Topical application of *Salvia officinalis* hydroethanolic leaf extract improves wound healing process

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Salvia officinalis L. (common sage) is a popular herb in the mint family, Lamiaceae. To our knowledge, literature regarding the wound healing properties hydroethanolic extract of *Salvia officinalis* is scarce. Here, we tried to evaluate the *in vitro* antioxidant properties and *in vivo* wound healing activity of the hydroethanolic extract of *S. officinalis*. About 105 healthy Wistar rats were inflicted with wound by excision and incisionand were randomly divided into five experimental groups: Group I, as control; Group II, received placebo; groups III-V treated with 1, 3 and 5% *S. officinalis* hydroethanolic leaf extract, respectively. Thehydroethanolic leaf extract of *Salvia officinalis* showed the highest total flavonoid and phenolic content and antioxidant capacity. Topical application of *S. officinalis* extract, especially higher dose, significantly (P <0.05) increased the percentage of wound contraction, a period of re-epithelialization, breaking strength ratio and upregulated hydroxyproline content versus control group. Additionally, *S. officinalis* significantly (P <0.05) increased the new vessel formation and Fibroblast distribution. Our results showed that *S. officinalis*, especially *S. officinalis* 5%, were significantly promoting wound healing effect and can be considered as an appropriate compound for clinical application in wound care.

Keywords: Antioxidant activity, Common Sage, Herbal

In regenerative medicine, the main challenge is improving tissue regeneration in the body through therapeutical manipulations and manage scar formation. The main purpose of this phenomenon is to make a balance in tissue regeneration and scar formation[1].

Traditional remedies based on plant sources too remarkably promote the wound healing process at one or more stages[2]. Sage (*Salvia* spp.) is a popular herb in the mint family. It is the largest genus of the *Lamiaceae* family, with approximately 900 species, including annual, biennial or perennial herbs along with woody subshrubswith immense medicinal potential[3,4]. In Turkish folk medicine, some *Salvia* species have been used as herbal tea[4,5] and also to cure wounds, inflammation and skin ailments[6,7]. Extract of dried roots and rhizomes of *Salvia miltiorrhiza* Bunge has been reported to have protective effect on myocardial ischemia-reperfusion injury[8]. Pharmacological and phytochemical potential of *Salvia gesneriiflora* Lindl. and *Salvia hispanica* L. have been demonstrated by Aberto *et al.*[9]. Earlier studies have shown the antibacterial[10], antidiarrheal[5], anti-inflammatory[6,11] antinociceptive[11] antioxidant[10,12],

antiproliferative[11] diuretic[6] and immunomodulatory[13] activities of *Salvia officinalis*. Further, its usage in traditional phytotherapy for bronchitis, cold, dental care, fever, liver, kidney and stomach ailments, midgrade depression, throat ache, women reproductive system and wounds and ulcers is also known[5,6]. *S. officinalis* is one of the medicinal herbs used in healthcare products such as band aid[14]. In Morocco, women during pregnancy take sage flowers infusion for getting back in shape[15]. Süntar *et al.*[7] reported that *Salvia cryptantha* had a significant effect on wound contraction and tensile strength.

To our knowledge, only few studies deal wtih the wound healing activity of various *Salvia* species. Thus, in this study, we tried to evaluate the wound healing activity of *Salvia officinalis* in topical application. We explored the effects of different dose of hydroethanolic extract of *S. officinalis* leaves on excision wound model (contraction ratio, period of re-epithelialization and histopathological changes), linear incision and dead space wound models in a rat model.

Materials and Methods
Plant material and extract preparation
Salvia officinalis was collected from the central district of the region of Urmia, West Azerbaijan

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province, Iran in July 2013 (latitude: 37 34', longitude: 44 58'). The plant was authenticated by Agricultural and Natural Resources Research Center, Hamadan, Iran. Around 600 g of fresh plant material (leaves) was dried naturally on laboratory benches at room temperature (23-24ºC) for six days until crisp and powdered in an electric blender. Then, 150 g of the plant powder was suspended in 600 mL of hydroethanolic solution for 96 h at room temperature. The mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No 1). The filtrate was placed in an oven to dry at 40ºC. The clear residue obtained was used for the study. The obtained extracts were kept at −15°C until further use[2].

