Wound healing traits of *Chelidonium majus* and *Valeriana officinalis* hydro-alcoholic extracts on surgical wounds in Wistar rats

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Abstract

The study objective was assessment of wound healing traits of *Valeriana officinalis* and *Chelidonium majus* hydro-alcoholic (HA) extracts on surgical wounds in Wistar rats. The HA root extracts were separated using percolator and 96 degree alcohol in desiccator device. In addition, 24 Wistar rats (six months and 200 gr) were divided into three groups including control, *V. officinalis* and *C. majus*. The wound creation (2 cm diameter) was developed by initial intraperitoneal injection of anesthetic drugs (5% ketamine and 5mg/kg of diazepam) and hair shaving. After 24 hrs of wound creation, the treatment using ointment containing 5% of each *V. officinalis* and *C. majus* HA extract was implemented for 21 days. The wound imaging on days 4, 7, 14 and 21 was performed using a digital camera. Additionally, histopathologic examination of wounds was conducted at 4, 7, 14 and 21-day intervals. The microscopic and macroscopic observations revealed significant higher wound healing rates in treated groups compared to the control. The histopathologic examinations inferred the sufficient angiogenesis, existence of

collagen and fibroblast cells and decrease in the inflammatory cells. Moreover, wound contraction was observed in treated groups. Noticeably, the *C. majus* HA extract treated the wounds more efficiently. The wound healing of Wistar rats using HA extracts from *V. officinalis* and *C. majus* was promising though more investigations are required. Additionally, *C. majus* HA extract had higher healing effect compared to that of *V. officinalis*. It is proposed to evaluate the cytotoxic levels of extracts and formulate them in future studies to achieve more efficient and rapid healing of wounds. In addition, combination of extracts from various herbal medicines and with synthetic drugs can be studied for wound healing.

Key words: Wound healing, *Valeriana officinalis, Chelidonium majus*, hydro-alcoholic extracts, *in vivo*

1. Introduction

The skin, the largest organ of the body, has considerable roles, including protection of the mechanical barrier, regulation of body temperature, protection against ultraviolet rays and foreign factors, hence playing a pivotal role in the body's immunity (1-3). Skin wounds are a type of tissue disintegration which destroy the skin integrity, thus making the body's immune system vulnerable (4). Wound healing is an intricate and relatively time-consuming process considering the immune system response to a wound in each living organism. It continues until the complete repair of the damaged tissue. Despite major advances in wound healing, healing remains a significant challenge. The cellular and molecular mechanisms behind the healing mainly include increase in the edema and inflammatory cells and collagen production by dermal cells (5, 6). Wound healing using synthetic drugs has high costs and leaves side effects and scar. In addition, surgical wound infections possibly delay in the healing process (7). Therefore, development of natural-based therapies is promising for wound healing.

Belonging to the *Papaveraceae* family, *Chelidonium majus* (*C. majus*) is a one-year herbaceous plant growing in the temperate and subtropical regions of the Northern Hemisphere (8). *C. majus* is highly diverse and rich in various alkaloids, as well as other materials such as mucilage, pectic resin materials, a coloring material called glycoxanthine, chelidonic acid, and also other in-organic acids, mineral salts, especially calcium, magnesium and ammonium phosphates (9-11). Other non-alkaloid compounds contents recently found in this herb include caffeic acid esters. The main bioactive compounds of *C. majus* include alkaloids, flavonoids and phenolic compounds (12, 13).

It has also demonstrated anti-oxidant and hepato-protective effects which promoted liver detoxification and stimulated bile production. Topical applications such as creams or ointments, have been used to treat various skin conditions like warts, eczema, and psoriasis. However, it should be used with caution and under professional guidance due to its potential toxicity (14).

Valeriana officinalis (V. officinalis) belongs to the *Valerianacea* family native to Asia, Europe and America continents. The herb has demonstrated relief effects. *V. officinalis* rhizome contains several compounds including essential oil and sesquiterpenoids, valeric acid, amino acids (arginine/GABA or GABA/glutamine and tyrosine) and alkaloids. The anti-oxidant properties have also important role in the pharmacologic effects. Sedative and relieving effects include remedy of anxiety and insomnia. It has been found to have a calming effect on the nervous system, promoting relaxation and improving sleep quality (15, 16). Moreover, muscle relaxation includes relief of muscle tension and spasms (17). Mild pain relief has been also observed in mild analgesic effects, helping to alleviate pain, particularly headaches and menstrual cramps. The aim of this study was assessment of the wound healing traits of *V. officinalis* and *C. majus* HA extracts on surgical wounds of Wistar rats.

