Effects of methanolic extract of *Verbascum inulifolium* Hub.-Mor. on incisional and excisional skin wounds in diabetic and non-diabetic rats

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The genus *Verbascum*, commonly known as mullein, traditionally used in folk medicine, is known for its anti-inflammatory, antioxidant and antibacterial activities. *Verbascum inulifolium* Hub.-Mor. (Scrophulariaceae), is an endemic species from Turkey. Here, we examined the healing effect of methanol extract of the aerial parts of *V. inulifolium* on incisional and excisional skin wounds in diabetic and non-diabetic rats. Ointments of the extract were prepared in two concentrations (0.5 and 1% (w/w)) and applied topically on wound models once daily throughout for 7 and 14 days. During the experiments, wounds were visually observed, photographically documented and wound areas were measured. After 7 and 14 days treatments, animals were sacrificed and measurements of hydroxyproline level and biomechanical analysis were performed. Histopathology of the wound area was evaluated considering features of re-epithelialization, the granulation tissue thickness, angiogenesis, presence of inflammation, number of mast cells. Outcomes of this study revealed that the ethanolic extract of the aerial parts of *V. inulifolium* enhances the healing process of skin tissue in both non-diabetic and diabetic wounds. The best wound healing activity was observed in incisional wound treated with 0.5% (w/w) concentrated ointment (99.7%) and in excisional wound treated with 1% (w/w) concentrated ointment (98.1%) on day 14 according to macroscopic results.

**Keywords:** Diabetes mellitus, Mullein, Re-epithelialization, Velvet plant, Wound healing

Wound healing is a complicated and integrated physiological, histological and biochemical process in which a variety of cells like inflammatory cells and epithelial cells are involved and also enzymes and growth factors are needed with several signal pathways. The regular healing process in healthy individuals takes place at an optimal speed, whereas in patients suffering from Diabetes mellitus (DM) it is commonly delayed or even fully impaired. Impaired wound healing in DM is a main clinical problem, such as disruption of homeostasis, acceleration in inflammation process and also inhibition of proliferation of cells due to high blood glucose, reduction in collagen production, etc.

According to the World Health Organization (WHO), approximately 80% of the human population uses plant-based medicines for health care. There are several reports on medicinal plants influencing various phases of the wound healing process, fresh wounds as well as chronic ones, including coagulation, inflammation, fibroplasia, collagenation, epithelization and wound contraction. In spite of widespread use of local plants in wound healing, only a few of them have been studied for their potential against incision and excision wound models in rats. Traditional remedies often lack in scientific evidence viz. systematic observations, methodologies and time-test. In case of traditionally used wound healing agents, only a few controlled trials have been demonstrated for clinical efficacy.

*Verbascum* L. (Scrophulariaceae), commonly called mullein or velvet plant, is a genus representing...
about 361 taxa worldover, and its species are spread over the temperate regions in the Northern Hemisphere. Out of 251 species, Turkey holds 195 which are endemic and more than 100 hybrids13-15. *Verbascum inulifolium* Hub.-Mor. is an endemic species spread in South Anatolia13,14. Various parts of *Verbascum* spp. including leaves, flowers and roots are used to treat several illnesses, such as rheumatism, eczema, respiratory disorders in Turkish traditional medicine16. In Anatolia, infusions of the genus are used for sweating, expectorant, sedative and urine enhancer while others are used for drying wounds, in anal fistula and urogenital organs for purulent conditions17. *Verbascum* spp. contain some secondary metabolites including iridoids, flavonoids, saponins, phenylethanoids, neolignan glycosides, alkaloids16,18-20, steroids18-20, fatty acids, musilage and carotenoids20. Biological activities, such as antiviral, antibacterial, antifungal16,18-20, cytotoxic, immunomodulator, antineoplastic, antibiotic, antioxidant18,19, antitumor, anti-inflammatory, neuroprotective and anthelmintic16 have also been reported.