Antioxidant activity
DPPH radical scavenging assay
Diphenylpicrylhydrazyl (DPPH) free radical inhibition was assessed using previous method[2]. Microtiter plates of 96-wells were used and five different concentrations of each sample assessed. A 100 mg/mL of DPPH solution in methanol was used and to minimize experimental error, all experiments were done in triplicates. Solutions incubated at 25ºC for 45 min (heidolph titramax 1000 and incubator 1000, Germany) and absorbance recorded at 517 nm using power wave XS Micro plate spectrophotometer (Bio-Tek Instruments, Inc.). The percent of free radical inhibition (In %) calculated using the formula:

$$In\% = \frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100$$

where A_{blank}: absorbance of control reaction (containing all reagents except the test compound) and A_{sample}: absorbance of the test compound. Finally, concentration of solution in 50 % inhibition (IC_{50}) was calculated from inhibition percentage of plotted graph against each sample concentration.

FRAP radical scavenging assay
Ferric reducing ability of plasma (FRAP) assay is an antioxidant power test based on reduction of ferric tripyridyltriazine (Fe^{+3}-TPTZ) to ferrous tripyridyltriazine (Fe^{+2}-TPTZ). Production of Fe^{+3}-TPTZ generates blue color at 593 nm absorbance[2]. FRAP reagent was prepared just before each experiment by mixing solutions A, B and C in the ratio of 10:1:1 (A: Acetate buffer 300 mM pH 3.6, B: 10 mM TPTZ [2, 4, 6-tripyridyl-s-triazine] in 40 mM HCl, C: 20 mM FeCl3. 6 H_2O). A 20 µL of sample mixed with 200 µL FRAP reagent, held for 10 min at room temperature and recorded at 593 nm absorbance

using Power wave XS Microplate spectrophotometer (Bio-Tek Instruments, Inc.). Different concentrations of $FeSO_4.7H_2O$ (200, 400, 800, 1200 and 1600 µM) were used as standard solution where reacted with TPTZ reagent and absorbance plotted against various ferrous ion concentrations. The results expressed as µM of Fe^{2+} equivalents per mg of dried extract. L-ascorbic acid was used as standard antioxidant.

Determination of the total flavonoid content
Total flavonoid content was assessed using previous method. The aluminum chloride test applied to determination total flavonoid content of extracts. The flavonoid content was expressed as mg of quercetin equivalents per gram of dried extract[2].

Determination of total phenolic content
Total phenolic constituents were assessed using previous method. Total phenolic constituents of *sample* extract were determined by modified methods using Folin-Ciocalteu reagent and Gallic acid (ranging from 0-1000 mg/L) as standard phenolic compound[2].

Biological activity test
Animals and study design
Healthy white Wistar male rats weighing approximately 200 g and 9 wk of age were used in the present study. Two weeks before and during the entire experiments, the animals were housed in individual plastic cages (50×40×20 cm) with an ambient temperature of 23±3ºC, stable air humidity and a natural day/night cycle. Animals were handled on a regular daily basis for 2 wk prior to the study in order to acclimatize them with testing area and experimental condition. Rats had free access to chew food and freshwater. The procedures were carried out based on the guidelines of the Ethics Committee of the International Association for the Study of pain[2] and the current laws of the Iranian government for laboratory animal care. The University Research Council approved all experiments.

Formulation of topical wound application forms
Four variants of the topical application ointment were prepared. All the variants consisted base formulation comprising commercial Eucerin (25%) and Vaseline (75%) in about 1:3 proportions. All rats randomly were labeled by none toxic color and divided into five groups. After surgical wound creation, Group I served as control: had no received any administration. Group II rats received Placebo (base formulation). Groups III-V were applied with

1, 3 and 5% of *Salvia officinalis* hydroethanolic extract mixed with base formulation (*S. officinalis*), respectively[2]. The ointments were topically applied once a day, starting from the day of operation, on the wound area until the wound healed completely. All rats were monitored for any wound fluid or any evidence of infection or other abnormalities, until complete epithelialization.