2. Materials and Methods

2.1.Preparation of HA extracts

The dried root parts of *V. officinalis* and *C. majus* were obtained and powdered. The HA extracts were separated using percolator and 96 degree alcohol during 24 hrs and kept in rotary device to concentrate the extract and next was dried in desiccator device (18).

2.2.Animals

Totally, 24 Wistar rats with mean age of six months and 200 gr weight were adopted and maintained at same nutritional and environmental conditions (12 hrs of light and 12 hrs of darkness) for three weeks. The mice were divided into three groups including control, *V. officinalis* and *C. majus* HA extracts. The round wound creation (cutting of dermis and epidermis) using cutting device (2 cm diameter) was performed by a specialist following initial intraperitoneal injection of anesthetic drugs (1:1 ration of 5% ketamine and 5 mg/kg of diazepam) and hair shaving. After 24 hrs of wound creation, the treatment using ointment containing 5% of each *V. officinalis* and *C. majus* HA extract was implemented daily (one time per day) for 21 days. The

wound imaging on days 4, 7, 14 and 21 was performed using a digital camera. Additionally, histopathologic examination of wounds was conducted on 4, 7, 14 and 21-day intervals following the euthanization of mice using thiopental sodium. The following formula was used for calculation of wound closure: Wound Closure (%) = [(initial wound area on day 0 – wound area on indicated day)/ wound area on day 0]*100 (19).

2.3.Histopathologic examination

After the tissue sections taking on 4, 7, 14 and 21 days, the slide preparation of parafinized tissue was subjected to Microtom device to take slides with 5 µm thickness. Then, the hematoxylin-eosin dye was utilized for the tissue staining. For the observation of collagen fibers, the Masson's trichrome staining method was performed in which the collagen fibers give blue color and the nucleus gives purple color in a red background. A light microscope was applied for the assessment of slides.

2.4.Data analysis

The data was analysed using SPSS version 21 and the comparison of groups was implemented using unpaired t-test. A p value<0.05 was defined as a significant finding. The histopathologic examinations were analysed using ANOVA test and LSD for three groups' comparison.

3. Results

3.1.Macroscopic examination of wounds

The wound closure percentage was measured according to the following formulae:

% Wound closure = wound size-initial wound size/initial wound size

Accordingly, the wound closure percentage was significantly higher in *V. officinalis* and *C. majus* compared to those of control values at the days 14 (p=0.01 and p=0.001, respectively) and 21 (p=0.006 and p=0.005, respectively). The analysis of wound closure area (mm^2) inferred a significant difference between each *V. officinalis* and *C. majus* group and negative control at 14 (p=0.018 and 0.001, respectively) and 21 (p=0.001 for each test group) days. Therefore, a marked level of wound healing was developed regarding test groups at day 21 (**Figures 1 and 2**).

Figure 1. The wound healing process among control, *V. officinalis* and *C. majus* during days 0-21

Figure 2. The mean wound closure area (A) and percentage of wound closure (B) in various groups

3.2.Histopathologic examinations

Using Masson's trichrome and H&A staining, in the control group at the early days of wound creation (day 4), the predominance of edema cells particularly of neutrophils and macrophages and lack of angiogenesis and fibrocytes and fibroblasts and disruption of dermis and epidermis without formation of collagen fibers was observed. However, in each *C. majus* or *V. officinalis* group, a negligible difference in terms of improvement was observed. In the *C. majus* group, higher gradual improvement rates were observed at days 7 and 14 compared to that the control in terms of decrease in the wound area and number of edema cells, enhancement of angiogenesis and fibrocytes and fibroblasts and collagen formation, epidermis layer formation and skin keratinized layer formation highlighting higher healing process. In *V. officinalis* and *C. majus* groups, at the day of 21, the formation and integrity of collagen fibers was insignificantly different from those of day 14, while edema cells were comparably decreased and the angiogenesis and load of fibrocytes and fibroblasts were increased insignificantly. In the tested groups using H&A staining at the day 21, the epidermis was formed, edema cells was decreased considerably, and the fibrocytes and fibroblasts have been enhanced with higher numbers and the formation and integrity of collagen fibers have been enhanced compared to that of day 14. Noticeably, higher levels of improvement factors was observed in the *C. majus* group with more rapid healing process. The scoring of collagen formation and fibrocytes and fibroblast cells was considered 1-5. Based on this, significant decrease in the severity and area of wound was observed in *C. majus* group compared to the control ($p=0.035$) at the day 21. There was no significant difference among other groups at other days. Additionally, significant decrease in the angiogenesis score was observed between each *C. majus* and *V. officinalis* and control group ($p=0.009$). The fibrocytes and fibroblast cells number scores were significantly enhanced in *C. majus* compared to those of control at 14 (0.009) and 21 days (0.035). Furthermore, a significant increase ($p=0.016$) in number of fibrocytes and fibroblast cells was observed in *C. majus* compared to that of *V. officinalis*. The formation and integrity of collagen fibers were also significantly higher in *C. majus* compared to that of the control group ($p=0.035$).

