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Possible healing effects of *Salvadora persica* extract (MISWAK) and laser therapy in a rabbit model of a caustic-induced tongue ulcers: histological, immunohistochemical and biochemical study

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Abstract

Caustic ingestion is a potentially detrimental event that can cause serious devastating damage on contact with tissues. Local exposure is associated with severe pain, swelling and ulceration. Caustics-induced oral ulcers can be painful enough to compromise the patient's quality of life. Treatment of oral ulcers is crucial in clinical practice. Albeit, some ulcers do not respond adequately to the conventional treatment. The current study was conducted to evaluate the potential healing effects of topical *Salvadora persica* (SP) extract, low-level laser (LLL) and high-level laser (HLL) therapies in a rabbit model of caustic-induced tongue ulcers and explore the underlying mechanisms. Fifty male rabbits with a caustic induced tongue ulcers were included in the study. Rabbits were equally divided into four groups: positive control (ulcer) group, SP, LLL and HLL groups in addition to the negative control (healthy) group. All treatments were given thrice weekly for 14 days. Results showed that acetic acid-induced tongue ulcers caused extensive structural tongue damage secondary to overexpression of apoptotic BAX, pathological angiogenesis indicated by VEGF overexpression, marked collagen fibers deposition as well as upregulation of tissue pro-inflammatory TNF- α and upregulation of tissue anti-inflammatory IL-10. The healing potential of topical SP, LLL and HLL therapy are mostly comparable. In conclusion, acetic acid-induced extensive tongue damage. Topical SP extract, LLL and HLL are equally effective therapies against caustics-induced tongue ulcers. However, we recommend SP extract, owing to its safety, non-invasiveness, availability and low cost.

Keywords Acetic acid · Caustics · Histo-morphometry · Laser · *Salvadora persica* · Tongue ulcers

Introduction

Caustic ingestion is a potentially damaging incident, however, international epidemiological data are rare secondary to under-reporting of caustic ingestion (Chirica et al. 2012, 2017). Corrosives are common in household and industrial chemicals. Caustics exposures in adults are usually intentional or unintentional in the setting of occupational exposure while exposures in children frequently occur by accidental ingestion (Wightman et al. 2016).

Caustics exposure may occur through the dermal, ocular, respiratory, and gastrointestinal routes with the most serious of these, resulting from ingestion (Fulton 2011). Furthermore, ingestion of the caustic material can cause serious devastating damage on contact with tissues. Local exposure is associated with severe pain, swelling and ulceration (Dargan 2016). Corrosive injury is associated with many life-threatening consequences (Hashmi et al. 2018). This case is

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a medical emergency with different clinical presentations and an extremely complex clinical progression. Corrosive ingestions can cause injury to the lips, oral cavity, pharynx, and the upper respiratory airways as well as extensive hemorrhage, gut perforation, aortic-enteric, gastro-colic fistulae, tracheal stenosis and tracheoesophageal fistula (Mamede and De Mello Filho 2002).

Oral ulcers are common pathological entities of the oral mucosa that can be painful enough to compromise the patient's quality of life. Thus, the treatment of oral ulcers seems very important in clinical practice (Bonavina et al. 2015). The dynamics of oral ulcers are complex involving the reduction of blood flow, cytokine production, cell death and impaired wound repair (Campisi et al. 2007; Oliveira et al. 2016). Therefore, many medical and palliative therapies for oral ulcers have been developed (Chamani et al. 2017). These current treatment options for oral ulcers include topical analgesic and anesthetic agents, corticosteroids, antibiotics, multivitamins, cauterization, immunosuppressive and combination therapies (Aggarwal et al. 2014).

Though, some ulcers do not respond adequately to the standard treatment and the long-term or repeated use of these therapies may evoke fungal infection, drug resistance or even life-threatening complications (Han et al. 2016; Silva et al. 2018). Thus, there is a critical need for an inclusive treatment with minimal possible complications. Medicinal plants have become a part of complementary medicine all over the world. Because of their potential health benefits, various plant extracts can be used effectively for therapeutic purposes (Al-Ayed et al. 2016). *Salvadora persica* (SP; commonly known as miswak) is a promising product with known antiplaque, analgesic, anticonvulsant, antibacterial, antifungal, antirheumatic, cytotoxic, deobstruent, carminative, diuretic, and astringent effects. Its pharmacological effects are attributed to its chemical constituents that include benzyl-isothiocyanate, saponins along with tannins, silica, a small amount of resin, trimethylamine and alkaloidal constituents (Al-Ayed et al. 2016; Khatak et al. 2010). On the other hand, laser therapy has become clinically accepted as part of physical therapy for many diseases. It has been tested as a novel treatment modality for patients with various dental and oral conditions (Biagi et al. 2015; Han et al. 2016). Consequently, this experimental study was designated to evaluate the potential healing effects of topical SP extract application and laser therapy in a rabbit model of a caustic-induced tongue ulcers as well as the underlying mechanisms.

Materials and methods

Chemicals

Carbopol 934P (Prod No. GRM6761) and glycerol (Prod No. GRM081) were purchased from Himedia, (India).

Triethanolamine (Prod No. 90279) was purchased from Sigma-Aldrich, (Germany). Green sticks of *Salvadora persica* (miswak, arak) were obtained from local markets in Makkah Al Mukarramah, (Saudi Arabia).

Animals

Fifty male rabbits weighing 1500–1700 g and aged approximately three months were included in the current study. Animals were held at the Laboratory Experimental Animal House Unit at-Azhar University (Girls), Cairo, Egypt. After getting the approval of the Ethical Committee for the experimental use of laboratory animals (*Approval Number IORG0010305*), rabbits with the aforementioned criteria were selected and housed within clean stainless-steel cages under the recommended optimum conditions of the Institutional Guideline for Care and Use of experimental laboratory Animals. Animals were acclimated for one week under standard laboratory conditions before starting the experiment. The room temperature was kept at 21 ± 2 °C, 12 h dark/light cycle, with humidity of relatively $56 \pm 5\%$ and free access to food and water. The whole experiment was carried out according to the International Principles of Laboratory Animal Research. The study was designed to minimize the number of animals required for the experiment and the study procedures were conducted in accordance with the guidelines of Helsinki Declaration for use of animals in biomedical research.

