Original Article

Comparative Study of the Efficacy of Low-Level Laser Therapy and Myrrh Oil on Healing of Oral-Induced Mucosal Ulcer in Rats

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Abstract

Background: Oral mucosa refers to the mucous membrane that lines the tissues within the oral cavity. Wound healing is an essential physiological process that involves the collaboration of numerous cell strains and their products to restore a lesion caused by a local aggressor. Low-level laser therapy (LLLT) is the application of light to a living system to stimulate tissue regeneration, reduce inflammation, and alleviate pain. Myrrh is an herbal product that was used in the past in treatment of wounds. **Objectives:** This study seeks to determine the efficacy of LLLT and Myrrh oil in promoting mucosal ulcer healing in rats. **Materials and Methods:** Fifty-four adult male albino rats (Rattus norvegicus) of about 250–300 mg of weight and age of about 2–3 months were used in this experimental study. The traumatic ulcer with 8 mm diameter, and 1 mm in depth was made on the right cheek mucosa by using round diamond bur where the control group left healed normally. The animals were sacrificed by overdose of general anesthesia at the end of healing periods 1, 3, and 7 days after ulceration. **Results:** Histological findings of myrrh oil and LLLT groups showed more epithelization; reduce inflammation, increase angiogenesis, and decrease healing time in comparison to control group. Histochemical finding showed significant difference of collagen fibers synthesis and extracellular matrix formation in myrrh oil and LLLT group in comparison to control group. **Conclusion:** The study revealed that the myrrh oil and LLLT were more effective in the enhancement of induced ulcer healing when compared to the control group.

Keywords: Histological evaluation, low-level laser therapy, myrrh oil, wound healing

Introduction

The three forms of oral mucosa have different histological, clinical, and functional characteristics. Masticatory mucosa, lining, or movable mucosa and specialized mucosa that can be either keratinized or nonkeratinized.[1] Histologically, the oral mucosa is composed of three layers: the oral epithelium, a stratified squamous type, which is either nonkeratinized and contains Melanocytes, Langerhans cells, lymphocytes, and Merkel cells, or keratinized and includes stratum basale, stratum spinosum, stratum granulosum, and stratum corneum. Lamina propria is a layer of underlying connective tissue consist of loose connective tissue (papillary layer) and a deeper layer of dense, irregular connective tissue known as (reticular layer). Submucosa is dense, irregular connective tissue that might not be present, and the lamina propria binds the mucosa to either bone or muscle.[2]

The growth factors that promote cell proliferation during the simple linear process of wound healing integrate dynamic changes involving soluble mediators, blood cells, the creation of the extracellular matrix, and the proliferation of parenchymal cells.[3] The following steps of biochemical events in wound healing can be identified Hemostasis, inflammatory reaction, cell proliferation, and synthesis of the elements which make up the extracellular matrix, called remodeling.[4]

In Saudi Arabia, the well-known plant myrrh is frequently used as a homemade medicine.[5] It is known as "mur" in Arabic, which means bitter. It is the myrrh tree's gum, and

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the oil it contains is known as oleoresin. It is known as "Mur Makki" because it notably originates from Mecca.[6] Myrrh has been used in the treatment of wounds in the past, and studies have shown that it can hasten the healing process due to faster wound contraction, re-epithelization, early neovascularization, and increased collagen density, which also has anti-inflammatory[7] and antibacterial properties.[8]

Biostimulation has been caused by low-level laser irradiation in clinical settings.[9] According to studies, laser therapy (photobiostimulation) can treat a variety of illnesses and physical ailments at the cellular and molecular levels.[10] Numerous pathological disorders have been discovered to be significantly improved by phototherapy, including the reduction of inflammation and the stimulation of nonheating wound healing.[9] At the molecular, cellular, and tissue levels, Low-level laser therapy (LLLT) has positive effects. It has been discovered that LLLT therapy at different intensities has a stimulating effect on cellular activities.[11] LLLT has the ability to inhibit cell apoptosis, stimulate mitochondrial activity, accelerate cell turnover, recruit new cells, promote cell growth, and alter cellular metabolite levels.[12]

Materials and Methods

Fifty-four animals were randomly divided into three equal groups: Myrrh oil group, LLLT group, and control groups (n = 18 for each group). All the groups were subdivided into three healing intervals 1, 3, and 7 days (six rats from each group for each interval). A round diamond bur with 15,000 rpm speed was used to induce traumatic ulcer on the right cheek mucosa 8 mm in size.