Acute skin irritation test

The test was carried out based on Farahpour *et al.*[2]. About 500 mm^2 areas on the dorsal fur of each rat was shaved and prepared aseptically. The *S. officinalis* formulations were applied. After 4 hr, the skin of each animal observed for inflammation and or any signs.

Wound healing models

Circular Excision Wound Model

In this wound model, 75 healthy white Wistar rats used for wound contraction ratio, period of re-epithelialization and histopathological change studies. Animals were anesthetized by intraperitoneal (IP) administration of ketamine 5%, 90 mg/kg (Ketaset 5%; Alfasan, Woerden, The Netherlands) and xylazine hydrochloride 2%, 5 mg/kg (Rompun 2%, Bayer, Leverkusen, Germany). The fur was prepared aseptically and the predetermined area was marked on the back of animals. Each rat was fixed on the surgery table in ventral posture. Following surgical preparation, a circular surgical full thickness wound was made, 314 mm^2 diameters, on the anterior-dorsal side of each rat. Wound contraction percentage and wound closure time were used to assess wound-healing property[2].

The wound area was measured by immediate placing of a transparent paper over the wound and tracing area of this impression was calculated using the graph sheet. The wound healing percentage was calculated by Walker formula after measuring the wound size[2]. The percentage of wound healing was computed at the beginning of experiments on days 3, 6, 9, 12, 15, 18, and 21 days postoperative.

$$\% \text{ of wound closure} = \frac{\text{wound area on day zero - wound area on day X}}{\text{wound area on day zero}} \times 100$$

X= number of days.

Incision wound model

In this wound model, 30 animals were randomly divided into five experimental groups with six rats in each: control (Group I), placebo (Group II), 1 (Group III), (Group IV)3 and 5% (Group V) *S. officinalis*-treated groups. All animals were anesthetized with the method mentioned above. A 4-cm length incision was made through the skin and cutaneous muscle at a distance about 1.5 cm from the middle on the right side of the depilated back. The wound was closed at 0.5 cm intervals using 3/0 nylon (Dafilon, B/Braun, Germany). Ointments were applied once daily for 9 days. On day 9, sutures were removed and the tensile strength of healed wounds was measured on day10 using Strongraph mechanical test frame (Toyoseiky Tensile Testing Unit, Model R3, Japan)[2]. Tensile strength was calculated using the following formula:

Tensile strength = breaking strength (g) / cross sectional area of skin (mm^2)

Dead space wound model and hydroxyproline content estimation

Animals were randomly divided into five experimental groups (n=6 in each). Group I was considered as control. Group II drenched phosphate buffered saline as placebo. Groups III-V treated with 1, 3 and 5% of *S. officinalis* leave extract ointment, respectively. The dead space wound was created using subcutaneous implanting of polypropylene tubes (2.5×0.5 cm) in the lumbar region on the dorsal side. All animals were gavage the extracts for 9 days post-injury. On day 10 post-wound induction, granuloma tissue created on the implanted tube was carefully dissected and used to determine breaking strength and estimation of hydroxyproline content[2].

Histopathological Study

Animals were anesthetized with the same way mentioned above and specimens from skin were taken on 3, 6, 9, 12, 15, 18 and 21 days after surgery. Sample tissues, excised along with 1 to 2 mm surrounding normal skin in a depth of approximately 3 mm, were pinned on a flat cork surface and fixed in neutral-buffered formalin 10%. Then the sample tissues were routinely processed, paraffin wax embedded, sectioned at 5 µm and stained with hematoxylin and eosin (H&E) and Masson's Trichrome stains for further examination using microscopy (Olympus CX31RBSF attached cameraman) to assess the predominant stage of wound healing. Three parallel sections were obtained from each specimen. The number of MNC, PMN, fibroblastic aggregation and angiogenesis (the number of blood vessels and capillary buds) were quantitatively evaluated in 5 per high power fields (HPFs) (×400). Acute hemorrhage, congestion, edema, epithelialization, collagen production and

density were also evaluated qualitatively and calculated manually. They were analyzed in 5 per high power fields (HPFs) ($\times 100$)^2.

