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Original Article

Wound Healing Traits of *Chelidonium Majus* and *Valeriana Officinalis* Hydro-Alcoholic Extracts on Surgical Wounds in Wistar Rats

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ABSTRACT

The objective of this study was to assess the 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. Additionally, 24 Wistar rats (six months old, 200 g) were divided into three groups: control, V. officinalis, and *C. majus*. Wound creation (2 cm in diameter) was developed by initial intraperitoneal injection of anesthetic drugs (5% ketamine and 5mg/kg of diazepam) and hair shaving. 24 hours after wound creation, treatment was initiated using ointment containing 5% of each *V. officinalis* and *C. majus* HA extract, applied for 21 days. Wound imaging on days 4, 7, 14 and 21 was performed using a digital camera. Histopathologic examination of the wounds was conducted at 4, 7, 14 and 21-day intervals. Microscopic and macroscopic observations revealed significantly higher wound healing rates in treated groups compared to the control. Histopathologic examinations indicated sufficient angiogenesis, existence of collagen and fibroblast cells and reduction in the inflammatory cells. Moreover, wound contraction was observed in the treated groups. Noticeably, the *C. majus* HA extract treated the wounds more efficiently. The wound healing in Wistar rats using HA extracts from *V. officinalis* and *C. majus* was promising though more investigations are required. Additionally, *C. majus* HA extract demonstrated 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 applications.

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1. Introduction

The skin, the largest organ of the body, plays several critical roles, including mechanical barrier protection, 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 represent a form 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 involving the immune system response to a wound in each living organism. It continues until complete tissue repair is achieved. Despite major advances in wound healing, healing remains a significant challenge. The cellular and molecular mechanisms involved primarily include increased edema and inflammatory cells and collagen production by dermal cells (5, 6). Wound healing using synthetic drugs is often costly and leaves side effects and scar. In addition, surgical wound infections possibly delay the healing process (7). Therefore, the development of natural-based therapies offers a promising alternative for wound healing. Belonging to the *Papaveraceae* family, *Chelidonium majus* (*C. majus*) is a one-year herbaceous plant thar grows in the temperate and subtropical regions of the Northern Hemisphere (8). *C. majus* is highly diverse and rich in various alkaloids, as along with other materials such as mucilage, pectic resin materials, a coloring material called glycoxanthine, chelidonic acid, inorganic acids, mineral salts, especially calcium, magnesium and ammonium phosphates (9-11). Recently identified non-alkaloid compounds 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) a member of the *Valerianacea* family,is native to Asia, Europe ,and America continents. The herb is known for its relief effects. The rhizome of *V. officinalis* 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 play an important role in the pharmacologic effects. Sedative and relieving effects include relief from anxiety and insomnia. It has been shown to have a calming effect on the nervous system, promoting relaxation and improving sleep quality (15, 16). Additionally, muscle relaxation includes relief of muscle tension and spasms (17). Mild analgesic effects have been also observed, helping to alleviate pain, particularly headaches and menstrual cramps. The aim of this study was to assess 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 over 24 hours , then concentrated using rotary device and subsequently dried in desiccator (18).

2.2. Animals

A total of 24 Wistar rats , with mean age of six months and an average weight of 200 gr , were adopted and maintained under same nutritional and environmental conditions (12 hours of light and 12 hours of darkness) for three weeks. The mice were divided into three groups : control, *V. officinalis* and *C. majus* HA extracts. 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.

Twenty-four hours after wound creation, treatment using ointment containing 5% of each *V. officinalis* and *C. majus* HA extract was applied once daily for 21 days. Wound imaging on days 4, 7, 14 and 21 was performed using a digital camera. Additionally, histopathologic examination of the wounds was conducted at 4, 7, 14 and 21-day intervals following the euthanasia 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 tissue sectioning on days 4, 7, 14 and 21, parafinized tissue slides were prepared using a microtom device to obtain 5 µm thick sections . Hematoxylin-eosin dye was utilized for tissue staining. To observe collagen

fibers, Masson's trichrome staining method was performed in which collagen fibers appear blue , nucleus appear purple and the background is red. A light microscope was applied for the assessment of slides.

2.4. Data analysis

The data was analyses using SPSS version 21.Group comparison was performed using unpaired t-test With a *p* value<0.05 considered statistically significant. Histopathologic examinations were analyzed using ANOVA and LSD test for comparison among the three groups.

