictures using a fluorescent microscope. Two independent readers assessed live/dead cells in several fields for each treatment which was done at least in triplicate, and the average of the two readers was taken. The nine treatments were also tested for the healing and migration of tenocytes using the scratch assay. The migration process was assessed within 24 hrs. and 48 hrs. by measuring the closing of the scratch that was done in the middle of plate [distance between the scratch edges] for each treatment.

2.7. Statistical analysis

All data were analyzed using IBM SPSS version 25. Data were expressed as means within one standard error. Data normality was tested for all experiments based on the Kolmogorov Smirnov test. ANOVA one-way analysis was used for normally distributed data. P-value < 0.05 was considered significant.

3. Results

3.1. Optimization of Inula viscosa extract concentration

The results of testing diluted five concentrations of 0.1 %, 0.5 %, 1 %, 5 %, and 10 % in DMEM from a stock solution of *Inula viscosa* extract of 100 mg/ml in DMSO, using Alamar blue test on a 24 well culture plate over 48 hrs. with seeding of 200,000 tenocytes/well, are shown in Fig. 1.

Results showed that the only concentration of *Inula viscosa* extract that was comparable to control in terms of cells' metabolic activity, proliferation, and viability was 5 %, so it was used in our experiments. Results revealed a dose-dependent increase in cell viability in all concentrations 0.1 %, 0.5 %, 1 %, to 5 % which is the only concentration that is comparable to the control, and at 10 %

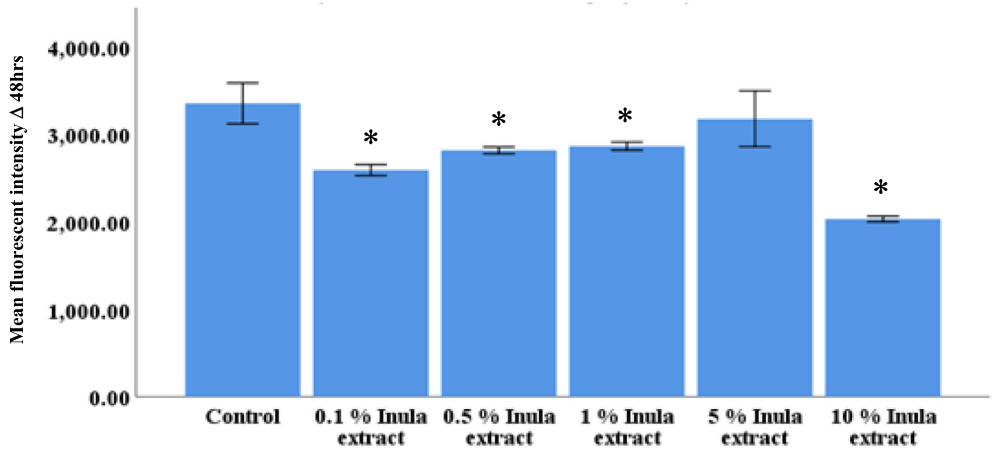

Fig. 1. Alamar blue viability test for sheep tenocytes over 48 hrs. in 24 well plates then transferred to black 96 well plate. Optimization of the best concentration of *Inula viscosa* extract to be used. 5 % was chosen since it is comparable to the control. The concentrations were taken from a stock solution containing 100 mg/ml in DMSO and diluted in DMEM media. * P-value < 0.05 considered significant from control.

