Wound Healing Activity of Extracts of *Malva sylvestris* and *Stachys lavandulifolia*

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Abstract

The flowers of *Stachys lavandulifolia* Vahl (Lamiaceae) and *Malva sylvestris* Linn (Malvaceae) are traditionally used to treat various skin disorders, antimicrobial and anti-inflammatory. The chloroform extract of *M. sylvestris* flowers and aqueous extract of *S. lavandulifolia* flowers were used to evaluate the wound healing activity at 200 mg/kg/day dose. Wounds were induced in Wistar rats divided into four groups as following; Group-I was treated with cold cream. Groups-II and -III were treated with cold cream containing of extracts, Group-IV received the standard drug (nitrofurazone). The efficacy of treatment was evaluated based on wound area and histopathological characteristics. The extract-treated animals by *M. sylvestris* showed significant reduction in the wound area when compared with other groups. Also, histopathological studies of the tissue obtained on days 6th, 9th and 16th from the extract-treated by *M. sylvestris* showed increased well organized bands of collagen, more fibroblasts and few inflammatory cells.

Keywords: Wound healing, Medicinal plants, *Stachys lavandulifolia*, *Malva sylvestris*

1. Introduction

Approximately one-third of all traditional medicine in use are for the treatment of wounds and skin disorders, compared to only 1-3% of modern drugs (Mantle et al., 2001). Reports about medicinal plants affecting various phases of the wound healing process, such as coagulation, inflammation, fibroplasia, epithelization, collagenation and wound contraction are abundant in the scientific literature (Asif et al., 2007; Hemmati & Mohammadian, 2000; Khalil et al., 2006; Nayak et al., 2007). A survey of the ethnobotanical studies, carried out in Iran, indicated the use of several of plant species by the inhabitants of the area, especially by those habiting the rural areas for wound healing purpose (Ghasemi Pirbalouti, 2009a; Ghorbani, 2005; Zargari, 1990).

Malva sylvestris Linn (Malvaceae), known locally as “Panirak”, is a medicinal plants in Iran whose flowers are used for the treatment of various ailments, including cold, cough and burn and cut wound healing in rural areas of Iran (Ghasemi Pirbalouti, 2009a; Ghorbani, 2005; Zargari, 1990). Fluidextract of *M. silvestris* flowers and leaves are used as a valuable remedy for cough and inflammatory diseases of mucous membranes (Farina, 1995). A new anthocyanin, malvidin 3-(6″-malonylglucoside)-5-glucoside has been characterized in both wild and cultivated forms of *M. Sylvestris* (D’Amelio, 1999; Takeda et al., 1989). The malvone A (2-methyl-3-methoxy-5, 6-dihydroxy-1, 4-naphthoquinone) is reported (Cutillo et al., 2006; Veshkurova et al., 2006)

Stachys lavandulifolia Vahl (Lamiaceae) a well-known traditional herb used in tribal medicine of Iran is locally known as “Chaye-e-Kohi or Lolopashmak”. The decoction of the flowers is being used by the tribal people for treatment of skin infection, menorrhagia and anti-bacterial (Ghasemi Pirbalouti, 2009b; Zargari, 1990).

No systematic studies have yet been carried out on the clinical evaluation of the wound healing potency of *M. sylvestris* and *S. lavandulifolia* so its effects were investigated using wound are and histopathological characteristics in rats.

2. Materials and Methods

2.1 Plant material and extract preparation

The flowers of *M. sylvestris* and *S. lavandulifolia* were collected from mountain areas of Zagross, district of Chaharmahal va Bakhtiari, Iran, during May–June, 2008. Their identity was confirmed and voucher specimens were deposited at the spice, aromatic and medicinal plant research centre (SAMPRC), Islamic Azad University, Iran (Mozaffarian, 1996). Powdered flowers of *M. sylvestris* (300 g) were Soxhlet-extracted with 80% chloroform (Merck, Germany) for 6 h (yield: 6%). The flower powder (200 g) of *S. lavandulifolia* was refluxed with 750mL of double distilled water for 2 h at 70 °C. The infusions were filtered and concentrated under rotary vacuum (model Zirbus 302®) for about 1 h (yield: 5.5%).

2.2 Animals

Male Wistar rats (180-200 g) of 2-3 months were used. The animals were housed in standard environmental conditions of temperature (22 ±3ºC), humidity (60 ±5%) and a 12 h light/dark cycle. During experimental time rats were given standard pellet diet (Pastor Institute, Iran) and water *ad libitum*.

2.3 Wound healing activity

Wound induction and evaluation extracts for properties wound healing before the beginning of the wound healing experiments, the dorsal skin of the Wistar rats were shaved. Animals were anesthetized with 1.5mg/kg *i.p*. of Ketamin and Xylazine. A full thickness of the excision wound (circular area about 150 mm^2 and 2 mm depth) was created along the markings using toothed forceps, a surgical blade and pointed scissors (Khalil et al., 2006).