3. Results

3.1. Macroscopic Examination of Wounds

The wound closure percentage was measured using the following formulae:

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

The wound closure percentage was significantly higher in the *V. officinalis* and *C. majus* compared to control group on days 14 (p=0.01 and p=0.001, respectively) ,and 21 (p=0.006 and p=0.005, respectively). Analysis of wound closure area (mm^2) showed 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).

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 ,was observed,along with a lack of angiogenesis, fibrocytes, and fibroblasts ,and disruption of the dermis and epidermis without collagen fibers formation. However, in both the *C. majus* or *V. officinalis* group, a negligible difference was noted in terms of improvement. In the *C. majus* group, higher rates of gradual improvement were observed at days 7 and 14 compared to the control in terms of decrease in the wound area and number of edema cells, enhancement of angiogenesis and fibrocytes , fibroblasts, and collagen formation, epidermis layer formation ,and skin keratinized layer formation, highlighting higher healing process.

By day 21, in both *V. officinalis* and *C. majus* groups, collagen fiber formation and integrity were not significantly different from day 14, although edema cells had comparably decreased and angiogenesis and load of fibrocytes and fibroblasts were slightly increased. In the tested groups, H&A staining on day 21 showed epidermis formation, a considerable reduction in edema cells and an increase in fibrocytes and fibroblasts, along with enhanced collagen fiber formation and integrity compared to day 14. Notably, higher levels of improvement factors were observed in the *C. majus* group with more rapid healing process.

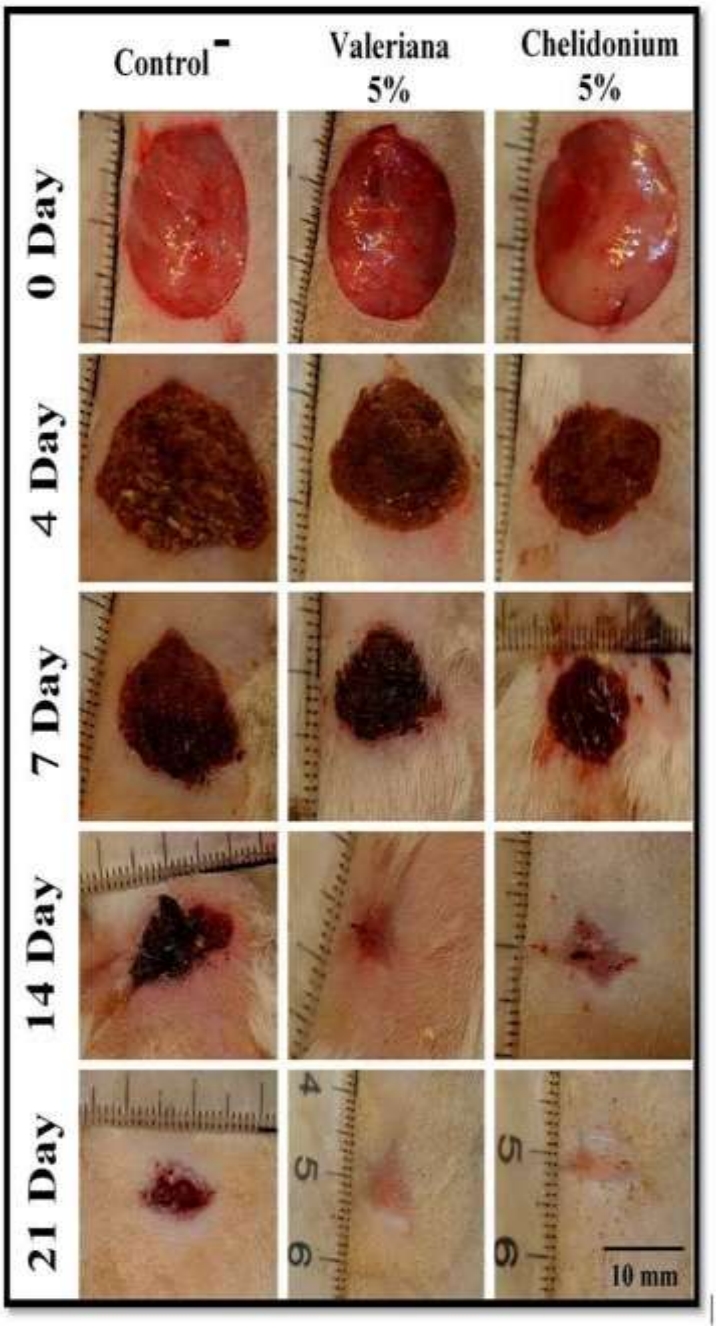

Figure 1. The wound healing process among control, *V. officinalis* and *C. majus* during days 0-21.