Experimental protocol

Induction of tongue ulcers

Tongue ulcers were induced according to a method described by Fujisawa et al. (2003). Forty rabbits were anesthetized by intra-peritoneal injection of 50 mg/kg sodium pento-barbital (Abbott Laboratories, Inc., Chicago, USA). Then 15 mL of 50% acetic acid-soaked filter paper (5.5 mm diameter) was pressed into the dorsal surface of the tongue for one minute to initiate a circular uniform ulcer.

Treatment groups

Negative control group, involved 10 healthy rabbits that received normal diet.

Rabbits with tongue ulcers were randomly and equally divided into four groups ($n = 10/\text{group}$): positive control (ulcer) group, *Salvadora persica* (SP) group, low-level laser (LLL) group, and high-level laser (HLL) group.

Animals of the positive control group were left for spontaneous healing. Rabbits of (SP) group received topically (SP) extract twice daily. Rabbits of (LLL) group received GaAlAs laser beam therapy, (830 nm, BTL 5000, UK) with

the following specifications; 5 J/cm², thrice weekly, using scanning method. Rabbits of (HLL) group received Nd: Yag laser beam therapy (1064 nm, design: HIRO 3.0, ASA srl company, Italy); 5 J/cm², thrice weekly, using scanning method. All treated animals received therapy for 14 days after ulcer initiation.

***Salvadora persica* (SP) extract preparation**

This involved two steps:

Step 1 Aqueous extracts preparation: Three kg of miswak (arak) green sticks were thoroughly washed with distilled water (DW), cut into small pieces and freeze dried for 24 h (Freeze Dryer: CHRIST; Germany). Dry sticks were crushed into fine powder by using a laboratory ball mill. The powder was macerated in DW with continuous stirring till complete exhaustion (supernatant discoloration). The extract was then filtered by using Whatman filter paper number 1. The filtrate was subsequently dried using the freeze drier. The obtained dried mass was kept in sterilized closed bottles and stored at - 20 °C for later work (Al-Ayed et al. 2016; Lebda et al. 2018).

Step 2 Gel formulation: Carbopol 934P (1g) and measured amount of the extract (to prepare 5% gel) were dispersed in 80 ml of DW with continuous stirring using magnet stirrer at 800 rpm for one hour (Hot Plat stirrer; IKA, Germany). Five mL of glycerol was added to the mixture with continuous stirring. The mixture was neutralized by triethanolamine, which was added dropwise. Mixing was continued to form a transparent gel. Gel formulation was prepared freshly for each treatment (Dev et al. 2019).

Histological technique and immunohistochemistry (IHC)

The animals were euthanized by an overdose of anesthesia (≥ 0.86 mg/kg sodium pentobarbital intraperitoneal); the tongues were dissected and fixed in 10% formalin and processed as paraffin blocks. These blocks were cut into 3–4 thick μm sections using rotary microtome (LEICA RM 2125; UK). Blocks were further stained with hematoxylin & eosin (H&E); routine stain of histological study and Masson trichrome (MT); special stain to assess collagen fibers (Bancroft and Layton 2013). Examination of the slides and histo-morphometric measurements were done using a light microscope (Olympus-Bx; 4500) connected to a digital camera (Nikon-Coolpix; 4500) at objective lens magnifications of $\times 10$, and $\times 40$.

Immuno-histochemical staining was performed by using 3–4 μm , formalin-fixed, paraffin-embedded tissue sections with the use of specific antibodies against VEGF (ab27278, abcam, polyclonal, diluted at 1:100) and BAX (HPA027878, Sigma-Aldrich, polyclonal, diluted at 1:100).

The standard avidin–biotin peroxidase technique was applied using diaminobenzidine (DAB) for visualization and Mayer's hematoxylin for counterstaining. Negative controls were prepared with omission of the primary antibody (Ramos-Vara et al. 2008).

Histo-morphometry

Histo-morphometric assessment were done by using Image-Pro Plus program (version 6.0; Media Cybernetics Inc., Bethesda, Maryland, USA), in the Pathology department, Faculty of Medicine, Cairo University. Optical density of positive immunostaining for VEGF and BAX was measured. Five randomly selected high-power microscopic fields within and around the ulcer area were examined to measure the optical density (Zhao et al. 2009; Zhuang et al. 2013). The area percentage of MT-stained collagen fibers was similarly assessed at $\times 20$ magnification. The results were expressed as mean area % of collagen/ μm^2 .

Tissue levels of tumor necrosis factor- α (TNF- α) and interleukin-10 (IL-10) assay by ELISA

Tissue levels of TNF- α and IL-10 were assessed by the commercial ELISA kits (RayBiotech, USA, Cat No. ELH-TNF α -CL-1 for TNF- α , Cat No. ELH-IL10-CL-1 for IL-10) according to the manufacturer's recommendations.

Tissue lysate of the tongue was prepared by using $2\times$ Cell Lysis Buffer (Ray-Biotech, Cat No. EL-lysis). The lysate was prepared by homogenizing 50 mg of tongue tissue with 500 μL $1\times$ Lysis Buffer. The homogenate was transferred to microcentrifuge tube and centrifuged at 14,000 rpm for 20 min at 4 °C. Tissue lysate was aliquoted and stored at - 80 °C. Total protein concentration was determined by Pierce™ BCA protein assay kit, (ThermoScientific, USA, Cat#: 23,227). The lysate was diluted to a final protein concentration 1.000 $\mu\text{g}/\text{mL}$ and then stored at - 20 °C till ELISA assay performance.