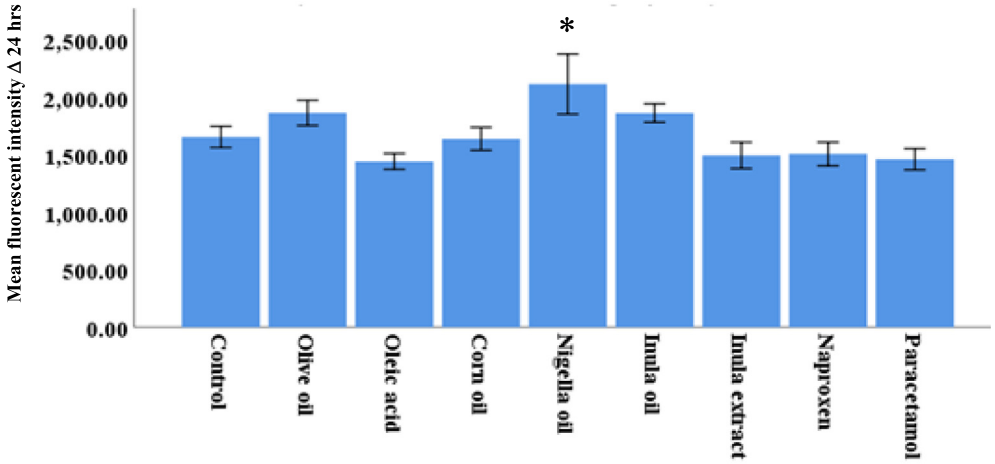

Fig. 2. Alamar blue viability test over 24 hrs in 96 well plate. Bars are represented with standard errors. * P value < 0.05 considered significant from control.

concentration, it decreased significantly. All other *Inula viscosa* extract concentrations 0.1 %, 0.5 %, 1 %, and 10 % were significantly lower than control cells in metabolic activity, proliferation, and viability [p values were, 0.002, 0.026, 0.041, and 8.43×10^{-7} respectively].

3.2. Effects of natural products VS synthetic drugs on tenocytes

The effects of six naturally occurring products [olive oil, oleic acid, corn oil, *Nigella sativa* oil, *Inula viscosa* oil, and *Inula viscosa* extract] compared with synthetic anti-inflammatory drugs [naproxen sodium and paracetamol] are shown in Fig. 2.

Results showed that the only treatment that has increased cell metabolism, proliferation, and viability significantly compared to control over 24 hrs. is *Nigella sativa* oil [p value is 0.020]. Both olive oil and *Inula viscosa* oil increased cell migration, proliferation, and viability compared to control but not significantly.

3.3. Effect of oleic acid for 48 hrs. VS LED alone and with the combination on tenocytes

The effects of oleic acid over 48 hrs. compared to LED alone and with the combination are shown in Fig. 3.

Oleic acid, LED, and a combination of both increased cell metabolism, proliferation, and viability significantly over 48 hrs compared to control [P values were 0.000019, 0.007, and 0.036 respectively].

3.4. FDA/PI staining quality assurance

The quality of FDA/PI staining was assured using 50 % DMSO treatment for cells as the negative control, all cells were killed by this toxic concentration and all cells took propidium iodide and appeared red compared to control viable cells which took FDA stain and appeared in green color. Results are showed in Fig. 4.

3.5. FDA/PI staining for natural products treated cells vs synthetic drugs

The effects of natural products compared to synthetic drugs on tenocytes viability are done in triplicates for each treatment and read by two independent readers and the average of their readings are quantitively shown in Fig. 5.

FDA-PI viability test showed a significant increase in the viability of the tenocytes treated with olive oil compared to control [p value is 0.037], while viability was decreased significantly with