Statistical analysis

Experimental results were expressed as means ± SEM. Statistical analyses were performed using PASW 18.0 (SPSS Inc., Chicago, IL, USA). Model assumptions were evaluated by examining the residual plot. Results were analyzed using one-way ANOVA. Dunnett's test for pair-wise comparisons was used to examine the effect of time and treatments. $P < 0.05$ was considered significant differences.

Results

Antioxidant activity, total phenol and flavonoid contents

The results of IC50 in DPPH, Eq+ Fe^{2++}, total phenol and total flavonoid contents of hydroethanolic *Salvia officinalis* leave extract is presented in Table 1. A seen, IC50 in DPPH of *Salvia officinalis* extract was 7.12 compared to BHT (107.04). Also, Eq+ Fe^{2++} (m per mg extract) was 18318.0 ± 83.6 in comparsion to ascorbic acid 7740.2 ± 64.9. Furthermore, total flavonoid and phenolic compouns of *Salvia officinalis* extract was 116.71 ± 2.34 and 298.8 ± 4.3, respectevly.

Acute skin irritation test

The results obtained from acute skin irritation test, 4 h after applying ointment on the skin, there was no sign of inflammation in all animals during the test.

Wound contraction developed and re-epithelization period in *S. officinalis*-treated animals

According to the data (Table 2), there was no significant difference in wound contraction percentage between *S. officinalis*-treated group and non-treated group on days 3, 6 and 9 after wound induction ($P > 0.05$). Interestingly, wound contraction percentage significantly increased in the rat treated with 3 and 5% of *S. officinalis*-treated group compared to the other groups on days 12 and 15 ($P < 0.05$). As noticed, wound contraction percentage significantly increased in *S. officinalis*-treated (1, 3 and 5%) animals compared to the control group on days 18 and 21 after wound creation ($P < 0.05$). The positive effect of 1% *S. officinalis* is seen from day 18 which indicates for low therapeutic properties of 1% *S. officinalis*-treated group. Likewise, re-epithelialization period significantly increased in *S. officinalis*-treated rats compared to non-treated group ($P < 0.05$), but there was no significant effect between 1 and 3% of *S. officinalis* administrated rat ($P > 0.05$).

Linear incision wound model and dead space wound model changed depending on dose

Effects of hydroethanolic *S. officinalis* leaf extract ointment on the linear wound incision is presented in Table 3. According to the obtained data, a significant effect observed in *S. officinalis* administered groups compared to control and placebo groups. As observed,

Table 1—Antioxidant properties, total phenol and total flavonoid contents of of hydroethanolic *Salvia officinalis* Leaf extracts

	IC50 in DPPH inhibition assay (µg/mL)	Eq+ Fe^{2++} (m per mg extract)	Total flavonoid (µg eq Rutin/mg dried extract)	Total phenols (µg/mg dried extract)
Salvia officinalis	7.12	18318.0±83.6	116.71±2.34	298.8±4.3
BHT	107.04	-	-	-
Ascorbic acid	-	7740.2±64.9	-	-

Table 3—Effect of *Salvia officinalis* on the linear wound incision model in rat

Groups	Mean±SEM
Control	280.2±15.6
Placebo	289.96±17.7
S. officinalis 1%	386.03±12.67[a]
S. officinalis 3%	484.03±10.11[a]
S. officinalis 5%	527.51±37.11[b]

n= 6 animals in each group. Data are presented as the mean±SEM. There are significant differences between groups with different codes in a column (superscript letters [a,b]; $P < 0.05$ *vs.* control).