Plant based ointments and tincture drugs have been shown to give successful results in the treatment of ulcers, sores, burns and wounds21. Plants used for wound healing also exhibit antimicrobial and antiseptic effects22. In Turkey, *Verbascum* spp. is widely used in traditional medicine for the treatment of wounds17,23. However, there is no report on the efficacy of *V. inulifolium* in treating diabetic wounds. In the present study, we evaluated the wound healing effects of *V. inulifolium* (Vi) ointment [1% and 0.5% (w/w)] on incisional and excisional skin wound models in diabetic and non-diabetic rats by examining hydroxyproline levels, tensile strength, wound healing ratio and histopathology.

**Materials and Methods**

**Animals and experimental protocol**

Male Wistar rats of 200-250 g each at the same age from Dumlupınar University Laboratory of Animal Production, Kutahya, Turkey were used for the study (n: 112). They were housed in separate cages and fed with standard chow diet and water *ad libitum* with 12 h light/dark cycle at 21±3°C. Experimental protocol was subjected to scrutiny of Dumlupınar University Institutional Animal Ethics Committee for experimental clearance (Registration no: 2012/8.1). In this study, 112 rats were randomly divided into 8 groups comprising 14 rats each.

In each group randomly selected 7 animals used for 7-day application and 7 animals used for 14-day applications. Group details are as follows: Group 1 (G1): Non treated group*; Group 2 (G2): Placebo group-vehicle*; Group 3 (G3): 0.5% *V. inulifolium* treated non-diabetic group (0.5% Vi); Group 4 (G4): 1% *V. inulifolium* treated non-diabetic group (1% Vi); Group 5 (G5): Diabetic control group*; Group 6 (G6): Diabetic placebo group-vehicle*; Group 7 (G7): 0.5% *V. inulifolium* treated diabetic group (DM/0.5% Vi); and Group 8 (G8): 1% *V. inulifolium* treated diabetic group (DM/1% Vi).

*Data of control and vehicle groups of the previous study were used24.

**Induction of diabetes**

Diabetes was induced by a single 45 mg/kg intraperitoneal injection of Streptozotocin (STZ) (Sigma Chemical Co. (USA)). Blood glucose levels were measured using a rapid glucometer (Bayer, Germany). Once 3 days after STZ injection, animals with blood glucose levels above 300 mg/dL defined as diabetic and used in the study.

**Plant material**

The fresh parts of *V. inulifolium* were collected from its natural habitat C4 Icel Silifke Castle, Mersin, Turkey at 110-120 m altitude during the month of June, 2014. The collected plant material was identified by Dr. S Guzel and the dried voucher specimens were kept in Herbarium of Ankara University, Faculty of Pharmacy, Ankara, Turkey (AEF 23713).

**Extraction and Ointment processing**

Air-dried aerial parts of *V. inulifolium* were cleaned properly then powdered mechanically. The powdered plant material (2 g) was extracted with methanol (250 mL) using Soxhlet apparatus. Whatman No: 1 filter paper was used for filtration procedure and the filtrate was evaporated for removing solvent under reduced pressure by using vacuum evaporator (Heidolph-Rotar TLR 1000, Germany) at 35-40°C. The extract was stored in dark at 4°C until further use. Simple ointment base was prepared according to the method based on Suntar et al.25 by using glycol stearate: propylene glycol:liquid paraffin (3:6:1). Then, extracts of 0.5% and 1% (w/w) of *V. inulifolium* were added, respectively in this mixture to prepare the ointments. The non-diabetic placebo and diabetic placebo-vehicle groups were treated with the mixture...
of glycol stearate: propylene glycol:liquid paraffin in the ratio of 3:6:1. During the experiments each ointment was served as 0.5 g topically on wound models once daily for 7 and 14 days.

Wound models
Surgical applications for skin tissue wound models were performed under anesthesia induced by intraperitoneal injection of 10 mg/kg xylazine hydrochloride and 30 mg/kg ketamine hydrochloride. Before surgical application dorsal aspect of each anesthetic animal was shaved then wound area was cleaned using ethanol [70% (v/v)].

