IJBPAS, March, 2015, 4(3): 1219-1228

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

# TOPICAL APPLICATION OF LAVENDER OFfiCINALIS HYDROETHANOLIC EXTRACT, IMPROVES EXCISIONAL CUTANEOUS WOUND HEALING IN EXPERIMENTAL ANIMALS

**MOHAMMAD REZA FARAHPOUR[1]*, SAEED HESARAKI [2], ALIREZA SANATI FAZ[3], HASSAN MOHAMMADI FARD JOSHAGHANI[3]**

**1:** Department of Clinical Sciences, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran

**2:** Department of Pathology, Science and Research Branch, Islamic Azad University, Tehran, Iran

**3:** Department of Basic Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran

***Corresponding Author: E Mail: mrf78s@gmail.com; 57159-44867; Tel: +98 4414373676 Fax: +98 441 3460980**

**ABSTRACT**

*Lavender officinalis* has been used in traditional medicine for soothing insect stings, curing headaches, sedation, tranquilization and depression. In the current study, excision wound model were used for the assessment of wound healing activity of hydroethanolic extract of *Lavander officinalis* ointment (LOO) in rat. Three different doses of LOO were administered for excision wound models in rats. One hundred and twenty healthy white Wistar rats randomly allocated into 5 experimental groups. In group 1, animals had no received ointment. In group 2, rat received placebo (70% Vaseline + 30% Eucerin). The rats in groups 3, 4 and 5 treated with 1, 3 and 5% of LOO, respectively. According to the results, 5% of LOO significantly decreased wound contraction rate, whereas increased new vessel formation and fibroblast infiltration at the wound site compared to control group ($P<0.05$). Furthermore, the number of fibroblasts, new vessels and mononuclear cells significantly increased in 5% of LOO-treated rat compared to control group ($P<0.05$). These results suggest that the aerial part of *Lavender officinalis* hydroethanolic extract ointment has beneficial effect on excision wound healing activity in rats and it might useful for treating various types of wounds.

**Keywords: Lavender *officinalis*, Hydroethanolic Extract, Ointment, Wound Healing, Rat**

## INTRODUCTION

Wound healing is the physiological process for Derm and epidermis regeneration. When a tissue is wounded, an orchestrated complex biochemical event takes place to heal the damage. These events overlap in time and artificially categorized into 4 stages including; homeostasis, inflammation, proliferation and remodeling/maturation **[1]**.

Lavender officinalis belongs to *Lamiaceae* (Labiatae) family with more than 30 species, native to southern Europe and Mediterranean regions. It is well documented that various species of Lavender bears biological activities **[2, 3]**. Pharmacological reports revealed that Lavender essential oils contain cineole, linalool, linalyl acetate, polyphenols, flavone glucosides, triterpenes and diterpenes **[4, 5]**. Lavender species have been used in folk medicine for stress relief and sedation, tranquilization, soothing insect stings, curing headaches and wound healing **[6, 7]**. Also, it has anti-depressive, anxiolytic and calming properties **[7]**.

Despite therapeutic effects of *Lavander officinalis*, its role in experimental cutaneous wound healing is not fully studied. Therefore, the present study designed to investigate effect of hydroethanolic extract of Lavander officinalis ointment (LOO) on wound contraction rate, poly morphonuclear and mononuclear cell migration, new vessels formation, fibroblast infiltration, epithelialization and collagen synthesis in rats.

## MATERIAL AND METHODS

### Plant and Hydroalcoholic extract preparation

Fresh aerial part (flower and leaves) of *Lavender officinalis L.* was collected locally during June 2012 from Hamadan city, Iran (latitude: 36 46', longitude: 48 34'). The plant material was cleaned and dried naturally on laboratory benches at room temperature (23-24ºC) for 10 days until crisp and powdered using an electric blender. Then 200 g of plant material powder was suspended in 800 mL of aqueous ethanol solution, for 7 day at room temperature. The mixture was filtered via a fine muslin cloth followed by filter paper (Whatman No. 1). The solvent was completely evaporated under vacuum at 40ºC in a rotary evaporator. The hydroalcoholic extract was stored at −20˚C until used.