Table 2—Effect of *Salvia officinalis* on wound area and period of re-epithelialization in rat model in *rat*

Groups	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	re-epithelialization
Control	4.12±3.28	20.45±4.28	41.30±2.38	56.90±2.03	64.55±1.10	78.40±0.62	86.65±0.11	22.87±1.31
Placebo	7.75±2.01	20.74±3.84	44.55±4.84	63.75±1.83	77.60±1.05	81.45±0.29	89.25±0.12	21.83±1.24
S. officinalis 1%	7.75±3.90	30.35±3.37	51.80±5.57	87.85±3.86	93.10± 0.29	98.87±0.30[a]	100±0.00[b]	18.16±1.42[a]
S. officinalis 3%	8.59±4.57	33.35±6.05	67.40±5.07	92.70±1.03[a]	96.81±0.28[a]	100±0.00[b]	100±0.00[b]	17.16±1.53[a]
S. officinalis 5%	10.49±5.48	38.55±5.47	78.35±0.79	96.00±0.65[b]	100±0.00[b]	100±0.00[b]	100±0.00[b]	15.65±1.57[b]

n= 6 animals in each group. Data are presented as the mean±SEM. There are significant differences between groups with different codes in a column (superscript letters a, b; P < 0.05 *vs.* control).

S. officinalis-treated group significantly increased wound incision compared to non-treated group (P <0.05). Additionally, there was no significant effect between 1 and 3% of *S. officinalis*-treated groups (III and IV) (P >0.05) while 5% of *S. officinalis*-treated group significantly increased wound incision compared to the other groups (P <0.05).

Dead space wound model and hydroxyproline content estimation

The effect of *S. officinalis* on wound healing of dead space wound is shown in Table 4. As seen, different levels of *S. officinalis*-treated groups (3 and 5%) significantly increased hydroxyproline content in dead space wound compared to control group (P <0.05). Likewise, 1% of *S. officinalis*-treated group had no significant effect on hydroxyproline content in

dead space wound compared to control and placebo groups (P >0.05). Also, different levels of *S. officinalis*-treated group (1, 3 and 5%) significantly increased wet weight of the granulation tissue compared to both non-treated and placebo groups (P <0.05). According to the data, the 3 and 5% *S. officinalis*-treated groups (IV and V) significantly promoted dry weight of the granulation tissue compared to non-treated group (P <0.05).

Histopathology

The histological results for PMN, MNC, Fib infiltration and new vascular formation are presented in Table 5. The *S. officinalis*-treated groups (III-V) significantly diminished PMN infiltration compared to control group on 3 day postopertion (P <0.05). Following 7 days after wound induction, the PMN

Table 4—Effect of *Salvia officinalis* on wound healing of dead space wound model in rat

Groups	Dead space wound model		
	Hydroxyproline content (μg/mL)	Wet weight of the granulation tissue (mg)	Dry weight of the granulation tissue (mg)
Control	11.63±0.4	84.18±5.4	13.21±0.89
Placebo	12.38±0.22	84.88±5.88	13.54±0.76
S. officinalis 1%	13.58±0.4	97.98±2.73[*]	15.37±0.55
S. officinalis 3%	14.7±0.36[*]	117.4±4.26[*]	16. 83±0.57[*]
S. officinalis 5%	16.22±0.31[*]	125.83±3.3[*]	20.38±0.51[*]

n= 6 animals in each group. P <0.05 *vs.* control. Data are presented as the mean±SEM. There are significant differences between groups with different codes in a column (superscript letters a, b; P <0.05).

Table 5—Effects of *Salvia officinalis* on polymorphonuclear and mononuclear cells, new vessels and fibroblasts formation on subsequent wound healing in rat