Excision wound model
For monitoring wound contraction and wound closure time, the open excision type wounds were created by biopsy punch on the back region of rats with 1.5 cm in a circular manner with the removal of skin. After surgical applications the excision wounds were left open. The V. inulifolium ointments and the vehicles were topically applied once a day throughout 7 and 14 days. On day 8, randomly selected 7 animals in each group were sacrificed under anesthesia for evaluating 7-day applications. The remaining 7 animals in each group were continued to apply topically with V. inulifolium ointments and the vehicles for 14 days. On day 15, the animals were sacrificed under anesthesia for evaluating 14-day applications.

Incision wound model
For biomechanical tests, the incisional wounds were created from 2 cm posterior to the excision wounds with 4 cm in length then each 1 cm apart three surgical sutures were placed. The V. inulifolium ointments and the vehicles were topically applied once a day throughout 7 and 14 days. On the 7th post-wound day, the sutures of randomly selected 7 rats in each group were removed and on day 8 they were sacrificed under anesthesia for evaluating 7-day applications. The remaining 7 animals in each group were continued to apply topically with V. inulifolium ointments and the vehicles for 14 days. On day 15, the animals were sacrificed under anesthesia for evaluating 14-day applications.

Macroscopic study
After wound creation on day 0, under anesthesia wounds were photographed by a camera (Spot Insight QE, Diagnostic Instruments, USA) and the rats with scarred skin tissue were individually placed in the cages. After 7 and 14 days treatment, wounds were photographed, wound areas on days 0, 7 and 14 were evaluated with SPOT Advanced (Diagnostic Instruments) program in order to determine progressive changes in the wound size. Briefly, the degrees of healing of the incision and excision wounds were evaluated by measuring the length and surface of wounds and then for each animal following equation was used to calculate wound healing ratio:

\[
\text{Wound healing ratio (\%)} = 100 \times \left(1 - \frac{\text{specific day wound size}}{\text{initial wound size}}\right)
\]

Biomechanical analysis
With termination of the topical applications, isolated incision wound (8×20 mm) tissues were placed in Ringer solution (pH 7.4, 15 min) and kept in −20°C until performing biomechanical analysis. Tensiometer (BIOPAC MP30 Acq. (Santa Barbara, USA) was used for measuring tensile strength. The cross section areas were determined and then the sections were subjected to a tensile test. Tensile strength of samples were measured by a biomechanical module (1 mm/s tensile stretching speed, 5 g/s stretching force, 2.5 kg max force) compatible with BIOPAC MP-30 Acq. (Santa Barbara, USA).

Measurement of hydroxyproline levels
Samples were weighed frozen, lyophilized and pulverized. Twenty-five microlitres from samples taken for hydrolisation and dissolved in the 1 mL of 50% (v/v) isopropyl alcohol. Chloramine-T was added to these samples 10 min later. Then they were incubated for 90 min at 50°C after adding 1 mL Ehrlich’s reagent. A colour change after the reaction was noted under 560 nm wave-length spectrophotometer (Shimadzu UV 1601, Shimadzu, Tokyo, Japan). Under the same conditions, hydroxyproline standards (0.2, 0.4, 0.6, 0.8, 1.2 and 1.6 µg) were also studied. Sample concentrations were calculated by the help of standard curve. Results were given as µg/mg dry wt. of tissues.

Histopathological evaluation
Histopathological evaluation was carried out for the V. inulifolium ointment and vehicle treated groups. The gross histopathological changes of the wound site were evaluated by Hematoxylin eosin (H&E) and also Toluidine Blue (TB) and Van Gieson (VG) staining. Tissues fixed in formalin were embedded in paraffin.
5 μm thick tissue sections were cut, stained and examined under a light microscope (Olympus, Japan).

Incisional and excisional biopsy samples of the subjects were evaluated for criteria as re-epithelialization, thickness of granulation tissue, angiogenesis, degree of inflammation, extent of dermal inflammation, number of mast cells, collagen deposition and presence of fibrosis. Scoring was given in Table 1.1-3.

Statistical analysis
Data analysis was performed by SPSS 16. Data represented as the mean ± SD for 112 rats and were evaluated using ANOVA test. Values of \( P < 0.05 \) and \( P < 0.01 \) were considered to be statistically significant.