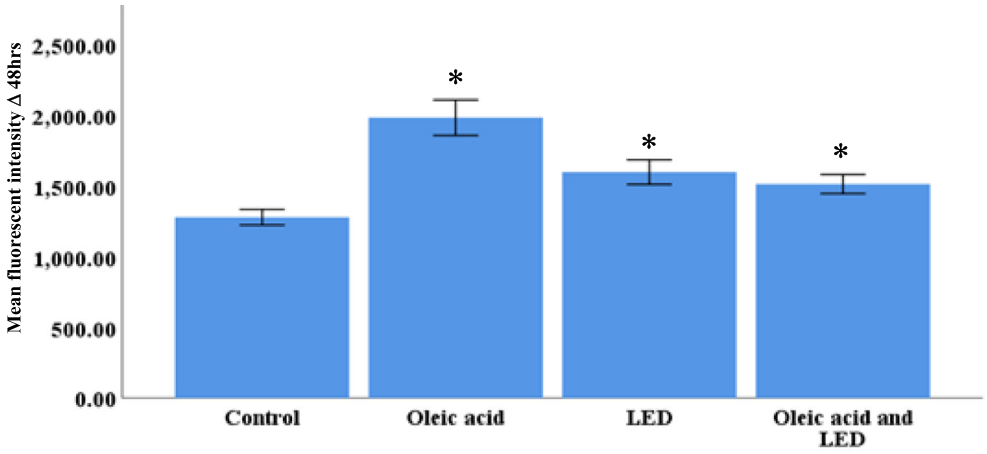

Fig. 3. Alamar blue viability test for sheep tenocytes over 48 hrs. in 24 well plate then transferred to black 96 well plate. Data Means are represented with standard errors. * P value < 0.05 considered significant from control.

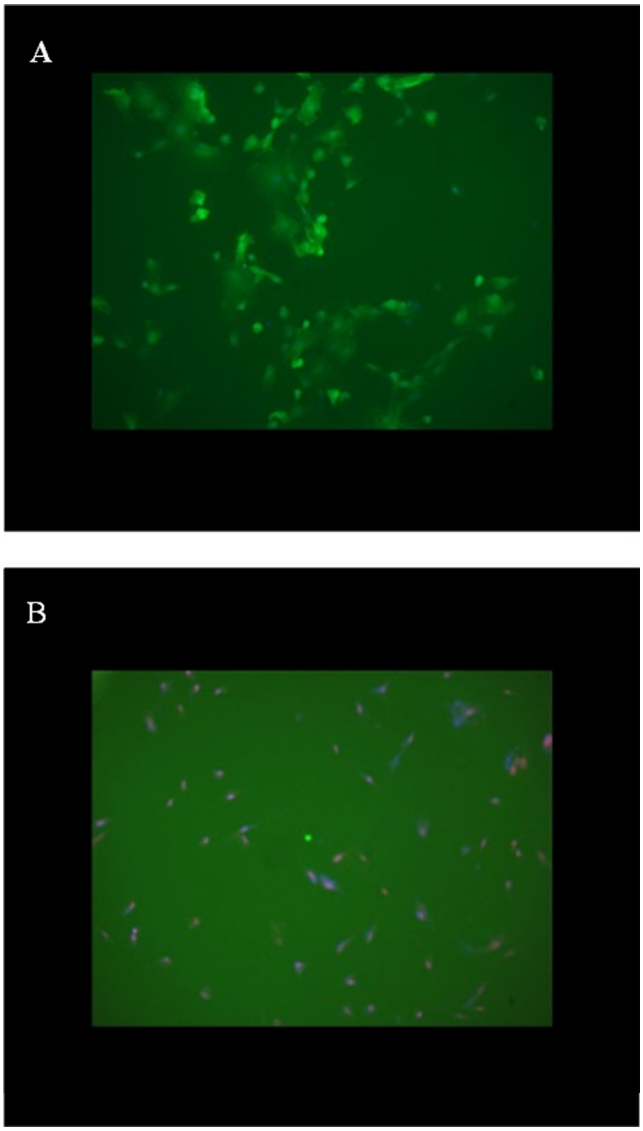

Fig. 4. Fluorescein diacetate [FDA]/propidium iodide PI [live/dead] stain on 6 well plate. A-Control: sheep tenocytes without treatment, most of the cells are viable and appear green with FDA stain. B-Negative control: sheep tenocytes are treated with a toxic agent that kills all cells [50 % DMSO], cells are dead and appear red with PI stain.

Inula viscosa oil and *Inula viscosa* extract [p values are 0.000001 and 0.000051 respectively].