### Experimental animals

One hundred and twenty (n= 24) healthy male Wistar rats weighing (190-200 g; 9 weeks of age) were used in the study. The animals were left in separate cages for five days at room conditions for acclimatization.

Animal kept in standard environmental condition at temperature of 22 ± 3°C, humidity (60 ± 5%) and a 12h light/dark cycle. All animals had *ad libitum* access to standard pellet diet and fresh water. The animals handled on a regular daily basis for 2 weeks prior to the study in order to acclimatize them to experimental procedure and minimize anxiety related testing inaccuracies. The procedures were carried out based on the guidelines of the Ethics Committee of the International Association for the Study of pain **[8]**. The University Research Council approved all experiments.

**Preliminary phytochemical assessment**

Aerial part (flower and leaves) of *Lavender officinalis* was undergone preliminary phytochemical evaluation in order to elevate major phytoconstituents in the extract formulation. The presence of phytochemicals, alkaloids, flavonoids, saponins, phenol and terpenoid were performed based on previous reports **[9, 10]**.

**Formulation of Topical Wound Application Forms**

In this study, 4 variants of the topical application were prepared comprising Eucerin (30%) and Vaseline (70%) as the base ointment formulation. After surgical wound creation, all rats were labeled by none toxic color and randomly divided into five groups (n=6). Group 1 (negative control) had no received any administration. Group 2 (positive control) received the base formulation (placebo). Groups 3, 4 and 5 treated with 1, 3 and 5g of *Lavender officinalis* hydroethanolic extract mixed with base formulation ointment (LOO), respectively. The ointments were topically applied once a day, starting from the day of operation, on the wound area until the wound completely healed.

**Wound healing activity**

**Circular Excision Wound Model**

The wound model is used to monitor wound closure time and wound contraction. After anesthesia induction with 5 mg/kg/IP Xylazine HCL 2% (Alfasan Co., Netherlands) and 50 mg/kg/IP ketamine HCL 10% (Alfasan Co., Netherlands) animals were fixed in a ventral position on a surgery table. The dorsal area skin from the scapula to the ileum was prepared aseptically. One circular full thickness surgical wound in 150 $mm^2$ area was created. Then the percentage of wound closure and epithelialization time were measured **[11]**.

**Wound Healing Measurement**

The percentage of wound healing was computed at the beginning of the experiment and on days 3, 6, 9, 12, 15 and 18 days post-operation. The wound area was measured using a transparent paper over the wound and tracing it out. The area of this impression was calculated using the graph

sheet. The wound healing percentage was calculated by the Walker formula **[12]**.

Percentage of wound size = wound size in day X / wound size in the first day ×100

Percentage of wound healing = 100 - Percentage of wound size

**Histopathological study**

Animals anesthetized with the same way mentioned above and specimens from skin were taken on 3, 6, 12 and 18 days after surgery. Sample tissues were excised along with 1 to $2^{mm}$ surrounding normal skin and in a depth of approximately $3^{mm}$ 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, stained with hematoxylin and eosin (H&E) and Masson's trichrome stains and examined under light microscopy (Olympus CX31RBSF attached cameraman) to assess the predominant stage of wound healing. Three parallel sections were obtained from each specimen. The number of poly morphonuclear cells (PMN), mononuclear cells (MNC), fibroblast aggregation and new vessels (NV) were evaluated in 5 per high power fields (HPFs) (¬×400). They were analyzed in 5 per high power fields (HPFs) (¬×100) **[13, 14]**.

**Statistical Analysis**

Data analyzed by one-way analysis of variance (ANOVA) using PASW 18.0 (SPSS, Inc., Chicago, IL, USA), and is presented as mean ± SEM. For treatment showing a main effect by ANOVA, means have compared by Dunnett's test. $P<0.05$ was considered as significant differences between treatments.