The animals were divided randomly into three groups of nine each. Group-I was treated with cold cream (Control). Groups-II and -III were treated topically with cold cream prepared from aqueous extract of *S. lavandulifolia* and *M. sylvestris* (200 mg/kg/day) respectively, Group-IV received the standard drug (nitrofurazone).

During the wound healing period and at the present time intervals, the wound area was traced manually and photographed. The wound area was calculated using AutoCAD Version 14 $_{\text{(Autodesk Company)}}$ software. At days 6^{th}, 9^{th} and 16^{th} the experiment was terminated and the wound area was removed from the surviving animals for histological examination. The excision skin biopsies were fixed in 4 % formaldehyde solution 48 h during the experimentation period.

2.4 Analysis of data

The relative burn wound area was statistically analyzed as mean ±S.D and statistically significance between treated and control groups were analyzed by means of Student's *t*-test. Data are significant; *P*-values ≤0.05 compared with control by the program "SAS $_{\text{ver 6.12 full}}$".

3. Results

The animals treated with the *M. sylvestris* showed a significant reduction in the wound area when compared with other groups (Table 1). The animals treated with the extract of *M. sylvestris* showed faster epithelialization than those treated with the standard drug and aqueous extract of *S. lavandulifolia*. The extract-treated animals by *M. sylvestris* showed about 99% reduction in the wound area when compared with nitrofurazone and *S. lavandulifolia* extract which were 95 and 92%, respectively (Table 1).

The study of the histological structure showed the tissue regeneration was grater in the skin wound treated with cold cream containing the *M. sylvestris* extract and following nitrofurazone ointment and *S. lavandulifolia* extract (Table 2 and Fig 1). The skin wound treated with cold cream (control) presented edema, monocyte cells and area with cellular necrosis that were not observed in the treated with herbal ointments and standard drug (Table 2 and Fig 1).

4. Discussions and Conclusions

Wound healing is a process by which damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of the area of the wound (Nayak et al., 2007). It is mainly dependent upon the type and extent of damage, the general state of health and the ability of the tissue to repair.

Despite the traditional uses *M. sylvestris* and *S. lavandulifolia* in wound healing process in Iran, there are no reported data available in the literature. *M. sylvestris* and *S. lavandulifolia* widely distributed plants of Iran are used for the infectious, anti-inflammatory, anti-microbial, skin disease and for wound healing properties according to several ethnobotanical surveys (Ghasemi Pirbalouti, 2009a,b; Ghorbani, 2005; Zargari, 1990).

In present study, results of wound area measurements, as shown in table 1, indicated a healing potential for the *M. sylvestris* extract. Statistically, area measurements showed that there is significance between the different groups.

Wound healing is a very complex, multifactor sequence of events involving several cellular and biochemical processes (Philips et al., 1991). The aims in these processes are to regenerate and reconstruct the disrupted anatomical continuity and functional status of the skin (Philips et al., 1991). The results in this study are in support that wound healing and repair is accelerated by applying *M. sylvestris* which was high-lighted by the full thickness coverage of the wound area by an organized epidermis in the presence of mature scar tissue in the dermis. This ability was especially obvious when the data were compared with the other groups. The results of histological evaluation showed that *M. sylvestris* significantly increased the rate of wound contraction and collagen turnover. Collagen, the major component which strengthens and supports extracellular tissue, is composed of the amino acid, hydroxyproline, which has been used as a biochemical marker for tissue collagen (Philips et al., 1991).

The preliminary phytochemical analysis of the flower extract by researchers showed the absence of anthocyanin, malvin, malvidin 3-(6″-malonylglucoside)-5-glucoside, malvaline, niacin and folic acid. Any one of the phytochemical constituents (malvone A: 2-methyl-3-methoxy-5, 6-dihydroxy-1,4-naphthoquinone) present in *M. sylvestris* may be responsible for antimicrobial activity (Cutillo et al., 2006; D'Amelio, 1999). It may be either due to the individual or additive effect of the phyto-constituents that hastens the process of wound healing. The exact component of the extract that is responsible for this effect, however, was not investigated. Further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

In conclusion, while plant based traditional medicine has been used throughout generations, the efficiency of such treatments requires experimental backup and scientific verification. In this study, two plant species presented were selected based on ethnopharmacological information, provided by local communities. This study confirms the wound healing activity of the flowers of *M. sylvestris*. Further studies need to be done to identify and separate the group of active constituents responsible for anti-inflammatory activity and wound healing activity from alcohol and petrol ether extracts.