The general anesthetic solution was administered through intramuscular injection (IM) of xylazine 2%, (0.3 mL/kg body weight [BW]) and ketamine 10%, (0.1 mg/kg BW). Before the surgery, all surgical tools and surgical towels were sterilized in an autoclave at 121°C and a pressure of 15 bar/cm^2 for 30 min. The digital vernia was used to measure the desired ulcer size, and a bur stopper was positioned on the surgical bur. Control group: the ulcer was treated once daily with single topical dose of 10 µL sterilized distilled water. LLLT group: laser group treated with daily application of (810 nm, 0.6 w, pulsating, 10 Hz) laser beam away from the ulcer, with slow movement of hand and about 30 s. Myrrh oil group: the ulcer was treated once daily with single topical dose of 10 µL of 1 µg/mL of myrrh oil by micropipette.[13] The animals were sacrificed by overdose of general anesthesia at the end of healing periods 1, 3, and 7 days after ulceration to prepare ulcer specimens for histological and histochemical analyses.

The specimens were fixed in 10% formalin solution, then embedded in paraffin and the samples were sectioned in thin slices (5 µm) for slide preparation. Finally, hematoxylin and eosin (H&E) staining was carried out for histological assessment under a light microscope.[14]

Histological analysis was done by counting the mean of the recovery ulcer size in all studied groups. Histochemical analysis was done by Vangieson stain to organize the collagen fiber deposition on days 1, 3, and 7 of healing periods in all studied groups. The mean was measured by ImageJ.exe, a 64-bit version of Java.[15]

Statistical analysis

The detailed description for each variable was made on the Statistical Package for Social Sciences (SPSS) version 25. Only serial numbers were related to the details of the participants and the collected data were managed on daily base. Data were expressed using mean, standard deviation, standard error, and 95% confidence interval, minimum and maximum levels. Because of not normally distributed data, Kruskal–Wallis test was used to assess the association between the studied nonnormally distributed variables instead of ANOVA test. A confidence level of 95% with P-value equal to or less than 0.05 was considered significant.

Ethical approval

The experiment was done in accordance with the animal experimentation ethical principles of College of Dentistry, University of Baghdad (No. 491).

Results

Wound contraction size

Table 1 showed descriptive statistics of mean of final ulcer size and mean percentage of recovery ulcer size in all the studied groups during the healing periods as well as multiple group comparison according to ulcer size by post hoc test. Table 1A illustrated the size of induced ulcers decrease with time in all the studied groups, and the least ulcer size was recorded in the oil group at 7 days. Also, Table 1A showed group comparisons of differences in ulcer size for all healing intervals by the Kruskal–Wallis test. The result revealed a nonsignificant group difference on the 1st day and a significant group difference on both the 3rd and 7th days. Table 1B illustrated an increase in the mean percentage value of recovery ulcer size in all the groups, and the lowest mean value was recorded in the oil group at 7 days. In addition, Table 1B showed group comparisons of differences in recovery ulcer size for all healing intervals by Kruskal–Wallis test. The result revealed a nonsignificant group difference on the 1st day and a significant group difference on both the 3rd and 7th days. While multiple group comparisons of ulcer size by post hoc test illustrated significant differences between the oil and control groups on both the 3rd and 7th days and also between the control and laser groups on the 3rd day, as shown in Table 1C.