4. Discussion

The skin acts as a physical barrier between the internal organs and the external environment. It helps protect the body from harmful substances, pathogens, UV radiation, and mechanical damage. The skin helps regulate body temperature by controlling the loss or retention of heat through processes like sweating or constriction of blood vessels. The skin plays a vital role in the body's immune defense system. It contains immune cells which recognize and combat pathogens, prevent infections and promote healing. Vitamin D produced in the skin is essential for bone health, immune function, and various other physiological processes (20). The skin assists in the elimination of certain waste products and toxins through sweating. Some substances can be absorbed through the skin, such as medications or certain chemicals. This property is utilized in transdermal patches for drug delivery (21).

Chemotherapy suffers from drawbacks for wound healing. Delayed wound healing, increased risk of infection, impaired collagen production, poor wound strength, skin sensitivity and irritation and increased risk of skin reactions. The of chemotherapy impact on wound healing can vary depending on the individual, the specific drugs used, the treatment regimen, and other factors. In recent years, there has been a great desire to investigate the effects of physiology and pharmacology of herbal extracts and the use of herbal medicines in the world (22). Factors such as less side effects, diversity of effective compounds in herbs, lower costs, and development of industries related to the cultivation of medicinal plants, creation of useful work and especially the proposal of the use of medicinal plants by the World Health Organization is the reason for the global approach to herbal medicines. Currently, in accordance with the progress of science and technology and the use of nanotechnology, medicinal plants are promising in therapeutic aims.

This study was carried out on *C. majus* and *V. officinalis* with the aim of accelerating wound healing following their local consumption and observing their reparative effects. These plants have demonstrated anti-microbial, anti-inflammatory, anti-fungal and antioxidant properties, and also improve the function of fibroblasts and fibrocytes and increase the amount of collagen resulting in positive effects on wound healing. We observed that in all the studied groups, including the control groups, the group treated with 5% *C. majus* ointment and 5% *V. officinalis* ointment, wound healing started after a few days. Wound contraction is known as a mechanism by which the edges of the wound are drawn towards the center and the size of the open wound is reduced. Wound

contraction is a basic process being essential for survival as it protects the organism from harmful environmental factors. The reduction of wound size was more rapid in two treatment groups compared to the control group. According to the statistical studies conducted on the dimensions of the wound during days 4, 7, 14, and 21, the process of wound closure in the groups treated with the HA extract of *C. majus* occurred more rapidly, highlighting its higher healing effects compared to *V. officinalis*. Similarly, the wound healing effects of clove extract nanofibers (Eugenol) exhibited acceptable in 21 days and histopathologic findings confirmed collagen production (2). Additionally, green synthesized copper nanoparticles inferred wound healing effects (3).

Various herbal medicines have demonstrated considerable wound healing effects such as *Aloe barbadensis miller* (reducing inflammation, promoting tissue regeneration) (23, 24), *Calendula spp* such as *Calendula officinalis* (anti-inflammatory and antimicrobial properties and reduction of scarring), *Symphytum officinale* (anti-inflammatory traits and stimulation of cell growth and repairing damaged tissues), various species of *Lavandula* (exerting antimicrobial, anti-oxidant and anti-inflammatory effects) (25, 26), *Matricaria chamomilla* and *Chamaemelum nobile* (anti-bacterial, relieving and anti-inflammatory properties) (27-29), tea tree species oil (antimicrobial properties), *Melaleuca alternifolia* and *Rosmarinus officinalis* L. (30). Major limitations of this study included low number of test groups, lack of molecular mechanism evaluation of wound healing and effect of combined herbal medicines on the wound healing. Moreover, the cell cytotoxicity of extracts on normal cell lines was not evaluated. The wound healing of Wistar rats using HA extracts from *V. officinalis* and *C. majus* was observed to be time- and concentration-dependent. *C. majus* HA extract had higher healing effect than that of *V. officinalis*. It is proposed to evaluate the cytotoxic levels of extracts and formulate them in future studies to achieve more efficient and rapid healing of wounds. In addition, combination of extracts from various herbal medicines and with synthetic drugs can be studied for wound healing.