**RESULTS**

**Preliminary phytochemical assessment**

The phytochemical analysis of *Lavender officinalis* aerial is presented in **Table 1**. According to the data, *Lavender officinalis* aerial contains alkaloids, flavonoids, tannins, saponins and terpenoids **(Table 1)**.

**Rate of Wound enclosure**

The data of wound closure are expressed in **Table 2**. Wound sizes were measured at 3, 6, 9, 12, 15 and 18 days of post-surgery. According to the data, a significant decrease in wound size observed on day 7 post-operative ($P<0.05$). Also, wound closure time was faster in LOO-treated rats compared to control and placebo groups. Furthermore, on day 18, the wound in the treatment approximately enclosed in LOO-treated animals in comparison to control group ($P<0.05$).

**Histopathological findings**

The effects of LOO on PMN, MNC, new vessel formation and the fibroblast cell proliferation count are presented in **Figures 1-5**.

As seen in **Figure 1**, PMN significantly decreased by 3% and 5%-LOO treated

animals compared to control group on day 3 and 6 postoperative (P<0.05). As seen in **Figure 2**, administration of LOO, specially 3% and 5% doses, significantly increased MNC filtration to the wound site (P<0.05). According to the results **(Figures 3 and 4)**, administration of LOO, specially 3% and 5% doses, significantly increased NV formation by 5%-LOO treated animals compared to control group on day 6 postoperative (P<0.05). As noticed **(Figures 5)**, in LOO treated animals, specially 3% and 5% doses, significantly increased Fibroblast distribution compared to control group on days 6 and 12 post injury (P<0.05).

**Table 1. Phytochemical Screening of Secondary Metabolites of hydroethanol aerial part extract of Lavender officinalis aerial part.**

| Secondary metabolites | hydroethanol extract |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Terpenoid | + |
| Saponin | + |
| Phenol | + |

**Note: + = present, - = absent**

**Table 2. Effect of *Lavender officinalis* hydroethanolic extract ointment (LOO) on circular excision wound contraction area ($mm^2$) and period of epithelialization.**

| Groups | Wound area in days | | | | | | Period of epithelialization (days) |
|---|---|---|---|---|---|---|---|
| | Day 3 | Day 6 | Day 9 | Day 12 | Day 15 | Day 18 | |
| Control | 155.69±1.7 | 95.49±0.34 | 52.24±1.31 | 43.78±0.73 | 29.18±1.26 | 22.59±0.96 | 18.86±1.44 |
| Placebo | 152.37±0.63 | 92.49±0.34 | 50.64±0.84 | 40.57±1.92 | 26.91±0.83 | 20.58±0.84 | 18.13±1.36 |
| LOO1% | 144.29±3 | 82.77±1.69 | 41.23±1.09 | 29.94±0.91* | 19.93±0.57* | 11.9±0.71 | 17.03±0.75 |
| LOO3% | 130.53±1.05 | 77.73±1.59* | 36.58±0.84* | 21.04±0.29* | 14.77±1.5* | 5.2±0.53* | 16.4±0.66* |
| LOO5% | 125.02±1.58 | 66.34±0.73* | 29.28±0.33** | 12.37±0.5** | 8.01±0.7** | 0.9±0.33** | 14.1±0.38** |

**n= 6 animals in each group. Valued are expressed as mean ± SEM. The treated groups are compared by Student t test with the control group. *P<0.05 vs Control.**

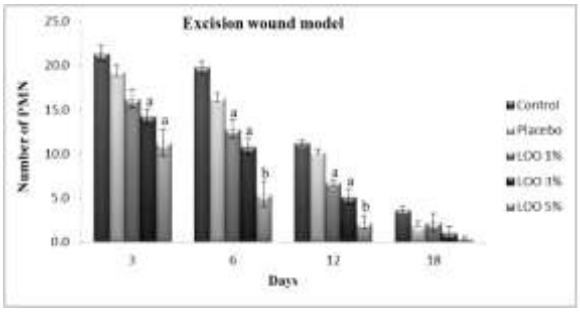


**Figure 1: Data are presented as means ± SEM; The number of poly morphnuclear cells (PMN) infiltration at the wound site. There are significant differences between groups with different codes in a raw (superscript letters a and b; P < 0.05)**