Table 1: A: Descriptive statistics of mean of final ulcer size, B: mean percentage of recovery ulcer size in studied groups in all healing periods, and C: multiple group comparison according to ulcer size by post hoc test

Day	Group	*N*	Mean	SD	Mean rank	Chi-square	*P* value
A							
Day 1	Oil	6	7.68	0.1	9.17	0.541	0.763
	Laser	6	7.73	0.2	10.75		
	Control	6	7.62	0.3	8.58		
Day 3	Oil	6	6.08	0.4	5.42	11.401	0.003*
	Laser	6	6.28	0.3	7.75		
	Control	6	7.03	0.3	15.33		
Day 7	Oil	6	1.6	0.9	6.5	6.902	0.032*
	Laser	6	1.92	0.4	7.92		
	Control	6	2.97	0.7	14.08		
B							
Day 1	Oil	6	4	1.8	9.83	0.541	0.763
	Laser	6	3.3	2.7	8.25		
	Control	6	4.8	3.8	10.42		
Day 3	Oil	6	24	4.4	13.58	11.401	0.003*
	Laser	6	21.5	4	11.25		
	Control	6	12.1	3.7	3.67		
Day 7	Oil	6	80	10.8	12.5	6.902	0.032*
	Laser	6	76	5.1	11.08		
	Control	6	62.9	9.1	4.92		
C							
Day	**Groups**	**Mean difference**	***P* value**				
Day1	Oil/laser	1.42	NS				
	Oil/control	7.58	NS				
	Laser/control	6.16	NS				
Day 3	Oil/laser	−2.33	1.000				
	Oil/control	−9.91	0.004*				
	Laser/control	−7.58	0.041*				
Day 7	Oil/laser	−1.42	1.000				
	Oil/control	−7.58	0.040*				
	Laser/control	−6.16	0.134				

NS: not significant.

*Significant result

Inflammatory cells

Table 2A showed the inflammatory cells score lied between moderate (score 3) and severe (score 4) for all studied groups in three healing intervals. The result showed the highest mean value of inflammatory cells recorded in the 1st day in the myrrh oil group and the lowest mean value in the same group on the 7th day duration. Table 2B showed group comparisons of the difference in the mean of inflammatory cells for all healing intervals by the Kruskal–Wallis test. The result revealed significant group differences in the 1st and 7th days and nonsignificant group differences in the 3rd and 4th days. While Table 2C showed multiple group comparison differences in the mean of inflammatory cells by post hoc test, the significant difference between the oil and control groups on the 1st and 7th days of healing intervals.

Epithelial thickness

Table 3A showed an increase in the mean value of epithelial thickness in all the groups with time; the lowest mean value was recorded in the control group on the 1st day, and the highest mean value was recorded in the laser group on the 7th day of healing intervals. A highly significant difference was observed between the studied groups on days 1, 3, and 7 of healing intervals (*P* value 0.000). Table 3B showed multiple comparisons between the groups according to epithelial thickness in all healing periods by using the post hoc test. The result showed a highly significant difference in epithelial thickness between the laser and control groups and between the oil and control groups in the 1st, 3rd, and 7th days of healing intervals.

Table 2: A: Distribution according to score of inflammatory cells,[16] B: comparison of the studied groups by Kruskal–Wallis test as well as C: multiple group comparison according to inflammation by post hoc test

Day	Group	Score	Frequency, $N = 6$	Percent (%)	Mean	SD
A						
Day 1	Oil	0	0	0	32.1	8.9
		1	0	0		
		2	1	16.7		
		3	5	83.3		
	Laser	0	0	0	29.6	9.6
		1	0	0		
		2	3	50		
		3	3	50		
	Control	0	0	0	22.8	1.3
		1	0	0		
		2	6	100		
		3	0	0		
Day 3	Oil	0	0	0	22.9	3.2
		1	0	0		
		2	4	66.7		
		3	2	33.3		
	Laser	0	0	0	25.2	3.3
		1	0	0		
		2	2	33.3		
		3	4	66.7		
	Control	0	0	0	24.8	1.4
		1	0	0		
		2	4	66.7		
		3	2	33.3		
Day 7	Oil	0	0	0	19.4	3.4
		1	0	0		
		2	5	83.3		
		3	1	16.7		
	Laser	0	0	0	23.1	2.9
		1	0	0		
		2	4	66.7		
		3	2	33.3		
	Control	0	0	0	26.6	2.1
		1	0	0		
		2	2	33.3		
		3	4	66.7		