Day	Group	PMN	MNC	New vessels	Fibroblast
	Control	62.4±4.1 [a]	12.1±0.54 [a]	2.5±2.1 [a]	21.5±1.52 [a]
	S. officinalis 1%	42.7±5.1[b]	19.2±0.95 [b]	5.2±0.62 [b]	39.3±1.63 [b]
3	*S. officinalis* 3%	39.8±4.2 [b]	23.1±0.77 [b]	6.5±1.01 [b]	47.8±2.19 [b]
	S. officinalis 5%	22.1±2.6 [bc]	26.1±0.45 [bc]	7.8±1.29 [bc]	55.2±2.38 [bc]
	Control	50.2±3.4 [a]	14.3±0.4 [a]	3.2±0.28 [a]	35±1.26 [a]
7	*S. officinalis* 1%	33.2±1.4 [b]	15.8±0.71 [a]	4.1±0.71 [b]	58±2.12 [b]
	S. officinalis 3%	29.91±0.4 [b]	16.5±1.6 [a]	4.7±0.29 [b]	60± 2.41 [b]
	S. officinalis 5%	15.12±1.8 [c]	19.9±0.81 [b]	5.6±0.68 [bc]	89±2.25 [bc]
	Control	32.1±1.7 [a]	9.12±0.67 [a]	2.8±0.81 [a]	57±2.62 [a]
	S. officinalis 1%	18.4±1.4 [b]	12.4±0.58 [a]	1.3±0.56 [b]	79±3.91 [b]
14	*S. officinalis* 3%	15.81±0.3 [b]	12.8±1.41 [a]	2.5±0.88 [a]	85±4.33 [b]
	S. officinalis 5%	8.92±0.6 [c]	14.32±1.09 [a]	3.6±1.03 [a]	99±4.81 [bc]
	Control	12.9±0.9 [a]	3.3±0.1 [a]	1.1±0.29 [a]	28±1.87 [a]
21	*S. officinalis* 1%	5.11±0.3 [b]	3.8±0.81 [a]	0.98±0.32 [a]	35±2.72 [b]
	S. officinalis 3%	3.99±0.5 [b]	3.1±0.88 [a]	1.9±0.68 [b]	42±3. 42 [b]
	S. officinalis 5%	1.1±0.11 [c]	4.3±0.79 [a]	2.2±1.09 [b]	49±3.55 [bc]

n= 6 animals in each group. PMN: polymorpho nuclear cells, MNC: mononuclear cells. Data are presented as the mean±SEM. There are significant differences between groups with different codes in a column (superscript letters [a, b, c]; P <0.05 *vs.* control).

distribution was significantly lower in *S. officinalis*-treated group versus to those in non-treated animals. Comparing different doses of *S. officinalis* showed that high dose-administrated *S. officinalis* exerted significantly (P <0.05) better results compared to lower doses (1% and 3%) on day 14 after operation (Fig. 1).

In this study, administrating 5% from *S. officinalis* significantly diminished MNCs infiltration compared to the other groups on day 3 postperation (P <0.05). Whereas no significant differences were observed between control group and 1 or 3% *S. officinalis*-treated rats (P >0.05). Compared to control group, the *S. officinalis*-treated animals exhibited significantly (P <0.05) lower MNCs on day 7 after wound induction. However, no significant differences were observed between treated and non-treated animals for MNCs on days 14 and 21 days after wound induction (P >0.05).

In contrary, administration of different levels of *S. officinalis*-treated group significantly increased NV formation compared to control group during to the postoperative days (P <0.05). As noticed, the 5% *S. officinalis*-treated group (V) had more potential but not significantly on NV formation compared to the other *S. officinalis*-treated groups (III and IV) on days 3 and 7 post wound creation injury (Fig. 1). Also, dose of 1% *S. officinalis* had no significant effect on NV (P >0.05), whereas 3 and 5% of *S. officinalis*-treated groups significantly improved NV compared to non-treated group on day 21 (P >0.05).