3.6. Scratch assay at zero and after confluency

Pictures at different time points were captured at zero time, 24 hrs., and 48 hrs. a picture of zero time and another after the closure of scratch is shown in Fig. 6.

3.7. Quantitative assessment of scratch line over 48 hrs. Using ImageJ software

The mean closed areas of nine treatments including natural products and synthetic drugs effects on the migration of tenocytes are shown in Fig. 7.

Inula viscosa oil, *Inula viscosa* extract, and paracetamol increased cell migration and closure of the scratch line over 48 hrs, significantly compared to control [P values 0.012, 0.000010, and 0.014 respectively], while oleic acid significantly decreased cell migration and closure of scratch line over 48 hrs when compared to control.

4. Discussion

Inula viscosa extract is the only treatment that was extracted and prepared in our lab and was not bought commercially, therefore, its toxicity on cells was determined empirically using different concentrations, while the rest treatments were commercially available and their proper concentrations were determined from previous work in literature. The optimum concentration of *Inula viscosa* extract was determined to be 5 % because it was the only concentration that did not decrease the metabolic activity, proliferation, and viability of cells. Preparations of olive oil, corn oil, *Nigella sativa* oil, and *Inula viscosa* oil, were similar to the preparation of 50 µm of oleic acid used in previous work (Al-Shudiefat et al., 2013). The preparation of paracetamol was similar to the preparation of 10 mM naproxen which was used previously (Aybar et al., 2004). LED specification treatment was adapted from a previous study (Alzyoud et al., 2019). Two different assays were used for viability test, namely Alamar blue and FDA/PI stains, which detect viability with different mechanisms, therefore they gave extensive study about the effect of different treatments on viability of sheep tenocytes using different techniques. The only treatment in Alamar blue assay on black 96 well plate with black bottom over 24 hrs. that increased metabolic activity, proliferation, and cell viability

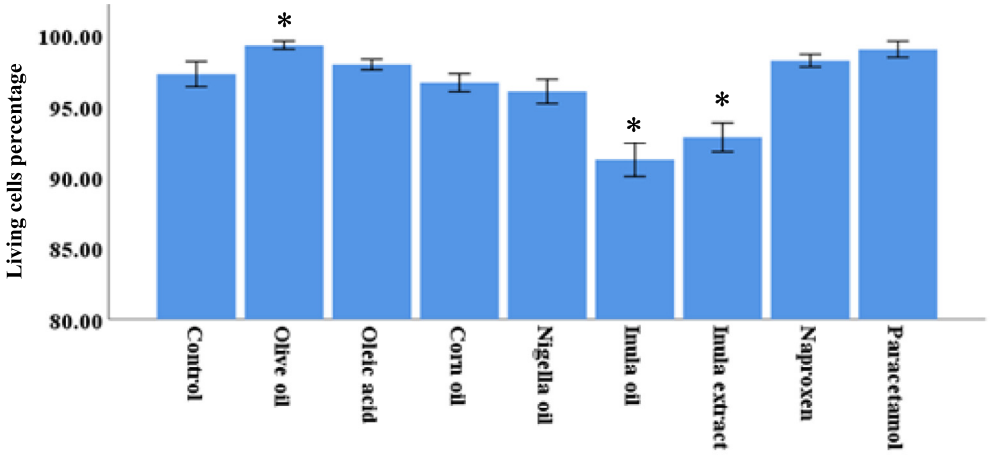

Fig. 5. FDA-PI viability test for sheep tenocytes. Treatments were repeated at least in triplicate. Two independent readers counted living cells [green] and dead cells [red] cells in each field and the means of the two readings were calculated. Values represent means ± SE. * P value < 0.05 considered significant from control.