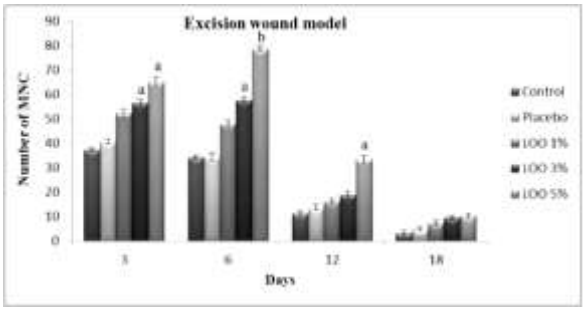


**Figure 2: Data are presented as means ± SEM; The number of mononuclear cells (MNC) infiltration at the wound site. There are significant differences between groups with different codes in a raw (superscript letters a and b; P < 0.05)**

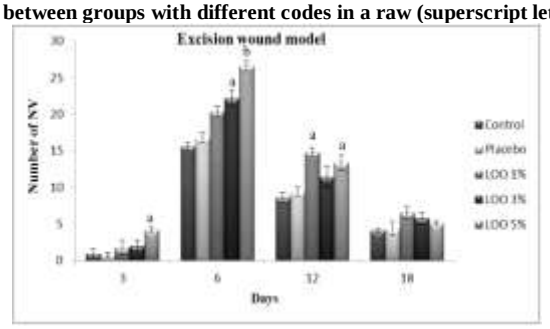


**Figure 3: Mean ± SEM number of the number of new vessel formation (NV) at the wound site. There are significant differences between groups with different codes in a raw (superscript letters a and b; P < 0.05)**

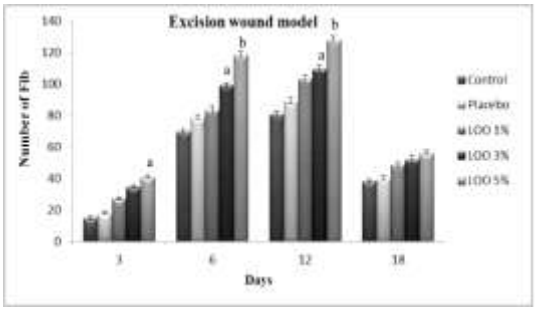


**Figure 4: Mean ± SEM number of fibroblast distribution (Fib) at the wound site. There are significant differences between groups with different codes in a raw (superscript letters a; P < 0.05)**

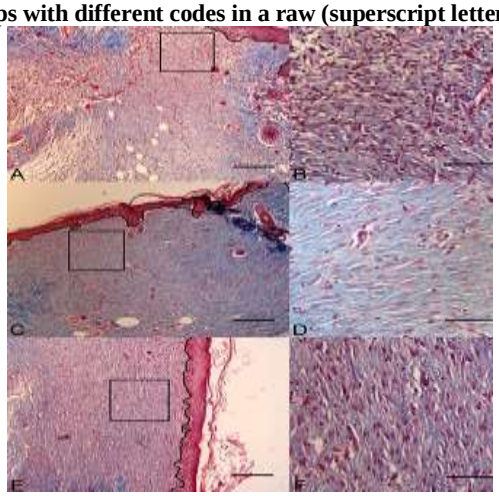


**Figure 5: Histopathological characteristics of rat skin on the 18th day after wound creation in excision wound model. A cross section from the skin after 18 days; (A, B,) Negative control group: the regenerated epidermal tissue is marked with continuous line in Figure A. Note the higher magnification of the healed tissue with moderate epithelial regeneration and collagen bundles regularity. (C, D) treated group with 3% of the Lavender officinalis hydroethanolic extract ointment: the regenerated epidermal tissue is marked with continuous line in Figure C. Note, the higher magnification of the healed tissue with well epithelial regeneration and remarkable collagen bundles regularity. (E, F) treated group with 5% of the Lavender officinalis hydroethanolic extract ointment: the regenerated epidermal tissue is marked with continuous line in Figure E. Note, the higher magnification of the healed tissue with high epithelial regeneration and remarkable collagen bundles regularity. Masson trichrome staining, A, C, E: 100×; B, D, F: 400×.**