Based on our data, a significant (P <0.05) distribution of fibroblasts was observed in the *S. officinalis*-treated animals compared to control group (Fig. 2). Additionally, group V (5% of *S. officinalis*-treated) had a slight better effect, but not
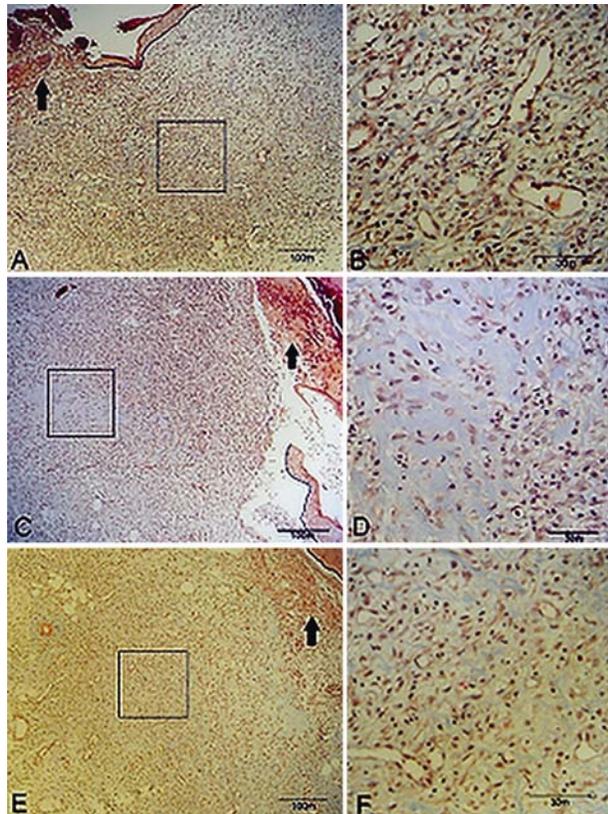

Fig. 1—Cross section from the wound area after 7 days. (A, B) Control group; (C, D) 3% *Salvia officinalis*-treated group; (E, F) 5% *S. officinalis*-treated group. The necrotic is shown by arrow. Note, the cross sections from higher magnification of the marked region with faint granulation tissue formation, impressive leukocytes infiltration and edema in control group. At the same time, see well-formed granulation tissue formation with remarkable reduction in the leukocytes infiltration and edema, along with remarkable fibroblast migration in *S. officinalis*-treated groups (*Masson trichrome staining*).
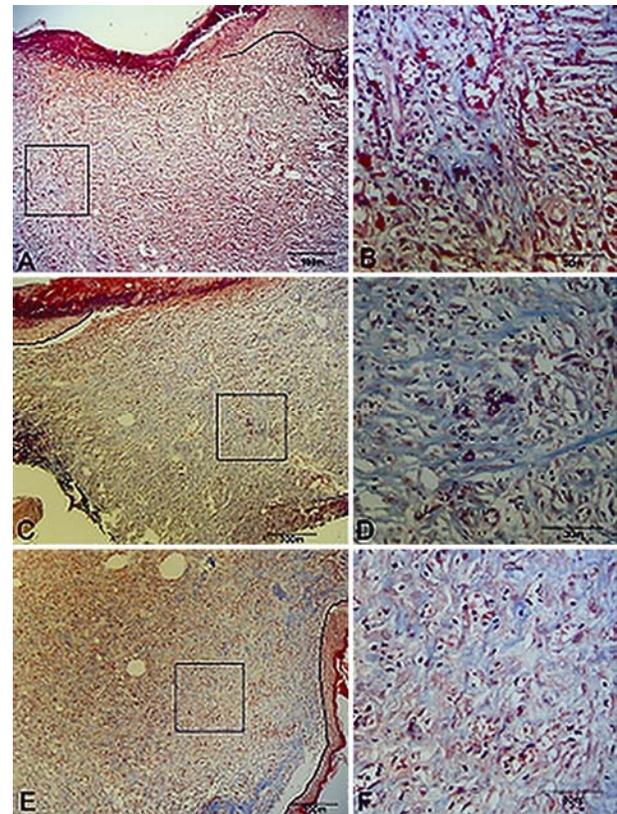

Fig. 2—Cross section from the wound area after 14 days. (A, B) Control group ; (C, D) 3% *Salvia officinalis*-treated group ; (E, F) 5% *S. officinalis*-treated group. The necrotic is shown by arrow. Note, the cross sections from granulation tissue with higher magnification of the marked region with less fibroblast distribution and collagen formation in control group. At the same time, see well-formed granulation tissue formation with remarkable in collagen formation in *S. officinalis*-treated groups, specially in 3% *S. officinalis*-treated group (*Masson trichrome staining*).

more significant than the other two treated group levels (1 and 3%) ($P >0.05$).