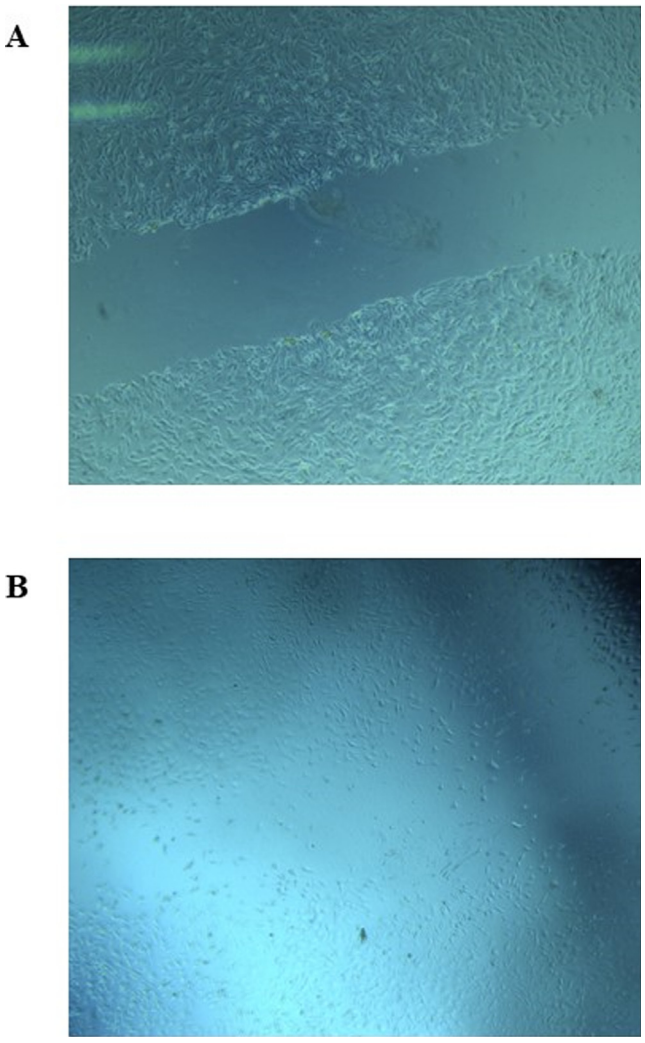

Fig. 6. Scratch assay test on 24 well plate. A- the scratch line was made using a 100 µL pipette tip with 90 % confluent sheep tenocytes B-closing of the scratch line after 48 hrs.

were *Nigella sativa* oil [Fig. 2], and this may be due to the several beneficial properties of *Nigella sativa* oil, which include anticancer, antioxidant, antimicrobial, analgesic and anti-inflammatory effects (Hadi et al., 2016). Although the other treatments including olive oil, oleic acid, *Inula viscosa* oil, and *Inula viscosa* extract have antioxidant and anti-inflammatory properties, they don't show significant enhanced metabolic activity, proliferation, and viability of tenocytes over 24 hrs., and this is a limitation of the study due to limited resources to repeat it for longer period like 48 hrs. with suitable conditions. This opinion is strengthened by the results in Fig. 3, in which cells were treated with oleic acid for 48 hrs. on 24 well plate with a larger media volume still show the exponential phase of growth and significant increase of metabolic activity, proliferation, and viability of tenocytes. And this result coincides with a previous study that oleic acid prevented tumor necrosis factor alpha-induced cell death and restored viability to normal (Al-Shudiefat et al., 2013). Oleic acid alone, LED alone or their combination also showed a significant increase in metabolic activity, proliferation, and viability of tenocytes. In a previous study, a combination of LED and platelet-rich plasma increased tenocytes viability significantly, and LED alone was comparable to the control (Alzyoud et al., 2019). In FDA/PI Fig. 4, we used a negative control and control without treatment to assure that the staining procedure is working, the negative control gave red color to all cells, which means that all cells took PI dye, and they are dead cells, while control cells without treatment, took the FDA stain and appeared in green color, which means that most of the cells are viable and living/dead staining is working. Only olive oil increased tenocytes viability over 48 hrs. [Fig. 5], which is similar to a previous study that olive oil increased significantly keratinocytes viability when exposed to UV light and prevented skin damage (Salucci et al., 2017). Another study showed that olive oil mixed with beeswax and butter were improved wound healing in rats (Bayir et al., 2019). *Inula viscosa* oil and *Inula viscosa* extract decreased tenocytes viability significantly compared to control, and this finding was similar to a previous study that *Inula viscosa* decreased cell viability in cancer cells (Messaoudi et al., 2016). Despite *Inula viscosa* oil and *Inula viscosa* extract reduced proliferation, metabolic activity, and viability of tenocytes, they increased significantly alongside paracetamol, the migration of tenocytes and helping in the closing of the scratch line [Fig. 7]. And these results are in