Results

Macroscopic study
During the wound healing period and at the present time intervals (days 0, 7 and 14), non-diabetic and diabetic rat incisional and excisional skin wounds were photographed (Fig. 1 A & B); wound sizes were measured with Spot programme and wound healing ratio was calculated for each animal. As shown in Fig. 2 A & B, on day 7, wound healing ratios of \( V. \text{inulifolium} \) ointment groups (G3, G4, G7, G8) were found to be significantly higher than control and vehicle groups in both wound models (\( P < 0.05 \)).

In excisional wounds G3, G4 (\( P < 0.05 \)) and G7, G8 (\( P < 0.01 \)) and in incisional wounds G3, G4, G7 and G8 (\( P < 0.01 \)) were found significantly higher than control and vehicle groups on day 14. However, there was no significant difference between the rate of plant ointments [0.5% and 1% (w/w)].

Biomechanical analysis

Tensile strength in \( V. \text{inulifolium} \) ointments treated diabetic groups G7 and G8 on 7th day (\( P < 0.01 \)) was considered to be significant with a tendency to fall in the 14th day (\( P < 0.05 \)) when compared to control and vehicle groups. G3 on days 7 and 14 and G4 on days 7 (\( P < 0.05 \)) and 14 (\( P < 0.01 \)) were found significantly different than the control and vehicle groups (Table 2).

Measurement of Hydroxyproline levels

The hydroxyproline content in excisional skin wound tissue of G3 on 7th day and G4 on 7th and 14th days was seen to be statistically significant when compared to control groups (\( P < 0.01 \)) (Table 3). While in incisional skin wounds, G4 was found significant than control group (\( P < 0.01 \)) on both 7 and 14 days; G7 and G8 was found significant than diabetic control on day 7 (\( P < 0.05 \)).

<table>
<thead>
<tr>
<th>Periods</th>
<th>Non-Diabetic groups</th>
<th>Diabetic groups</th>
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<tbody>
<tr>
<td>G1</td>
<td>0.32±0.1</td>
<td>0.28±0.2</td>
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<tr>
<td>G2</td>
<td>0.33±0.1</td>
<td>0.31±0.6</td>
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<tr>
<td>G3</td>
<td>0.55±0.3*</td>
<td>0.72±0.6**</td>
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<tr>
<td>G4</td>
<td>0.44±0.1*</td>
<td>0.72±0.6**</td>
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<tr>
<td>14 Day</td>
<td>0.43±0.1</td>
<td>0.48±0.1</td>
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<td>0.48±0.1</td>
<td>0.61±0.3*</td>
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<td>0.60±0.2*</td>
<td>0.69±0.18*</td>
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[Measuring the average ± SD and Min-Max. *ANOVA analysis was performed. \( P < 0.05 \); **ANOVA analysis was performed. \( P < 0.01 \). (G1, Control; G2, Vehicle; G3, 0.5% Vi; G4, 1% Vi; G5, DM Control; G6, DM Vehicle; G7, DM/0.5% Vi; G8, DM/1% Vi)]
Fig. 1—Macroscopic view of excisional (A) and incisional (B) wounds for 0, 7 and 14 days. [G1, Control; G2, Vehicle; G3, 0.5% Vi; G4, 1% Vi; G5, DM Control; G6, DM Vehicle; G7, DM/0.5% Vi; G8, DM/1% Vi]

Fig. 2—Wound closure rates of excisional (A) and incisional (B) wounds in non-diabetic and diabetic rats (% contraction). [Measuring the average ± SD. Min-Max value intervals were given in parenthesis. *ANOVA analysis was performed. P<0.05; **ANOVA analysis was performed. P<0.01. (G1, Control; G2, Vehicle; G3, 0.5% Vi; G4, 1% Vi; G5, DM Control; G6, DM Vehicle; G7, DM/0.5% Vi; G8, DM/1% Vi)]
Histopathological evaluation

Histopathological scores of groups both in excisional and incisional wound models were shown in Tables 4 & 5. Histopathological studies showed that in all V. inulifolium ointments treated groups, epithelial regeneration was significant compared to control and vehicle groups on both 7 and 14 days ($P < 0.05$). At the same time, after 14 days, granulation tissue thickness of excisional wounds was significant in G3, G4, G7 and G8 groups compared to control and vehicles ($P < 0.05$).