**DISCUSSION**

To our knowledge, few studies done to investigate effects of *Lavender officinalis* essential oil cream on wound healing. *Lavender officinalis* is a member of the Lamiaceae family and contains high levels of phenolic compounds and flavone glucosides **[5]**. There are many reports that phenolic compounds, proanthocyanidins, flavonoids and flavone glucosides which are synthesized by plants have therapeutic effect such as wound healing, antioxidant, anti-inflammatory and antinociceptive **[15, 16]**.

Based on histopathologic findings of the present study, LOO-treated group had better wound healing than the control group. Macrophages are the principal cells in the inflammatory phase of wound healing **[1, 17]**. Macrophages attract to the wound area to phagocyte invested pathogens and release growth factors and cytokines. This phenomenon leads to migration of fibroblasts and myofibroblasts as well as promoting collagen synthesis in the wound area **[18, 19]**. As seen in **Figures 1 and 2**, LOO dose dependently decreased PMN and increased MNC in rats on day 3 post-surgery. Increased MNC in the wound site resulted in a decrease in PMN (inflammatory phase) that indicates the completion of the first phase of wound healing (inflammatory phase) and the beginning of the second stage of healing process. Based on our data, LOO ointment dose dependently increased MNC and fibroblasts in wound area. According to previous reports, anti-inflammatory effect might relate to caryophyllene oxide **[5]**, terpenoid oxide and cineole content of *Lavender officinalis* **[4, 20]**.

New vascular formation is needed to promote the proliferation phase in wounded tissue **[1, 21]**. As seen in figure 3, there was significant differences between 5%-LOO treated animals in neovascularization compared other groups on day 3. Fibroblasts by secreting collagen, play a major role in wound contraction and tensile strength **[1, 21, 22]**. According to the results of this study, LOO amplified fibroblasts concentration and maintained high during the experiment (especially 3 and 5%-LOO treated animals) **(Figure 4)**. This could indicate an increase in collagen synthesis in LOO treated animals which increases tensile strength of skin **(Table 2)**.

**CONCLUSION**

In conclusion, topical administration of the *Lavender officinalis* hydroethanolic extract ointment, especially higher dose, improved wound healing as an in vivo experimental wound models in rat. We think further studies needed to clarify pharmacological properties and physiological mechanisms by those phenolic compounds in the *Lavender*

*officinalis* hydroethanolic extract. Additionally, merit studies are needed to distinguish its potential for clinical use in clinical trials.

**CONFLICT OF INTEREST**

There are no conflicts of interests to declare.

**REFERENCES**

**[1]** Beldon P, Basic Science of wound healing. Surgery (Oxford), 2010, 28, 409-412.

**[2]** Cavanagh H, Wilkinson J, Biological activities of lavender essential oil. Phytotherapy Research, 2002, 16,301-8.

**[3]** Moon T, Wilkinson J, Cavanagh H, Antibacterial activity of essential oils, hydrosols and plant extracts from Australian grown Lavandula spp. International Journal of Aromatherapy, 2006, 16 (1),9-14.

**[4]** Goren AC, Topcu G, Bilsel G, Bilsel M, Aydogmus Z, Pezzuto JM, The chemical constituents and bioloopgical activity of essential oil of Lavandula stoechas ssp. Stoechas. Zeitschrift fur Naturforschung C-Journal of Biosciences, 2002, 57,797-800.

**[5]** Gabrieli C, Kokkalou E, A new acetylated glucoside of luteolin and two flavone glucosides from Lavandula stoechas ssp. stoechas. Die Pharmazie - An International Journal of Pharmaceutical Sciences, 2003, 58,426-7.