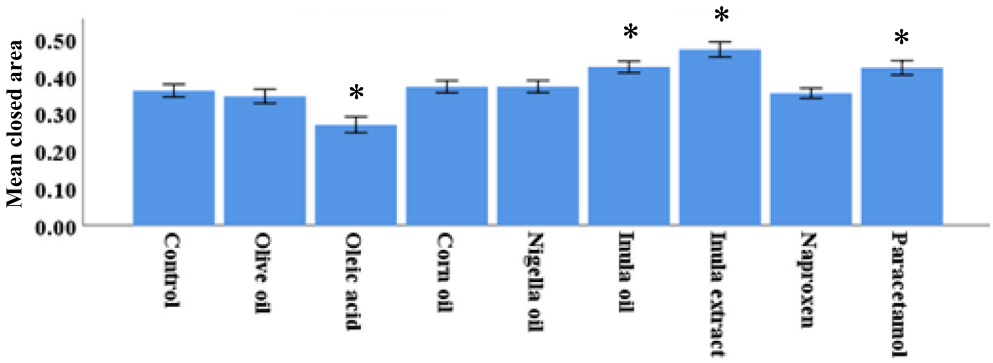

Fig. 7. Scratch assay over 48 hrs. on 24 well plate. Data are represented with standard errors. * P value < 0.05 considered significant from control.

agreement with previous work which revealed that *Inula viscosa* increased the migration of cells and wound healing in mice (Khalil, Afifi, & Al-Hussaini, 2007). Paracetamol enhanced tenocytes migration significantly and this result is similar to the findings published previously that wound healing is improved significantly by using paracetamol in athletes and this may be due to its anti-inflammatory properties (Bourne, 1980).

5. Conclusion

In conclusion, *Nigella sativa* oil increased the metabolic activity, proliferation, and viability of tenocytes significantly over 24 hrs., while oleic acid, LED, and their combination increased these activities significantly over 48 hrs. in Alamar blue viability test. Furthermore, Olive oil increased tenocytes viability significantly in FDA/PI assay. Study data suggest a potential role of *Nigella sativa* oil, olive oil, oleic acid, LED, and a combination of LED and oleic acid for the metabolic activity, proliferation, and viability of tenocytes, but not their migration. Moreover, *Inula viscosa* oil, *Inula viscosa* extract, and paracetamol increased tenocytes migration significantly but not their proliferation. Therefore, these natural compounds may be played as a good alternative natural way for tendinopathy therapy instead of synthetic drugs or other invasive procedures. One limitation of the study was that primary cell cultures are restricted by cell senescence. Future recommendations include investigation of olive oil, *Nigella sativa* oil, *Inula viscosa* oil, *Inula viscosa* extract, and corn oil effect on tenocytes for longer periods. Another recommendation is to explore *Inula viscosa* oil and oleic acid effects on tenocytes at the genetic level using 3D cell culture.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Note

Fig. 4 **and** Fig. 6 **should be colored (stained cells)**

Authors contributions

Conceived and designed experiments: ASA, JA. Performed the experiments: SA, YB, BA. Analyzed data: ASA, JA, ST. Contributed reagents/materials/analysis tools: YB, BA, SA. Wrote the paper: ASA, JA, ST.

All authors provided final approval of the final version of the manuscript

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