Angiogenesis, dermal inflammation ($P < 0.05$) and inflammation ($P < 0.05; P < 0.01$) values of G3 and G4 were found significantly different than excisional control and vehicle groups on day 14.

Histopathological evaluation of the excisional and incisional wounds of non diabetic and diabetic rat skin tissues on day 0, 7 and 14 was observed with H&E, TB and VG staining. Normal skin tissue structure was observed in control group. Histopathological studies showed well organization of collagen fibers, increase in fibroblast cells and new
blood capillaries formation in non-diabetic wound models of 14 days 1% *V. inulifolium* ointment treatment. However, in diabetic control and vehicle groups mast cell infiltration and presence of inflammatory cells were apparent. After 14 days, 1% *V. inulifolium* ointment treatment of diabetic groups, acceleration in dense connective tissue besides significant epithelial tissue formation were seen. Granulation tissue replaced to fibrous connective tissue and angiogenesis were observed in sections. Mast cells were also observed to be wandering in the connective tissue (Fig. 3 A-D).

**Discussion**

The wound healing process of skin involves several steps including angiogenesis, re-epithelialization, fibroblast activation and migration and endothelial cell proliferation, all along with inflammatory response and oxidative reaction in the damaged tissue\(^{31,34}\). *Verbascum* genus possesses strong antioxidant, anti-inflammatory also antimicrobial properties which makes it ethnomedicinally valuable for wound healing applications\(^{20,35}\). Previous studies reported that the genus had antimicrobial activity against some microorganisms including *Staphylococcus* sp., *Bacillus* sp., *Escherichia coli*, *Streptococcus pyogenes*, *Candida* sp. and *Aspergillus fumigatus*. Additionally, wound healing effect of *V. specioum* with antibacterial properties against *Staphylococcus* sp., *Bacillus* sp., *Escherichia* sp., *Streptococcus pyogenes*, *Candida* sp. and *Aspergillus fumigatus* was also observed\(^{20,22}\). Mucilage, iridoids, flavonoids, saponins, phenylethanoids, lignans, steroids and alkaloids were presented in *Verbascum* species\(^{18,20}\). Antioxidant, antibacterial and antifungal activities of the phenolic constituents such as flavonoids have been demonstrated in several studies. Flavonoids and phenylethanoids isolated from this genus had antimicrobial and anti-inflammatory activities\(^{20}\). Several species of *Verbascum* are used as sedative, diuretic, mucolytic and expectorant in folk medicine\(^{35,36}\). Mucilages are used for intestinal difficulties, saponins are related to expectorant and diuretic effects and also saponins, mucilages and alkaloids of the genus have anti-inflammatory and antimicrobial effects\(^{20}\).

Present study revealed that topical applications of ointments obtained from *V. inulifolium* extract [0.5% and 1% (w/w)] causes marked acceleration in wound healing on non-diabetic and diabetic incisional and excisional skin wound models compared to the control groups. Further, to substantiate the healing effects of *V. inulifolium* ointment on these wound models, we performed macroscopical, histopathological, biomechanical and biochemical analysis on 0, 7th and 14th days of treated wounds.