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Table 1. Effect of the treatments on wound healing in rats

Treatments	**Wound area relative (cm^2)**		
	6^{th}	**9^{th}**	**16^{th}**
S. lavandulifolia + Cold cream	0.78±0.22 **	0.39±0.21 *	0.117±0.4 *
M. sylvestris + Cold cream	0.87±0.05 **	0.84±0.05 *	0.23±0.09 **
Nitrofurazone	0.77±0.15 **	0.33±0.13 **	0.077±0.04 **
Control (Cold cream)	**1.38**	**1.132**	**0.23**

Each value represents mean ±S.D. *N= 9 animals.*

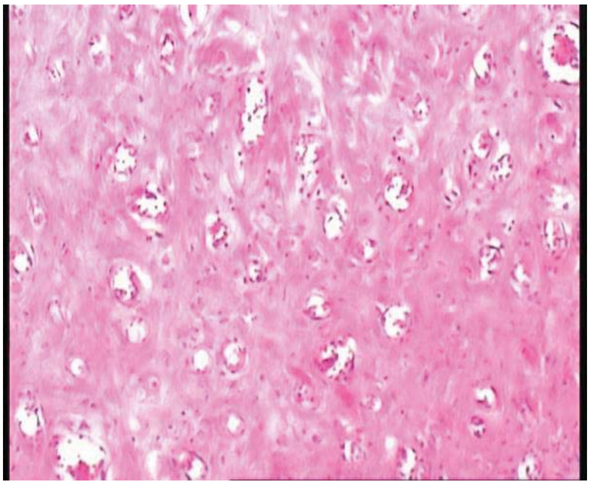
***: P≤ 0.01, *: P≤ 0.05* levels of significance.

Table 2. Effect of the treatments on the evolution of wounds in rats after 6, 9 and 16 days of topical application

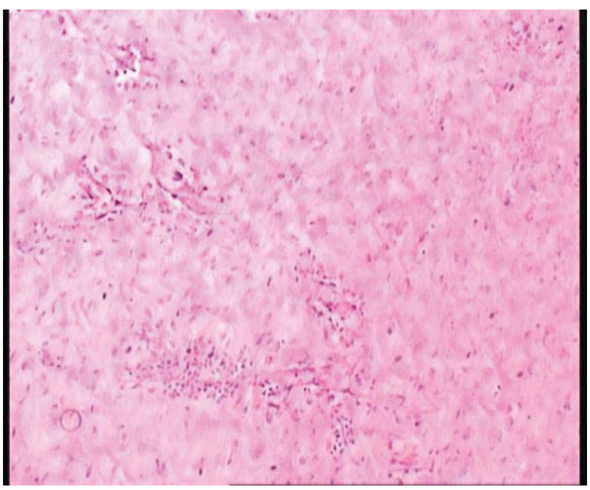
Treatments	***Inflammatory cells***			***Collagen fibers***			***Re-epithelization***			***Organization of the collagen***			***Necrosis***			***Fibrin***		
	6	***9***	***16***	***6***	***9***	***16***	***6***	***9***	***16***	***6***	***9***	***16***	***6***	***9***	***16***	***6***	***9***	***16***
Control **(Cold cream)**	+++	++	++	–	–	+	–	–	+	–	–	+	+++	++	+	+++	++	+
Standard drug **(Nitrofurazone)**	+	+	_	+	+	++	+	+	++	+	++	++	+	_	_	++	+	_
S. lavandulifolia	+	+	+	+	+	++	+	+	+	+	+	++	+	+	_	++	+	_
M. sylvestris	+	_	_	+	++	+++	+	+	+++	++	++	+++	+	_	_	+	+	_

+: slight, ++: moderate, +++: extensive, -: absent.

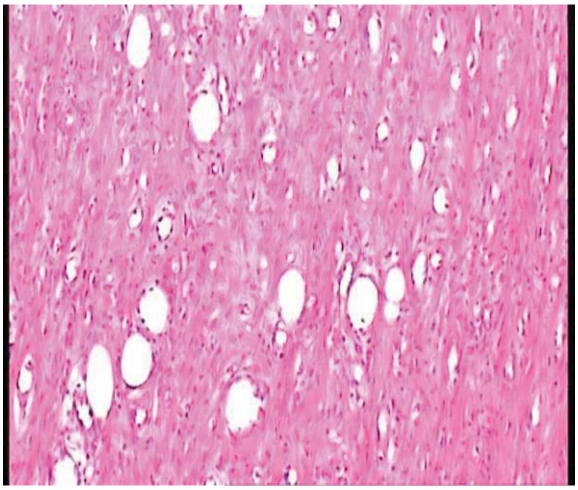
Figure 1. Flowers and areal plant of *Malva sylvestris* and *Stachys lavandulifolia*



Nitrofurazone (a)



M. sylvestris + cold cream (b)



Stachys lavandulifolia (c)

Figure 2. Histological evaluation after 16 days of wound creation in three groups (a, b & c)