B

Day	Group	Mean rank	Chi-square	*P* value
Day 1	Oil	13.17	6.712	0.035*
	Laser	10.08		
	Control	5.25		
Day 3	Oil	7.25	1.923	0.382
	Laser	11.5		
	Control	9.75		
Day 7	Oil	4.83	9.055	0.011*
	Laser	9.58		
	Control	14.08		

Table 2: Continued

C

Day	Groups	Mean difference	*P* value
Day1	Oil/laser	2.5	0.951
	Oil/control	9.3	0.031*
	Laser/control	6.8	0.350
Day 3	Oil/laser	−2.3	NS
	Oil/control	−1.9	NS
	Laser/control	0.4	NS
Day 7	Oil/laser	−3.7	0.367
	Oil/control	−7.2	0.008*
	Laser/control	−3.5	0.430

*Significant result

Table 3: A: Descriptive statistics of the mean of epithelial thickness on days 1, 3, and 7 and B: multiple group comparison according to epithelial thickness by post hoc test

Day	Group	*N*	Mean	SD	Mean rank	Chi-square	*P* value
A							
Day 1	Oil	6	190.8	21.3	12.9	18.536	0.000*
	Laser	6	187.3	34.9	11.5		
	Control	6	24.5	40.8	4.1		
Day 3	Oil	6	214.2	19.4	13.5	15.339	0.000*
	Laser	6	199.9	20.8	11		
	Control	6	55.7	22.5	4		
Day 7	Oil	6	301.6	46.3	11.4	13.262	0.000*
	Laser	6	310.6	14.9	11.3		
	Control	6	155.2	46.7	5.8		

B

Day	Groups	Mean difference	*P* value
Day 1	Oil/laser	3.5	0.614
	Oil/control	166.3	0.000*
	Laser/control	162.8	0.000*
Day 3	Oil/laser	14.3	0.118
	Oil/control	158.5	0.000*
	Laser/control	144.2	0.000*
Day 7	Oil/laser	−9	0.227
	Oil/control	146.4	0.000*
	Laser/control	155.4	0.000*

*Significant result

Collagen density

Table 4A illustrated an increase in the mean value of collagen density in all the groups on days 3 and 7, with the lowest mean value recorded in the control group at the 3rd day and the highest mean value recorded in the laser group on the 7th day of healing intervals. In addition, Table 4A showed group comparisons of the difference in collagen density in the 3rd and 7th days of healing intervals by the Kruskal–Wallis test. The result revealed significant group differences on the 3rd and 7th days of healing periods. Table 4B showed multiple comparisons between the groups according to collagen density in both healing periods by using the post hoc test. The result showed a highly significant difference in collagen density between the laser and control groups in 3 days and a significant difference in day 7 of healing periods for the same groups. Table 4C showed an increase in blood vessels in all the studied groups during healing intervals, but with a nonsignificant group difference where the highest value was recorded in the myrrh oil group on the 7th day and the lowest value was recorded in the control group on the 1st day.

Histological and histochemical findings

Figure 1 reveals the histological and histochemical findings of the present study. The histological view of the control group at 7 days after ulceration showed newly formed thin epithelium without rete ridges on the

Table 4: A: Descriptive statistics of the mean of collagen fibers in studied groups in all healing periods and B: multiple group comparison according to collagen density as well as C: descriptive statistics of mean of blood vessels in studied groups in all healing periods