Discussion

In this study, different wound models are used to evaluate the healing activity of hydroethanolic extracts of *Salvia officinalis* as a traditional remedy. For this purpose, the preliminary phytochemical assessment of hydroethanolic extract of *S. officinalis* were performed. Biochemical analysis results showed that the *S. officinalis* hydroethanolic extract contains high levels of phytochemicals such as phenolic and flavonoid compounds. It is reported that flavonoids and phenols[16,17] have antioxidant activity[18-21] and accelerate wound healing activity[2,22-25], by their free radical scavenging[25-28], astringent, anti-microbial properties[2,25,29,]. Also, flavonoids are responsible for activating anti-inflammatory system which acts against lipid peroxidation[28,30-32].

In contrast, it is revealed that poly morphonuclear cells (PMN) is one of the primery sources of reactive oxygen species (ROS)[2,33]. Administration of *S. officinalis* significantly decreased in PMN cells infiltration and subsequently inhibited RNA damage. Therefore, it seems the flavonoid content of the *S. officinalis* extract could considerably decrease the inflammation-induced oxidative stress and RNA damage in epidermal and dermal cells.

Mononuclear cells (MNC), specially Macrophage cells are important phagocytosis cells in the inflammatory phase of wound healing process[1,2,34]. The presence of macrophage cells in the wound area is an indicator for the beginning of the second phase of the healing process[35]. On day 3 post-operative, the number MNC significantly increased in 3 and 5% *S. officinalis*-treated groups, compared with non-treated group.

Vascular endothelial cells attracted to the wound site by macrophage cells[1,2,34]. In histological assessment of on day 3 after exision wound creation, significant increase was observed innumber of new blood capillaries formation in 3 and 5% *S. officinalis*-treated groups compared to the untreated group. On the other hand, macrophage cells release PDGF, TGF-β and collagen which atracts fibroblast and smooth muscle cells cells into the wound site[2,34]. In histological assessment on days 7 and 14 post wound creation, the number of fibroblasts significantly increased in 3 and 5% *S. officinalis*-treated groups compared to the untreated group. Our observations demonstrated that administration of *S. officinalis* diminished inflammatory phase and promoted proliferative stage by increased in fibroblast distribution.

There is a positive correlation on collagen synthesis and fibroblasts distribution[1,36,37]. Increased collagen content, cross-linkages between collagen fibers and extracellular matrix secreted by fibroblasts to granulation tissue[38]. This phenomenon increases tensile ratio and onset wound contraction[2,39,40]. Our analysis showed that wound contraction and breaking tensile strength ratio significantly accelerate in *S. officinalis*-treated animals, especially 3 and 5% treated animals.

Hydroxyproline is a direct estimation marker for collagen synthesis[41,42]. Therefore, increase in the collagen content of granulation tissue, collagen cross linking and subsequent maturation in collagen, increases hydroxyproline, wet and dry granulation tissue weight[43,44]. Our results revealed that hydroxyproline content significantly amplified in 5% *S. officinalis*-treated animals which sunsequently increased wet and dry granulation tissue weight.

Neovascularization is an essential factor for migration of epithelial cells from the margins to the central of wound[1,45]. Also, re-epithelialization decreases distance wound size[2,35,46]. Rapid re-epithelialization in the wound healing process is considered as a hallmark for well wound treatment[35,46]. In this study, the epithelization time significantly reduced in 5% *S. officinalis*-treated group. The surveillance of epithelial cells in groups treated with *S. officinalis* could be attributed to its antioxidant characteristics which prevented cellular damage mediated by oxidative stress and accelerated process of their migration.

In conclusion, our data suggested that different topical doses of the *Salvia officinalis* hydroethanolic leaves extract increased tensile strength, facilitated wound contraction, decreased healing time as well as increased collagen deposition by up-regulating macrophage and fibroblast cell distribution followed by promoted proliferative stage of the healing process. Findings of the current study suggest that hydroethanolic extract of *Salvia officinalis* leaves support wound healing activity, and thereby indicating the potential of this plant in reducing the healing time.

Acknowledgement

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