Statistical analysis

Continuous variables were presented as the mean \pm SD. Equality of variance was checked by Bartlett's test. One-way ANOVA or Welch's ANOVA depending on the equality of variance was used to detect statistical differences between groups. Post-hoc tests (Tukey test if equal variances were assumed; Tamhane's T2 test if equal variances were not assumed) were performed for multiple comparisons between the experimental groups. Differences were considered significant at $P < 0.05$. All statistical comparisons were two-tailed and performed using Graphpad Prism, Version 8.0 Software (GraphPad Software; SanDiego, CA, USA).

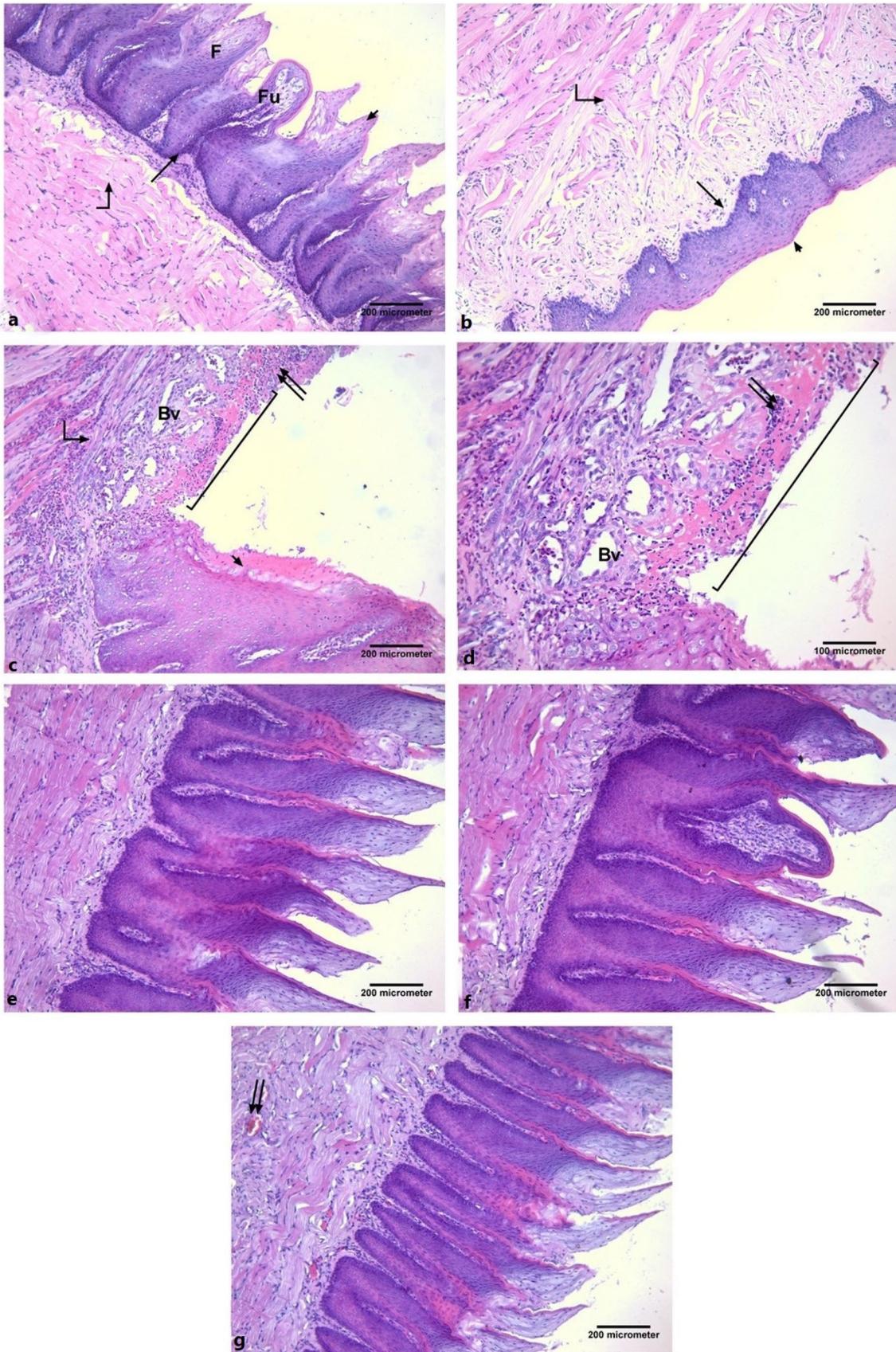


Fig. 1 Effects of topical *Salvadora persica* (SP) extract gel, low-level laser (LLL) and high-level laser (HLL) therapies on tongue histology following acetic acid injury. **a** A photomicrograph of the dorsal surface of a tongue of a rabbit from negative control group showing regular orientation of long conical shaped filiform papillae (F) with tapering ends. They have a core of connective tissue covered by keratinized epithelium (arrow head). A fungiform papilla (Fu) can be seen. The underlying lamina propria is formed of connective tissue (\uparrow). The lingual muscle fibers run in different directions (bent arrow). **b** A photomicrograph of ventral surface of a tongue of a rabbit from control group showing no lingual papillae and presence of thin regular keratin layer (arrow head). The underlying lamina propria is formed of connective tissue (\uparrow). The lingual muscle fibers run in different directions (bent arrows). **c** A photomicrograph of the dorsal surface of a tongue of a rabbit from ulcer group showing apparent non healed ulcer ($_$) lacking the epithelial covering. The epithelial covering adjacent to the ulcer area (arrow head) lacking the lingual papillae. The lamina propria shows excessive mononuclear cellular infiltration (double arrows) and multiple dilated blood vessels (Bv). The lingual muscle fibers run in different directions (bent arrows). **d** A higher magnification of the previous section showing apparent non healed ulcer ($_$) lacking the epithelial covering. The lamina propria shows excessive mononuclear cellular infiltration (double arrows) and multiple dilated blood vessels (Bv). **e** A photomicrograph of the dorsal surface of a tongue of a rabbit from SP group showing complete healing of the ulcer, the histological structure of the tongue is similar to that of the control group. **f** A photomicrograph of the dorsal surface of a tongue of a rabbit from LLL group showing complete healing of the ulcer, the histological structure of the tongue is similar to that of the control group. **g** A photomicrograph of the dorsal surface of a tongue of a rabbit from HLL group showing complete healing of the ulcer, the histological structure of the tongue is similar to that of the control group. Multiple congested blood vessels (double arrows) can be seen. [H&E; (a–c, e–g) $\times 100$, scale bar = 200 μm and (d) $\times 200$, scale bar = 100 μm]