Day	Group	*N*	Mean	SD	Mean rank	Chi-square	*P* value
A							
Day 3	Oil	6	37.1	15.9	8.83	6.327	0.042*
	Laser	6	49	13.1	13.67		
	Control	6	28.7	6.8	6		
Day 7	Oil	6	64.4	24.7	9.33	10.889	0.004*
	Laser	6	83.6	8.4	14.67		
	Control	6	51.5	9.3	4.5		
B							
Day	**Groups**	**Mean difference**	***P* value**				
Day 3	Oil/laser	−11.9	0.351				
	Oil/control	8.4	1.000				
	Laser/control	20.3	0.039*				
Day 7	Oil/laser	−19.2	0.251				
	Oil/control	12.9	0.351				
	Laser/control	32.1	0.003*				
C							
Day	**Group**	***N***	**Mean**	**SD**	**Mean rank**	**Chi-square**	***P* value**
Day 1	Oil	6	7.4	0.8	10.92	0.764	0.683
	Laser	6	7.3	1.4	9.33		
	Control	6	5.1	2	8.25		
Day 3	Oil	6	11.7	2.4	11.92	2.881	0.237
	Laser	6	10.7	3.7	9.83		
	Control	6	7.5	3.9	6.75		
Day 7	Oil	6	13.2	2.1	10.08	0.91	0.634
	Laser	6	12.7	3.8	10.58		
	Control	6	9.5	3.1	7.83		

*Significant result

ulcer surface [Figure 1A] (red arrow), H&E stain (×10). Vangieson stain of control group showed the formation of collagen fibers with granulation tissue in the lamina propria which contains congested blood vessels [Figure 1B] (red arrow), Vangieson stain ×40. The histological view of the ulcer treated by myrrh oil at 7 days revealed a well-defined keratinized squamous epithelium. Lamina propria showed granulation tissue with signs of collagen fiber remodeling, numerous blood vessels, and a reduction in inflammatory cell infiltration [Figure 1C] (red arrow), H&E stain (×10). Vangieson stain of myrrh oil group showed significant quality of collagen fibers in the positive reaction [Figure 1D] (red arrow), Vangieson stain ×40. The histological view of the ulcer treated by laser group at 7 days revealed easily identified epithelial layer and demarcated basal cell layer, differentiation of keratinocytes to keratin layers, a decrease in the number of inflammatory cells, and an increase in the number of blood vessels adjacent to granulation tissue [Figure 1E] (red arrow), H&E stain (×10). Vangieson stain showed a large area of defect occupied by new collagen fibers [Figure 1F] (red arrow), Vangieson stain ×40.

Discussion

Traumatic ulcers are wounds to the oral mucosa brought on by mechanical or physical trauma, including eating sharp foods, accidentally biting down while chewing, biting while speaking, being punctured by sharp objects, and having teeth that are broken, misshapen, or decayed.[17] Due to its effects on accelerating wound contraction, re-epithelialization, early neovascularization, and increased collagen density, myrrh can speed up wound healing.[7] LLLT can hasten the healing of wounds, speed up remodeling, and enhance the quality of the tissue that is healed.[18] The current study aimed to examine the impact of LLLT and myrrh essential oil on the healing of oral mucosal traumatic ulcers.

The mean percentage of recovery ulcer size grew with time in the current study across all analyzed groups, with the control group recording the lowest mean value as compared to the Myrrh oil and LLLT groups. This speeding up of tissue repair may be caused by LLLT's biostimulation effects, which enhance local circulation, cell proliferation, and collagen formation. Thus,

improved granulation, early tissue epithelialization, better fibroblast cell matrix formation, and quicker neovascularization of newly created tissue have all been noted.[19] Myrrh oil contains antibacterial properties, speeds up wound contraction, and prevents postoperative hemorrhage.[20]

In the current study, inflammatory cells were analyzed histologically and histomorphometrically to reveal varying degrees in each healing interval. The effects of LLLT on tissue regeneration, where it reduces anti-inflammatory response, may explain why the experimental groups showed a drop in their inflammatory response over time while the control group showed an increase.[19] Myrrh oil exhibits anti-inflammatory[7] and antibacterial properties[8] that are not present in the control group. This is in agreement with the study by Su *et al.*,[20] who used myrrh water extract (MWE), frankincense water extract (FWE), and combined of them extract (CWE) to evaluate the anti-inflammatory effects by utilizing the paw edema mice induced by formalin and carrageenan. The results showed that MWE and the CWE at the 3.9 g/kg, and 5.2 g/