### Table 5 — Histological scores of groups in incisional wound model

<table>
<thead>
<tr>
<th>Application periods</th>
<th>Groups</th>
<th>Re-epithelialization</th>
<th>Granulation tissue</th>
<th>Angiogenesis</th>
<th>Inflammation</th>
<th>Dermal inflammation</th>
<th>Mast cell</th>
<th>Collagen</th>
<th>Fibrosis</th>
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<td>Parameter (Mean ± Standard Deviation)</td>
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<tr>
<td>7 Day</td>
<td>Non-Diabetic</td>
<td>G1 1.71±0.95 0.86±0.38 2.57±0.79 2.57±0.79 2.29±1.25 34.43±10.83 2.14±0.69 0.00±0.00</td>
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<td>G2 2.29±0.95 1.29±0.49 1.86±0.90 2.29±0.94 2.86±0.38 23.14±8.36 1.29±0.49 0.29±0.49</td>
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<td>G3 2.57±0.53* 0.86±0.69 2.00±1.29 1.43±0.79* 2.29±1.11 12.71±2.21 2.43±0.98 0.29±0.49</td>
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<td>G4 3.00±0.00** 1.14±0.38 0.57±0.5** 1.29±0.76* 2.0±0.82* 19.86±5.15 2.43±0.98 0.00±0.00</td>
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<td>G5 1.71±1.38 1.00±1.15 1.43±0.79* 2.29±0.95 2.29±0.76 20.14±8.15 1.29±0.76 0.14±0.38</td>
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<td>G6 1.57±1.27 0.71±0.48 2.14±0.90 2.29±0.95 2.71±0.49 21.14±7.86 1.29±0.95 0.00±0.00</td>
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<td>G7 2.42±0.79* 1.29±0.58 1.57±0.53* 2.57±0.53 2.86±0.38 23.14±9.46 1.29±0.49 0.00±0.00</td>
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<td>G8 2.14±0.90* 1.00±0.58 1.43±0.53* 1.86±1.07 2.14±0.38 21.29±5.06 0.86±0.69 0.14±0.38</td>
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<td>14 Day</td>
<td>Non-Diabetic</td>
<td>G1 2.29±0.95 0.43±0.53 1.86±1.07 1.71±0.95 2.14±1.21 24.00±12.60 2.71±0.49 0.14±0.38</td>
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<td>G2 2.14±1.07 1.14±0.38 1.71±1.25 1.57±0.98 2.71±0.49 13.29±4.42 2.57±0.79 0.00±0.00</td>
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<td>G3 3.00±0.00* 0.43±0.53 1.43±0.53 1.00±0.0* 2.0±1.15* 16.00±4.83 2.57±0.54 0.14±0.38</td>
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<td>G4 3.00±0.00* 0.86±0.69 0.86±0.69* 1.29±0.49* 1.7±0.96* 14.85±4.71 3.00±0.0* 0.00±0.00</td>
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<td>G5 1.86±1.35 1.57±0.53* 2.00±0.82 1.86±0.90 3.00±0.00 20.86±9.25 2.14±0.90 0.00±0.00</td>
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<td>G6 2.00±0.82 2.14±0.69* 2.00±0.82 2.00±0.82 3.00±0.00 31.29±16.32 2.00±0.82 0.00±0.00</td>
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<td>G7 3.00±0.00* 0.71±0.49 0.86±0.38* 1.00±0.0* 2.86±0.38 23.14±9.46 2.71±0.49 0.29±0.49</td>
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<td>G8 3.00±0.00* 0.86±0.38 1.29±0.49 1.14±0.38* 2.71±0.49 18.43±5.19 2.00±0.82 0.14±0.38</td>
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</table>

[Measuring the average ± SD and Min-Max. *ANOVA analysis was performed. P <0.05; **ANOVA analysis was performed. P <0.01. (G1, Control; G2, Vehicle; G3, 0.5% Vi; G4, 1% Vi; G5, DM Control; G6, DM Vehicle; G7, DM/0.5% Vi; G8, DM /1% Vi)]
Fig. 3—Histological examination of H&E, TB and VG stained tissue sections. [Photomicrographs depict tissue sections of the wounds in control and *V. inulifolium* treated groups on days 0, 7 and 14 after wounding. (A) Non-diabetic groups on the 7th day; (B) Diabetic groups on the 7th day; (C) Non-diabetic groups on the 14th day; and (D) Diabetic groups on the 14th day. [e, epithelium; c, connective tissue; nv, neovascularization; co, collagen; asteriks, granulation tissue; m, mast cells; cp, capillaries; a, adipose tissue; h, hair follicles; and →, re-epithelialization. H&E: X40, TB and VG X400. (G1, Control; G2, Vehicle; G3, 0.5% Vi; G4, 1% Vi; G5, DM Control; G6, DM Vehicle; G7, DM/0.5% Vi; G8, DM/1% Vi)]
rds. Our results have shown that *V. inulifolium* improves the wound healing activity by its anti-inflammatory and epithelization induction effects as well as significant induction of fibroblast proliferation and collagen bundle synthesis.