Results

Comparison of SP with High or Low laser treatment effects on acid-induced tongue ulcers

Hematoxylin and eosin stained sections of tongue in negative control group, showed equally distributed prominent filiform papillae, uniform in size, shape and orientation with normal covering keratinized epithelium in the dorsal surface of the tongue as well as well-formed connective tissue and muscles. The ventral surface was smooth and devoid of papillae (Fig. 1a, b). Ulcer group revealed non-healed ulcer lacking the epithelial covering. The epithelial covering adjacent to the ulcer area had no lingual papillae. Multiple dilated blood vessels as well as mononuclear cells appeared to infiltrate the connective tissue in the ulcerated area (Fig. 1c, d). SP, LLL and HLL groups showed complete healing of the ulcer; the histological picture of the tongue in these groups appeared similar to that of the negative control group except that, there were multiple congested blood vessels in the lamina propria of the high dose laser treated group (Fig. 1e–g).

In order to assess the degree of collagen deposition, we stained sections of tongue in all study groups by Mallory's trichrome stain. In the negative control group, the collagen fibers appeared in the lamina propria and in between the muscle fibers (Fig. 2a, b). However, ulcer group displayed marked collagen fibers deposition in the lamina propria and in between the muscle fibers (Fig. 2c). SP, LLL and HLL groups revealed minimal collagen fibers deposition in the lamina propria and in between the muscle fibers which more or less were similar to that of the negative control group (Fig. 2d–f).

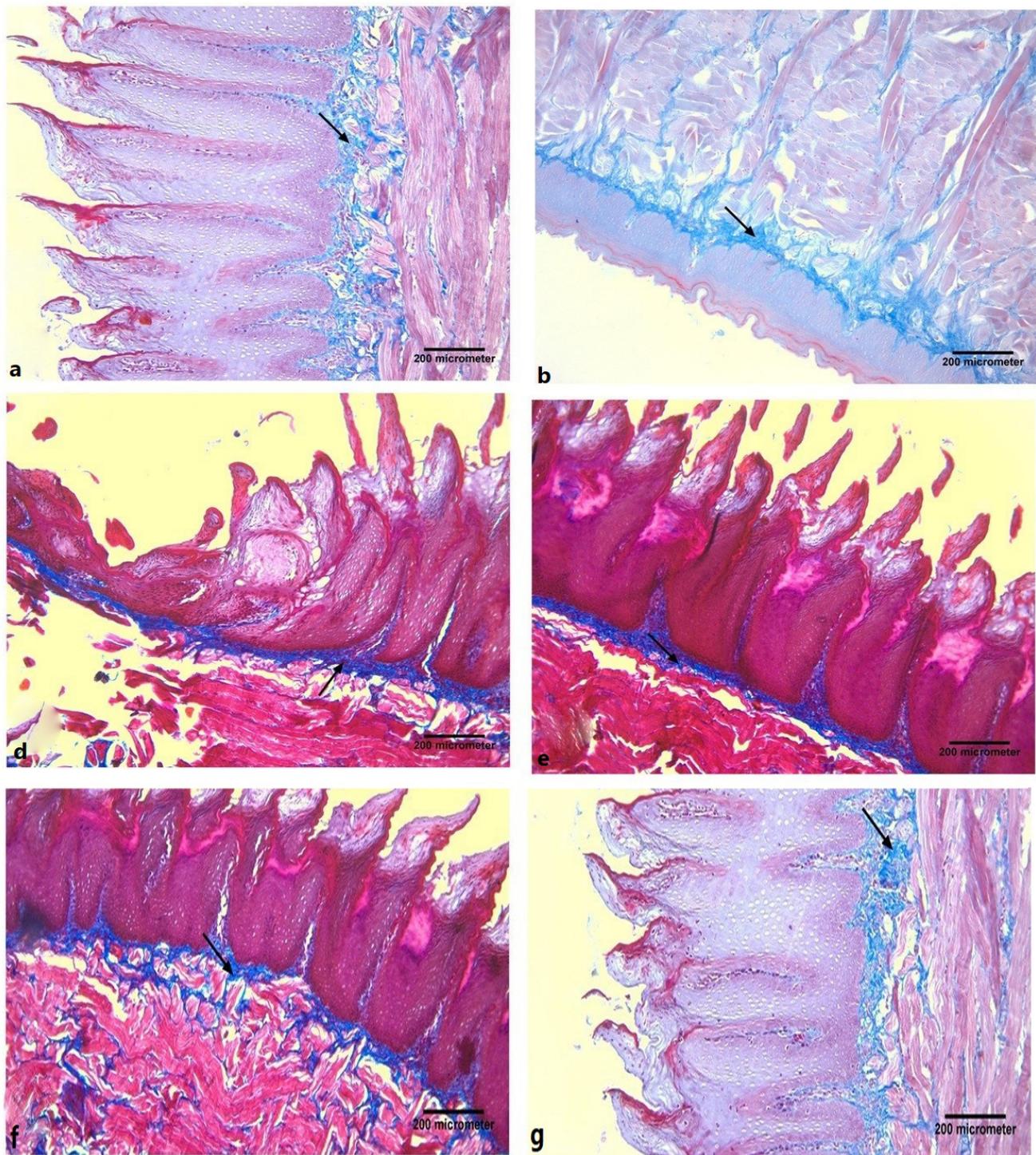
Distribution and immunoreactivity levels of BAX and VEGF