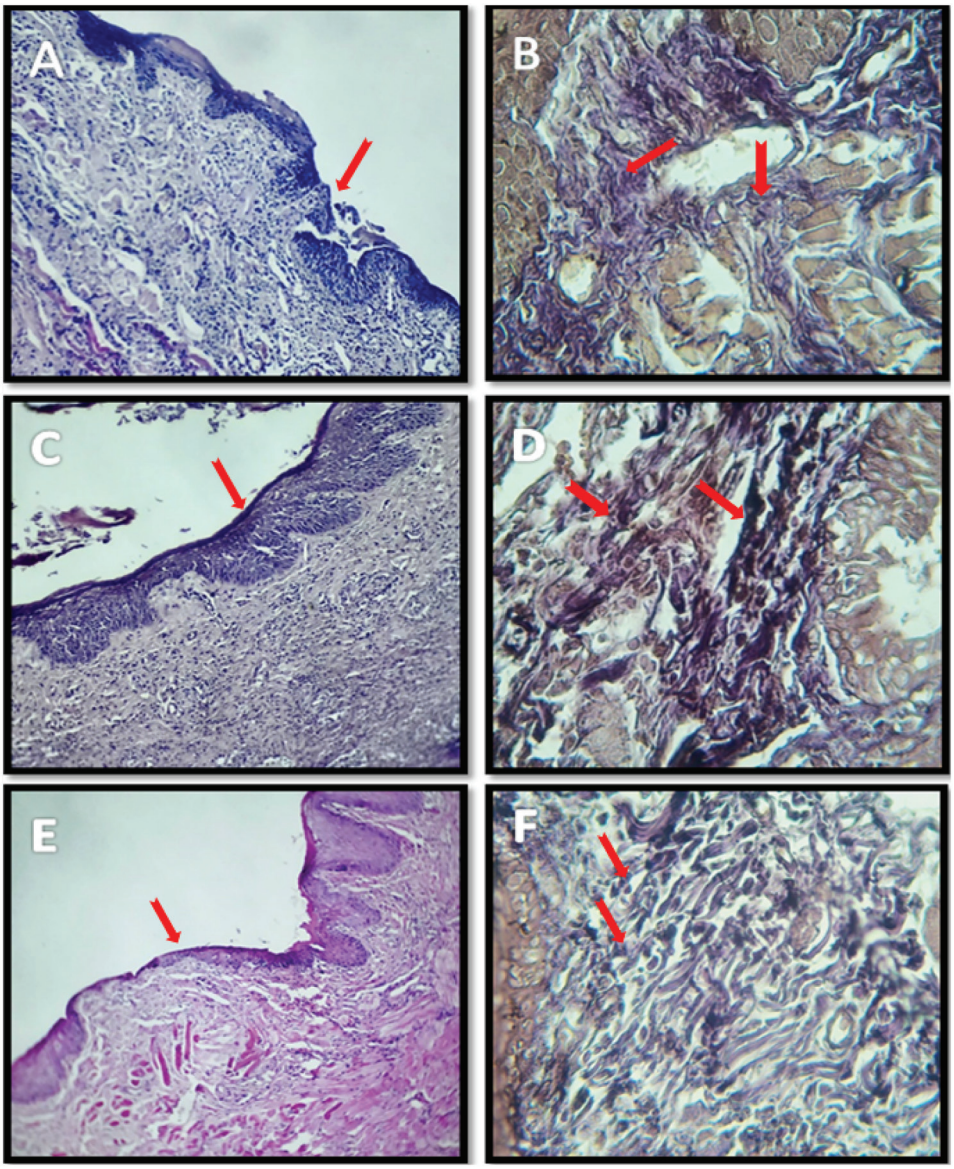

Figure 1: The histological and histochemical findings. A: The control group at 7 days after ulceration showed newly formed thin epithelium without rete ridges on the ulcer surface (red arrow), H&E stain (×10); B: Vangieson stain of the control group showed the formation of collagen fibers with granulation tissue in the lamina propria (red arrow), Vangieson stain ×40; C: ulcer treated with myrrh oil at 7 days revealed (red arrow), H&E stain (×10); D: Vangieson stain of myrrh oil group showed significant quality of collagen fibers in the positive reaction (red arrow), Vangieson stain ×40; E: ulcer treated by laser group at 7 days revealed an easily identified epithelial layer and a demarcated basal cell layer, differentiation of keratinocytes, a decrease in the number of inflammatory cells, and increase in the number of blood vessels (red arrow), H&E stain (×10); F: Vangieson stain showed a large area of defect occupied by new collagen fibers