Our macroscopic studies revealed that on the 7th and 14th days, wound healing in both incisional and excisional skin wounds of non-diabetic and diabetic groups were significant compared to control and vehicle groups. Results of the present study indicated that it was most significant in excisional skin wound of non-diabetic rats treated with 1% ointment of *V. inulifolium* on the 14th day (98.1%). However, incisional macroscopic wound healing was significant in non-diabetes on the 14th day treated with 0.5% *V. inulifolium* ointment (99.7%). In general, ointments of 0.5% and 1% *V. inulifolium* were found to be more significant in all treatments than the control and the vehicle groups in terms of wound surface closure (*P* < 0.05).

Hydroxyproline content is an indicator of wound healing process and measures the synthesis of collagen. Collagen is produced by fibroblast cells and allows tensile strength during wound repair. On the 7th day hydroxyproline content was increased in all treatment groups. Tensile strength defines the adequacy of subepidermal repair of the wound. Decreased tensile strength value in the diabetic animals is the reflection of decrease in collagen synthesis or imbalance in the synthesis and lysis. Our results here have shown that treatment of *V. inulifolium* ointment in both the doses significantly increased tensile strength in diabetics and non-diabetics.

Histopathological properties of the excisional and incisional wound models in non-diabetic and diabetic rat skin tissues on day 0, 7 and 14 were also observed in our study. Histopathological observations showed well organization of collagen fibers, increase in fibroblast cells and new blood capillaries formation in non-diabetic wound models of 14 days 1% *V. inulifolium* ointment treatment. Best wound healing was observed in this group. However, in diabetic control and vehicle groups mast cell infiltration and presence of inflammatory cells were apparent. It is an expected observation in diabetic samples as because of diabetic complications. But, after 14 days 1% *V. inulifolium* ointment treatment of diabetic groups, acceleration in dense connective tissue besides significant epithelial tissue formation were seen. Granulation tissue replaced to fibrous connective tissue and angiogenesis were observed in sections. Mast cells were also seen to wonder in connective tissue. It can be said that wound closure was much better in 1% *V. inulifolium* treated wounds on day 14 than on day 7. It is known that the healing process depends on collagen synthesis so, it is not surprising to observe thick granulation tissue in 1% *V. inulifolium* treated diabetic group on both 7 and 14 days.

*Verbascum* species growing in Turkey were studied by Suntar et al. and reported that methanol extracts of *V. olympicum* Boiss, *V. stachydifolium* Boiss et Heldr and *V. uschackense* (Murb.) Hub.-Mor. showed the highest wound healing ratio on both incision and excision wound models. Akdemir et al. demonstrated that *V. mucronatum* Lam. had remarkable antiinflammatory, antinociceptive and wound healing activities. Moreover, Demirci et al. investigated wound healing effect of methanol extract obtained from *V. speciesorum* Schrader and they found that the plant showed in vitro positive effects on angiogenesis and had beneficial effects on wound healing process. Previous studies on *Verbascum* spp. mentioned above support our results which showed healing effect of methanol extract of aerial parts of *V. inulifolium* with high rates of recovery in both diabetic and non-diabetic wounds; particularly, 1% (w/w) dose of *V. inulifolium*, significantly caused more closure of wound surface, more collagen accumulation and increased tension resistance compared to control groups.

In conclusion, following hydroxyproline measurements, biomechanical analysis, macroscopic and histopathological examinations and evaluations, findings of our study suggested that application of ointment prepared with methanolic extract of *Verbascum inulifolium* accelerated the cutaneous wound healing in both non-diabetic and streptozotocin-induced diabetic rats with incisional and excisional wounds. However, further studies on mode and mechanism of action are needed to recommend *V. inulifolium* as a wound healing agent.

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