In order to assess the degree of apoptosis and the pathological angiogenesis, we immunostained sections of tongue in all groups by BAX and VEGF immunostaining, respectively. In the negative control group, the tongue showed a weak positive cytoplasmic reaction for BAX in the epithelial cells and a peri-vascular weak positive immune reaction for VEGF (Figs. 3a, 4a). The ulcer group demonstrated an extensive positive cytoplasmic reaction for BAX in the epithelial cells as well as a peri-vascular strong positive immune reaction for VEGF (Figs. 3b, 4b). While the ulcer group treated with SP and low Laser therapies showed weak positive cytoplasmic reactions for BAX in the epithelial cells and peri-vascular weak positive immune reactions for VEGF (Figs. 3c, d, 4c, d). Additionally, HLL therapy elicited a mild positive cytoplasmic reaction for BAX in the epithelial cells and a peri-vascular mild positive immune reaction for VEGF (Figs. 3e, 4e). Mean optical density of lingual epithelial BAX expression was highly statistically significantly increased in ulcer group (1.89 ± 0.79) than negative control (0.31 ± 0.2 , $P = 0.001$), SP (0.43 ± 0.16 , $P = 0.002$), LLL (0.44 ± 0.18 , $P = 0.002$) and HLL (0.51 ± 0.21 , $P = 0.003$) groups, as shown in Figs. 5 and 6.

Similarly, mean optical density of perivascular VEGF expression was highly statistically significantly increased in ulcer group (1.76 ± 0.65) than negative control (0.28 ± 0.11), SP (0.32 ± 0.13), LLL (0.35 ± 0.14) and HLL (0.47 ± 0.19) groups, ($P < 0.001$, for each), as shown in Fig. 5.

Likewise, the mean area percent of collagen fibers disposition was highly statistically significantly increased in ulcer group (14.24 ± 1.34) than negative control (4.62 ± 1.02), SP (5.25 ± 1.12), LLL (5.89 ± 1.21) and HLL (6.01 ± 1.72) groups, ($P < 0.001$, for each), as shown in Fig. 5.

Morphometric parameters were more or less similar in negative control group and all therapies, as shown in Fig. 5.



Tissue pro-inflammatory (TNF- α) and anti-inflammatory (IL-10) biomarkers

In order to assess the inflammatory status of tongue tissue, we measured tissue levels TNF- α and IL-10. Mean tissue normalized level of TNF- α was highly statistically significantly increased in ulcer group (21.07 ± 2.08) than negative

control (10.31 ± 1.2), SP (11.23 ± 1.16), LLL (12.44 ± 2.01) and HLL (10.31 ± 2.21) groups, ($P < 0.001$, for each). Similarly, mean tissue normalized level of IL-10 was highly statistically significantly increased in ulcer group (10.36 ± 1.45) than negative control (5.28 ± 0.01), SP (6.22 ± 0.73), LLL (5.45 ± 1.14) and HLL (5.61 ± 0.01) groups, ($P < 0.001$, for each), as shown in Fig. 5.

Fig. 2 Effects of topical *Salvadora persica* (SP) extract gel, low-level laser (LLL) and high-level laser (HLL) therapies on collagen deposition in tongue tissue following acetic acid injury. **a** A photomicrograph of the dorsal surface of a tongue of a rabbit from negative control group showing collagen fibers deposition (arrow) in the lamina propria and in between the muscle fibers. Notice the collagen fibers appear blue. **b** A photomicrograph of the ventral surface of a tongue of a rabbit from negative control group showing collagen fibers deposition (arrow) in the lamina propria and in between the muscle fibers. **c** A photomicrograph of the dorsal surface of a tongue of a rabbit from ulcer group showing increase in collagen fibers deposition (arrow) in the lamina propria and in between the muscle fibers. **d** A photomicrograph of the dorsal surface of a tongue of a rabbit from SP group showing decrease in collagen fibers deposition (arrow) in the lamina propria and in between the muscle fibers. **e** A photomicrograph of the dorsal surface of a tongue of a rabbit from LLL group showing decrease in collagen fibers deposition (arrow) in the lamina propria and in between the muscle fibers. **f** A photomicrograph of the dorsal surface of a tongue of a rabbit from HLL group showing minimal decrease in collagen fibers deposition (arrow) in the lamina propria and in between the muscle fibers (Mallory's trichrome $\times 100$, scale bar = 200 μm). (Color figure online)

Discussion

Results of the current study proved the healing effects of topical SP extract application and laser therapy in a rabbit model of acetic acid-induced tongue ulcers. Our findings showed that the acetic acid-induced tongue ulcers caused extensive structural tongue damage, overexpression of apoptotic BAX, pathological angiogenesis indicated by VEGF overexpression, marked collagen fibers deposition as well as upregulation of tissue pro-inflammatory cytokine; TNF- α and upregulation of tissue anti-inflammatory cytokine; IL-10.

Acetic acid ulcer models are similar to human ulcers regarding the pathology and healing processes (Polo et al. 2012). Consistently, Kobayashi et al. (2001) stated that acetic acid injury has a multifactorial pathogenesis; such as, alteration in inflammatory cytokines, prostaglandins and growth factors. Our results coincided with the observations made by Lee et al. (2017) on the rabbit acetic acid-induced oral ulcer model, who has shown the partial healing effect in addition to the inflammatory cell infiltration. Proteins of Bcl-2 family play a key role in the regulation of apoptotic cell death. Some members of this family, such as BAX, can promote apoptosis and inhibit cell survival (D'Arcy 2019).

Results of the current study revealed over expression of lingual VEGF which is an essential regulator of angiogenesis attributing to the inflammatory signaling via TNF- α and IL-6 (Malaponte et al. 2015).