kg showed inhibition of formalin-induced paw edema with inhibition rates of 30.44%, and 23.50%, respectively, and this is due to the anti-inflammatory effects of myrrh extract. Also in agreement with the study by Kamil and Al-Ghaban,[13] who improved the anti-inflammatory effects of myrrh when compared to the control group by using myrrh oil to treat skin wound healing. This study agrees with Tabakoglu *et al*.,[19] in that it employed an 808nm GaAlAs diode laser to evaluate the healing of a circular wound in rat skin and discovered that the inflammatory response subsided over time after using LLLT.

The neovascularization in the current study was clearly assessed with some variation between the experimental (MO and LLLT) and control groups. In comparison to the control group, the oil group recorded the greatest mean value of blood vessels on the 1st and 3rd days, though it was close to the mean value of the LLLT group. All of the groups experienced angiogenesis growth on the 7th day, although the LLLT group had the greatest mean value when compared to the control group. Last but not least, the current study demonstrated that experimental groups (MO and LLLT) had more angiogenesis than the control group, which is agreement with the study by Al-Mobeeriek,[21] who treated an intraoral mucosal ulcer with myrrh powder diluted with sodium chloride and discovered that this improved the anti-inflammatory activity of myrrh when compared to the control group. Also, in agreement with the study by Allameh *et al*.[22] who used LLLT and fibroblastic growth factor injection on mucosal wound healing in rat experimental model and found that laser illumination can improve angiogenesis, cell proliferation, and collagen synthesis by increasing cell access to ATP.

Re-epithelization is an attempt to seal the wound during the healing process by basal and suprabasal cell migration and proliferation. The current study demonstrated that both control and experimental groups' re-epithelialization grew throughout time and peaked at a high mean value on day 7. Because of increased epithelial cell proliferation and progression, as well as an increase in the amount of neovascularization, fibroblast cells, and collagen fiber in the myrrh oil and LLLT groups, the re-epithelialization process occurred more quickly in the Myrrh oil group on days 3 and 7, which was nearly equal to LLLT groups and they slightly more than the control group. This supports earlier research[23] that used herbal mixture composed of Aloe Vera, Henna, *Adiantum capillus-veneris*, and Myrrh oil on wound healing in streptozotocin-induced diabetic rats and he found that the diabetic rats treated with myrrh herbal mixture produce more epithelization than nondiabetic rats.

Also in agreement with the study by De Abreu Freitas *et al*.[24] who used LLLT and microcurrent on the healing of skin burns in rats and he found that LLLT increased epithelial layer thickness, collagen fiber reorganization, and improvement in burn healing, and in agreement with the study by Ribeiro *et al*.[25] who conducted with 20 rats, using LLLT HE-NE (632.8 nm) with 10 mW of output power, observed an acceleration in epidermis formation, increased epithelial layer thickness, neovascularization, collagen fiber reorganization, and improvement in burn healing.

The proliferation and remodeling phase is when collagen fibers typically develop, and Van Gieson stain can distinguish between fine and coarse collagen fibers.[26] When compared to the control group, the experimental groups (MO and LLLT groups) significantly differed in the amount of collagen fibers deposited on days 3 and 7, with LLLT group recording the highest mean value. The increased number of blood vessels (occurring early in the healing process) and adequate migration of fibroblast to the site of wound resulted in the deposited collagen fibers providing strength and integrity to tissue matrix during wound healing process as compared with control group; this agrees with the results of the study by Gál *et al*.[27,28]

Conclusions

According to the results obtained in this study, it can be concluded that the local application of essential myrrh oil is potentially more effective in enhancing oral traumatic ulcer healing when compare with LLL treatment and the latter is potentially more effective than control group.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of patient consent

The experiment was done in accordance with the animal experimentation ethical principles of College of Dentistry, University of Baghdad (No. 491). This study done on animals according to the above ethical approval.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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