Ethics approval

Not applicable [Ethical Code: IR.FUMS.REC.1399.103].

Consent to publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

None

Funding

No funding was received for this study.

Authors' contribution

A.Y. conceptualized and designed the study. A.Z. and M.M. performed the work. A.G. and E.Z. analyzed the data and wrote the main manuscript draft. All authors approved the study.

Acknowledgements

This study has been performed and written by the authors.

References

1. Fernández-Gallego N, Sánchez-Madrid F, Cibrian D. Role of AHR Ligands in Skin Homeostasis and Cutaneous Inflammation. Cells. 2021;10(11).
2. Ashjazadeh MA, Jahandideh A, Abedi G, Akbarzadeh A, Hesaraki S. Histopathology and Histomorphological Study of Wound Healing Using Clove Extract Nanofibers (Eugenol) Compared to Zinc Oxide Nanofibers on the Skin of Rats. Arch Razi Inst. 2019;74(3):267-77.
3. Hakimzadeh S, Kosar M. Wound healing activity of green synthesized copper nanoparticles through cell proliferation-migration, antimicrobial effects, and nitric oxide triggering. Archives of Razi Institute. 2023:-.
4. Rousselle P, Montmasson M, Garnier C. Extracellular matrix contribution to skin wound re-epithelialization. Matrix Biol. 2019;75-76:12-26.
5. Wilkinson HN, Hardman MJ. Wound healing: cellular mechanisms and pathological outcomes. Open Biol. 2020;10(9):200223.
6. LeBlanc K, Langemo D, Woo K, Campos HMH, Santos V, Holloway S. Skin tears: prevention and management. Br J Community Nurs. 2019;24(Sup9):S12-s8.
7. Ratnayake P, Udalamaththa V, Samaratunga U, Seneviratne J, Udagama P. Therapeutic Potential of Skin Stem Cells and Cells of Skin Origin: Effects of Botanical Drugs Derived from Traditional Medicine. Stem Cell Rev Rep. 2022;18(6):1986-2001.
8. Krizhanovska V, Sile I, Kronberga A, Nakurte I, Mezaka I, Dambrova M, et al. The Cultivation of Chelidonium majus L. Increased the Total Alkaloid Content and Cytotoxic Activity Compared with Those of Wild-Grown Plants. Plants (Basel). 2021;10(9).
9. Popovic A, Deljanin M, Popovic S, Todorovic D, Djurdjevic P, Matic S, et al. Chelidonium majus crude extract induces activation of peripheral blood mononuclear cells and enhances their cytotoxic effect toward HeLa cells. Int J Environ Health Res. 2022;32(7):1554-66.
10. Gardin NE, Braga AJ. Preliminary outcome of Chelidonium majus (greater celandine) for COVID-19. Phytother Res. 2023.