In the present work, IL-10 level was markedly elevated in ulcer group than the treated groups. This could be explained according to Iyer and Cheng (2012), who stated that IL-10

is a potent anti-inflammatory cytokine that is generated secondary to the release of inflammatory mediators to prevent uncontrolled tissue damage.

Moreover, VEGF triggers angiogenesis, inflammation endothelium-dependent vasodilatation and augmented microvascular permeability (Cekmen et al. 2003). Similar results were reported by Mahmoud et al. (2019), who found a significant elevation of oral mucosal VEGF and the inflammatory endocan levels in patients with recurrent aphthous ulcers.

Wound healing is a vital but complex multidimensional process in human or animal, including consecutive yet overlapping stages, involving hemostasis/inflammation stage, proliferation stage, and finally remodeling stage (Lindley et al. 2016).

Our results demonstrated that the healing potential of topical SP application, LLL and HLL therapy in a rabbit model of a caustic-induced tongue ulcers quite equaled. In the present study, topical SP application and LLL therapy resorted the normal histological appearance of the tongue. However, HLL therapy was associated with lingual vascular congestion. An experimental seven-day-study of Lebda et al. (2018) to assess the protective effect of SP extract on a rat model of ethanol-induced gastric ulcer, revealed approximately normal gastric histological appearance following SP extract administration.

In accordance to our findings, Imran et al. (2015) has evaluated that skin wound healing in SP extract-treated rats confirming the progressive decrease in wound area with complete epithelialization and healing within 18 days compared to the control group (32 days). Comparable to our findings, a study of Nomani et al. (2017) attributed the anti-inflammatory activity of SP extract to the downregulation of (TNF- α) mRNA in a rat mode of inflammatory bowel disease (IBD).

Our results are consistent with the conclusion of Han et al. (2016). In their systematic review, they found that laser therapy is superior in alleviating oral ulcer pain and faster healing compared with placebo group or conventional therapy. Laser is a safe, non-invasive and effective treatment option, which provides thermal, chemical and mechanical effects (Alayat et al. 2018). Similar to our results, a review of Lins et al. (2010) reported the beneficial effects of the low-power lasers in wound healing in terms of anti-edema, anti-inflammatory, analgesic, endorphins release, controlling pain mediators and restoring neural function.

In agreement to our results, Ahmed et al. (2018) showed the therapeutic effects of low-level laser therapy for 14 days, in terms of normalization of dermal collagen fibers and diminished serum levels of the key pro-inflammatory mediators (IL-1 β and TNF- α) in non-diabetic and diabetic wounded rats.