**[6]** Ulubelen A, Gören N, Olcay Y, Longipinene derivatives from Lavandula stoechas subsp. stoechas. Phytochemistry, 1988, 27, 3966-67.

**[7]** Gilani AH, Aziz N, Khan MA, Shaheen F, Jabeen Q, Siddiqui BS, Herzig JW, Ethnopharmacological evaluation of the anticonvulsant, sedative and antispasmodic activities of Lavandula stoechas L. Journal of Ethnopharmacolology, 2000, 71, 161-7.

**[8]** Zimmermann M, Ethical guidelines for investigations of experimental pain in conscious animals. Pain, 1983, 16, 109-110.

**[9]** Sachin J, Neetesh J, Tiwari A, Balekar N, Jain DK, Simple evaluation of wound healing activity of polyherbal formulation of roots of Ageratum conyzoides Linn. Asian Journal of Research in Chemistry, 2009, 2(2), 135-8.

**[10]** Sasidharan S, Nilawatyi R, Xavier R, Yoga Latha L, Amala R, Wound Healing Potential of Elaeis guineensis Jacq Leaves in an Infected Albino Rat Model. Molecule, 2010, 15, 3186-99.

**[11]** Lai HY, Lim YY, Kim KH, Potential dermal wound healing

agent in Blechnum orientale Linn. BMC Complementary and Alternative Medicine, 2011, 11(1), 62.

**[12]** Walker HL, Mason AD, A standard animal burn. Journal of Trauma, 1968, 8, 1049-51.

**[13]** Akkol EK, Koca U, Pesin I, Yilmazer D, Evaluation of the Wound Healing Potential of Achillea biebersteinii Afan. (Asteraceae) by In Vivo Excision and Incision Models. Evidence-Based Complementary and Alternative Medicine, 2011, 474026.

**[14]** Karayannopoulou M, Tsioli V, Loukopoulos P, Anagnostou T, Giannakas N, Savvas I, Papazoglou LG, Kaldrymidou E, Evaluation of the effectiveness of an ointment based on Alkannins/Shikonins on second intention wound healing in the dog. Canadian Journal of Veterinary Research, 2011, 75, 42-8.

**[15]** Meotti FC, Luiz AP, Pizzolatti MG, Kassuya CA, Calixto JB, Santos AR, Analysis of the antinociceptive effect of the flavonoid myricitrin: evidence for a role of the L-arginine-nitric oxide and protein kinase C pathways. Journal of Pharmacology and Experimental Therapeutics, 2006, 316, 789-6.

**[16]** Nayak BS, Sandiford S, Maxwell A, Evaluation of the wound-healing activity of ethanolic extract of Morinda citrifolia L. leaf. Evidence-Based Complementary and Alternative Medicine, 2009, 6, 351-6.

**[17]** Brancato SK, Albina JE, Wound macrophages as key regulators of repair: origin, phenotype, and function. American Journal of Pathology, 2011, 178, 19-5.

**[18]** Albina JE, Mills CD, Henry WL, Caldwell MD, Temporal expression of different pathways of 1-arginine metabolism in healing wounds. The Journal of Immunology, 1990, 144, 3877-80.

**[19]** Singer AJ, Clark R, Cutaneous wound healing. The New England Journal of Medicine, 1999, 341, 738-6.

**[20]** Hajhashemi V, Ghannadi A, Sharif B, Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of Lavandula angustifolia Mill. Journal of Ethnopharmacology, 2003, 89, 67-1.

**[21]** Deodhar AK, Rana R, Surgical physiology of wound healing: a

review. Journal of Postgraduate Medicine, 1997, 43, 52-6.

**[22]** Topçu G, Ayral MN, Aydin A, Gören AC, Chai HB, Pezzuto JM, Triterpenoids of the roots of Lavandula stoechas ssp. stoechas. Die Pharmazie, 2001, 56, 892-5.