11. Sathasivam R, Yeo HJ, Park CH, Choi M, Kwon H, Sim JE, et al. Molecular Characterization, Expression Analysis of Carotenoid, Xanthophyll, Apocarotenoid Pathway Genes, and Carotenoid and Xanthophyll Accumulation in Chelidonium majus L. Plants (Basel). 2021;10(8).
12. Huang XY, Shao ZX, An LJ, Xue JJ, Li DH, Li ZL, et al. New lignanamides and alkaloids from Chelidonium majus and their anti-inflammation activity. Fitoterapia. 2019;139:104359.
13. Nawrot R, Warowicka A, Rudzki PJ, Musidlak O, Dolata KM, Musijowski J, et al. Combined Protein and Alkaloid Research of Chelidonium majus Latex Reveals CmMLP1 Accompanied by Alkaloids with Cytotoxic Potential to Human Cervical Carcinoma Cells. Int J Mol Sci. 2021;22(21).
14. Liu Y, Peng Y, Zhang Z, Guo X, Ji M, Zheng J. In vitro and in vivo studies of the metabolic activation of chelidonine. Chem Biol Interact. 2019;308:155-63.
15. Pinheiro ML, Alcântara CE, de Moraes M, de Andrade ED. Valeriana officinalis L. for conscious sedation of patients submitted to impacted lower third molar surgery: A randomized, double-blind, placebo-controlled split-mouth study. J Pharm Bioallied Sci. 2014;6(2):109-14.
16. Farah GJ, Ferreira GZ, Danieletto-Zanna CF, Luppi CR, Jacomacci WP. Assessment of Valeriana officinalis l. (Valerian) for Conscious Sedation of Patients During the Extraction of Impacted Mandibular Third Molars: A Randomized, Split-Mouth, Double-Blind, Crossover Study. J Oral Maxillofac Surg. 2019;77(9):1796.e1-.e8.
17. Feng Y, Dai W, Ke J, Cui Y, Li S, Ma J, et al. Protective effect of valerian extract capsule (VEC) on ethanol- and indomethacin-induced gastric mucosa injury and ameliorative effect of VEC on gastrointestinal motility disorder. Pharm Biol. 2022;60(1):1095-105.
18. Bhargava S, Malhotra H, Rathore OS, Malhotra B, Sharma P, Batra A, et al. Anti-leukemic activities of alcoholic extracts of two traditional Indian medicinal plants. Leuk Lymphoma. 2015;56(11):3168-82.
19. Sato H, Ebisawa K, Takanari K, Yagi S, Toriyama K, Yamawaki-Ogata A, et al. Skin-derived precursor cells promote wound healing in diabetic mice. Ann Plast Surg. 2015;74(1):114-20.
20. Navarro-Triviño FJ, Arias-Santiago S, Gilaberte-Calzada Y. Vitamin D and the Skin: A Review for Dermatologists. Actas Dermosifiliogr (Engl Ed). 2019;110(4):262-72.
21. Baker LB. Physiology of sweat gland function: The roles of sweating and sweat composition in human health. Temperature (Austin). 2019;6(3):211-59.
22. Maver T, Maver U, Stana Kleinschek K, Smrke DM, Kreft S. A review of herbal medicines in wound healing. International journal of dermatology. 2015;54(7):740-51.
23. Teplicki E, Ma Q, Castillo DE, Zarei M, Hustad AP, Chen J, et al. The Effects of Aloe vera on Wound Healing in Cell Proliferation, Migration, and Viability. Wounds. 2018;30(9):263-8.
24. Coelho FH, Salvadori G, Rados PV, Magnusson A, Danilevicz CK, Meurer L, et al. Topical Aloe Vera (Aloe barbadensis Miller) Extract Does Not Accelerate the Oral Wound Healing in Rats. Phytother Res. 2015;29(7):1102-5.
25. Samuelson R, Lobl M, Higgins S, Clarey D, Wysong A. The Effects of Lavender Essential Oil on Wound Healing: A Review of the Current Evidence. J Altern Complement Med. 2020;26(8):680-90.
26. Ben Djemaa FG, Bellassoued K, Zouari S, El Feki A, Ammar E. Antioxidant and wound healing activity of Lavandula aspic L. ointment. J Tissue Viability. 2016;25(4):193-200.
27. Niknam S, Tofighi Z, Faramarzi MA, Abdollahifar MA, Sajadi E, Dinarvand R, et al. Polyherbal combination for wound healing: Matricaria chamomilla L. and Punica granatum L. Daru. 2021;29(1):133-45.
28. Nezhad-Mokhtari P, Kazeminava F, Abdollahi B, Gholizadeh P, Heydari A, Elmi F, et al. Matricaria chamomilla essential oil-loaded hybrid electrospun nanofibers based on polycaprolactone/sulfonated chitosan/ZIF-8 nanoparticles for wound healing acceleration. Int J Biol Macromol. 2023;247:125718.

29. Kazemian H, Ghafourian S, Sadeghifard N, Houshmandfar R, Badakhsh B, Taji A, et al. In vivo Antibacterial and Wound Healing Activities of Roman Chamomile (Chamaemelum nobile). Infect Disord Drug Targets. 2018;18(1):41-5.
30. Labib RM, Ayoub IM, Michel HE, Mehanny M, Kamil V, Hany M, et al. Appraisal on the wound healing potential of Melaleuca alternifolia and Rosmarinus officinalis L. essential oil-loaded chitosan topical preparations. PLoS One. 2019;14(9):e0219561.