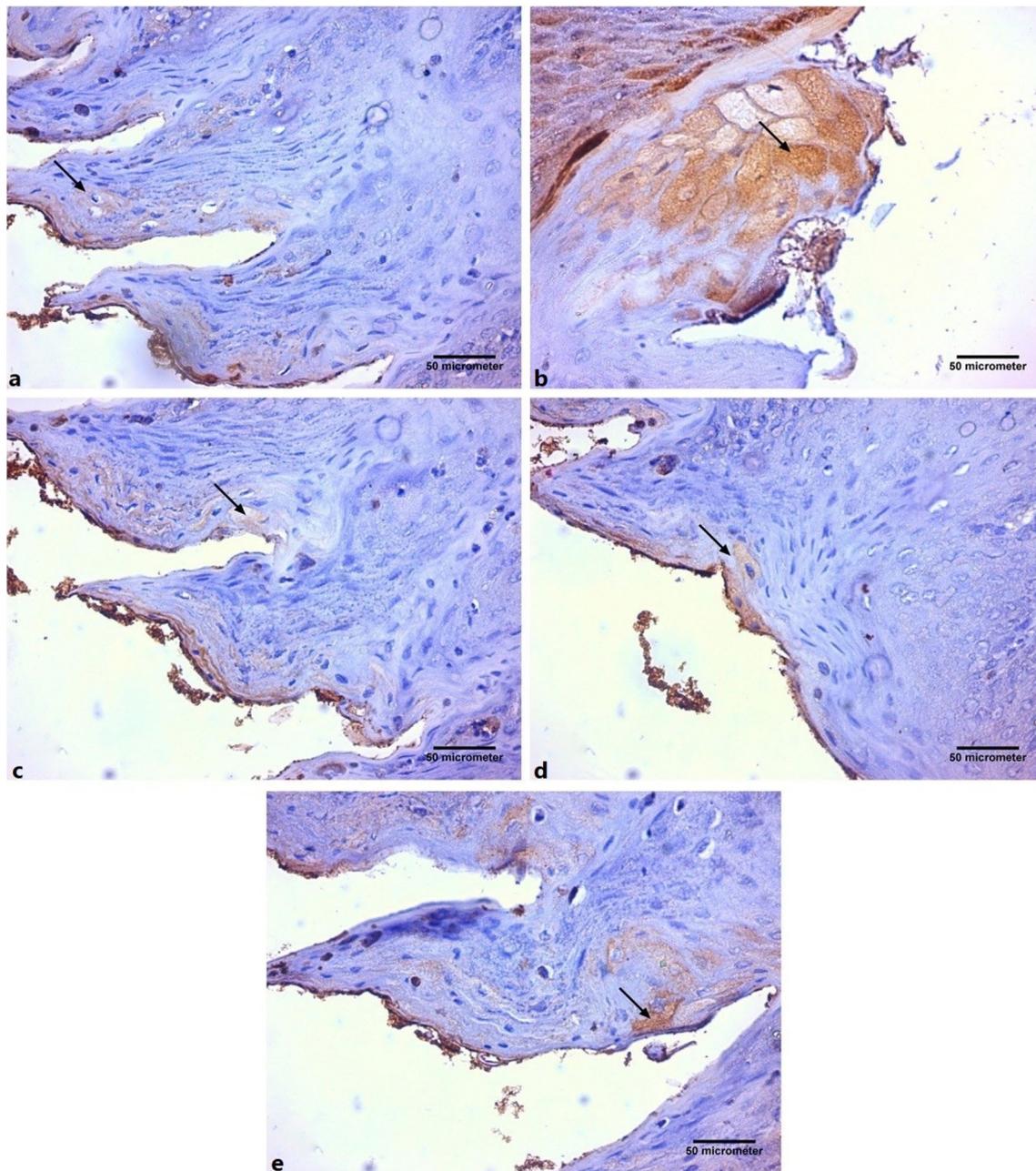


Fig. 3 Effects of topical *Salvadora persica* (SP) extract gel, low-level laser (LLL) and high-level laser (HLL) therapies on BAX immunoreaction in tongue tissue following acetic acid injury. **a** A photomicrograph of the dorsal surface of a tongue of a rabbit from negative control group showing a weak positive cytoplasmic reaction (arrow) for BAX in the epithelial cells. **b** A photomicrograph of the dorsal surface of a tongue of a rabbit from ulcer group showing a strong positive cytoplasmic reaction (arrow) for BAX in the epithelial cells. **c** A photomicrograph of the dorsal surface of a tongue of a rabbit

from SP group showing a weak positive cytoplasmic reaction (arrow) for BAX in the epithelial cells. **d** A photomicrograph of the dorsal surface of a tongue of a rabbit from LLL group showing a weak positive cytoplasmic reaction (arrow) for BAX in the epithelial cells. **e** A photomicrograph of the dorsal surface of a tongue of a rabbit from HLL group showing a mild positive cytoplasmic reaction (arrow) for BAX in the epithelial cells (Avidine biotin peroxidase stain with Hx counter stain $\times 400$, scale bar = 50 μm)

It should be noted that SP has several bioactive constituents (Aumeeruddy et al. 2018). However, we did not target an exact one. Our aim was to use the plant as a whole. In this way, we could offer an easily available remedy for

the community. We found several methods for the plant extraction but we selected the method that matched with our available resources.

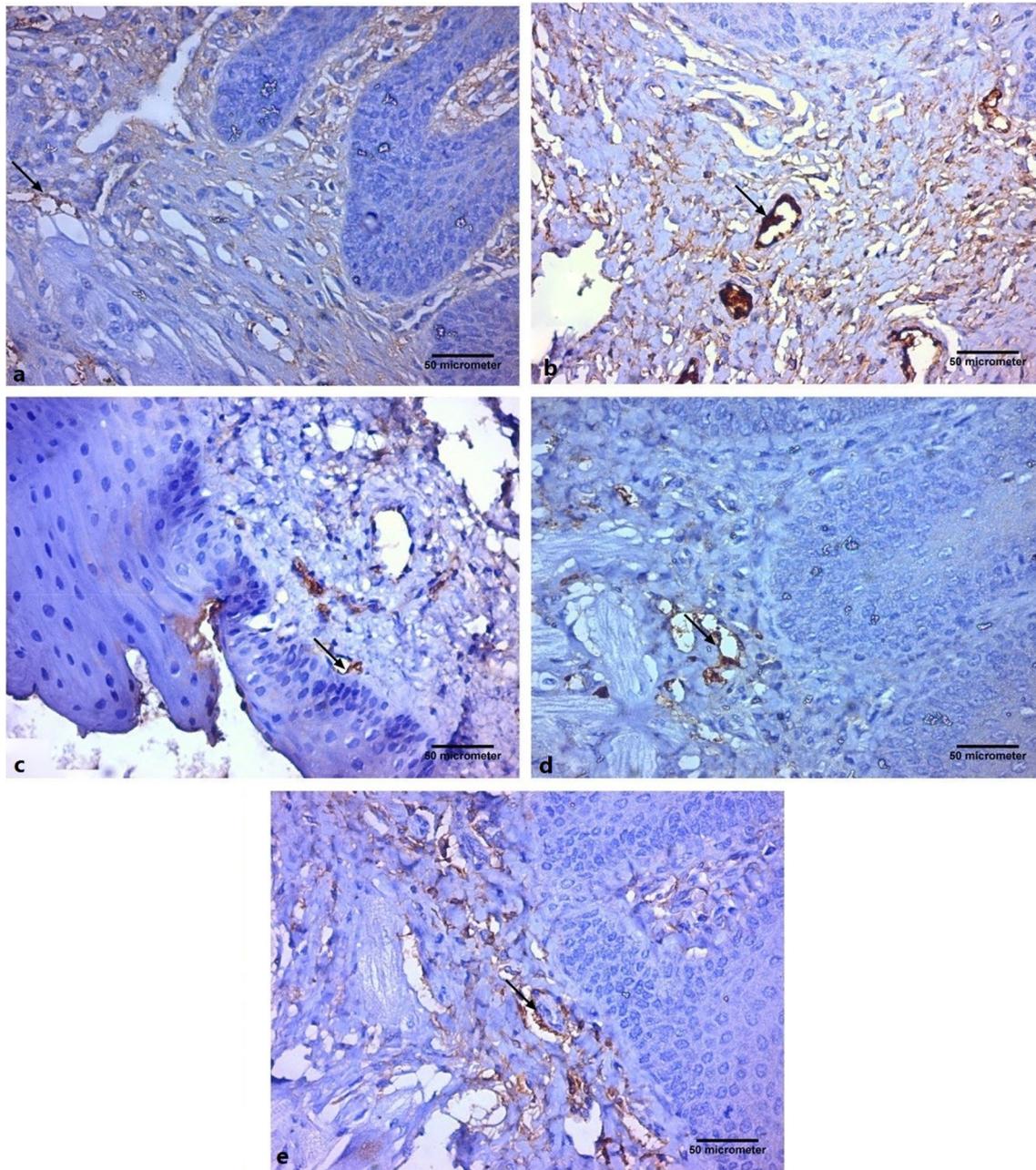


Fig. 4 Effects of topical *Salvadora persica* (SP) extract gel, low-level laser (LLL) and high-level laser (HLL) therapies on VEGF immunoreaction in tongue tissue following acetic acid injury. **a** A photomicrograph of the dorsal surface of a tongue of a rabbit from negative control group showing a weak positive immune reaction (arrow) for VEGF around the blood vessel. **b** A photomicrograph of the dorsal surface of a tongue of a rabbit from ulcer group showing a strong positive immune reaction (arrow) for VEGF around the blood vessel. **c** A photomicrograph of the dorsal surface of a tongue of a rabbit

from SP group showing a weak positive immune reaction (arrow) for VEGF around the blood vessel. **d** A photomicrograph of the dorsal surface of a tongue of a rabbit from LLL group showing a weak positive immune reaction (arrow) for VEGF around the blood vessel. **e** A photomicrograph of the dorsal surface of a tongue of a rabbit from HLL group showing a mild positive immune reaction (arrow) for VEGF around the blood vessel (Avidine biotin peroxidase stain with Hx counter stain $\times 400$, scale bar = 50 μm)

Conclusion

In conclusion, acetic acid induced prominent tongue damage. Topical SP extract, LLL and HLL are equally

effective therapies against caustics-induced tongue ulcers. However, we recommend SP extract, owing to its safety, non-invasiveness, availability and low cost.

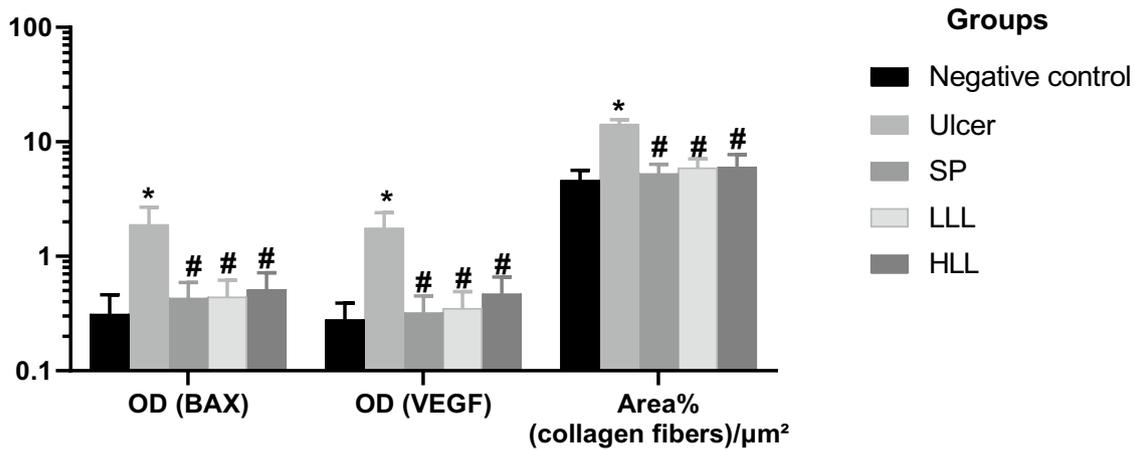


Fig. 5 Effects of topical *Salvadora persica* (SP) extract gel, low-level laser (LLL) and high-level laser (HLL) therapies on expression BAX, VEGF and area percent of collagen fibers deposition in the rabbit acetic acid-induced tongue ulcers model. Ten rabbits in each group.

*Significant vs negative control group, #Significant vs ulcer group ($P < 0.05$) by Welch's one-way ANOVA followed by post hoc Tamhane's T2 multiple comparisons test. OD optical density, BAX Bcl-2-associated X protein, VEGF Vascular endothelial growth factor

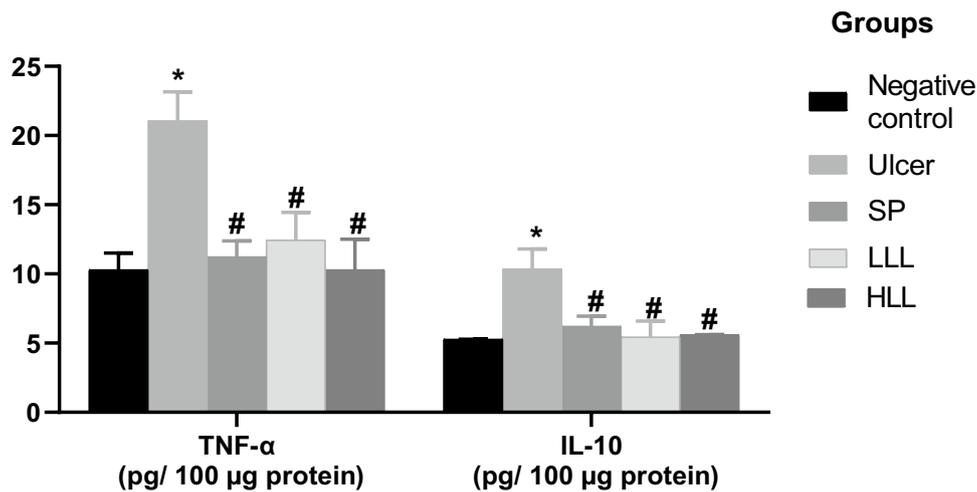


Fig. 6 Effects of topical *Salvadora persica* (SP) extract gel, low-level laser (LLL) and high-level laser (HLL) therapies on tumor necrosis factor- α (TNF- α) and interleukin-10 (IL-10) in the rabbit acetic acid-induced tongue ulcers model. Ten rabbits in each group. *Significant vs negative control group, #Significant vs ulcer group ($P < 0.05$) by a

one-way ANOVA followed by post hoc Tukey's multiple comparisons test or Welch's one-way ANOVA followed by post hoc Tamhane's T2 multiple comparisons test. TNF- α and IL-10 were normalized per 100 μg protein sample

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Compliance with ethical standards

Conflict of interest The authors report no declarations of interest.

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