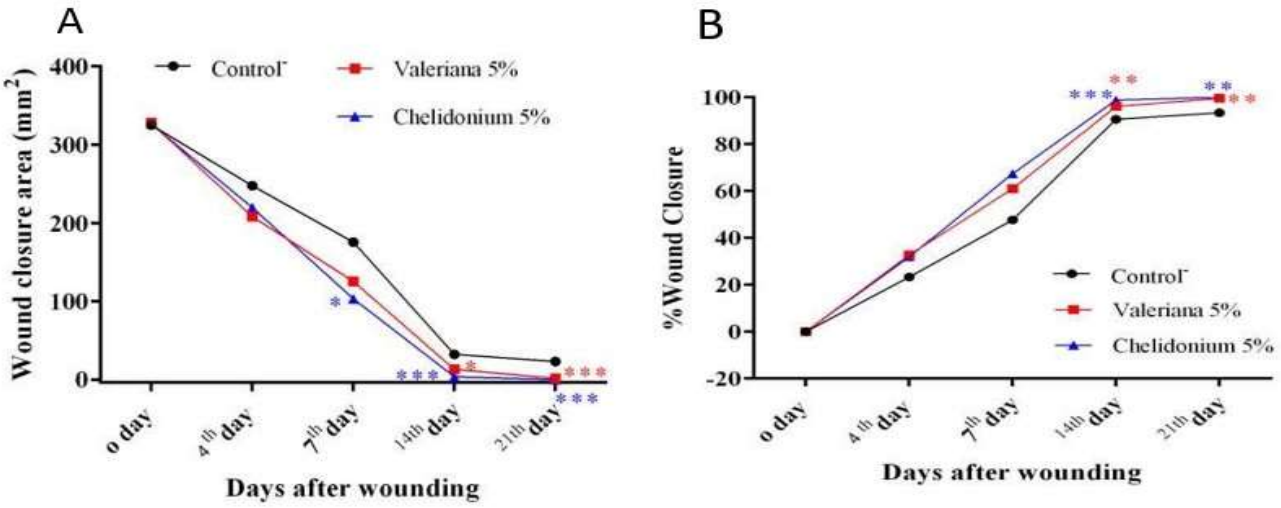

Figure 2. The mean wound closure area (A) and percentage of wound closure (B) in various groups.

Collagen formation and fibrocytes/ fibroblast cells was based on a scale of 1-5.

A 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,a significant decrease in the angiogenesis score was observed between both *C. majus* and *V. officinalis* groups compared to the control (p=0.009). Fibrocytes and fibroblast cells scores were significantly enhanced in *C. majus* compared to the control on days 14 (0.009) and 21 days (0.035). Furthermore, a significant increase in fibrocytes and fibroblast cells was observed in *C. majus* compared to of *V. officinalis* (p=0.016). Collagen fiber formation and integritywere 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 internal organs and the external environment. It helps protect the body from harmful substances, pathogens, UV radiation, and mechanical damage. It helps regulate body temperature by controlling the loss or retention of heat through processes like sweating or constriction of blood vessels, and plays a vital role in body's immune defense system. It contains immune cells that 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 presents several drawbacks for wound healingincluding delayed wound healing, increased risk of infection, impaired collagen production, reduced wound strength, skin sensitivity, irritation and increased risk of skin reactions. The impact of chemotherapy on wound healing can vary depending on the individual, specific drugs used, 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 fewer side effects, diversity of effective compounds in herbs, lower costs, and the 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 investigated the wound healing potential of *C. majus* and *V. officinalis* through topical application and evaluation of 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 began within 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 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) proved acceptable in 21 days and histopathologic findings confirmed collagen production (2).

Additionally, green synthesized copper nanoparticles confirmed 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 scar-reducing effects), *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 absence of combined herbal medicine testing. Moreover, the cell cytotoxicity of extracts on normal cell lines was not evaluated. Wound healing in Wistar rats using HA extracts from *V. officinalis* and *C. majus* was observed to be time- and concentration-dependent. *C. majus* HA extract demonstrated higher healing effect compared to *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.

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This study was conducted and written by the authors.

Authors' Contribution

Study concept and design: A.Y.
Acquisition of data: A.G, E.Z.
Analysis and interpretation of data: A.G, E.Z.
Drafting of the manuscript: A.Z, M.M.
Critical revision of the manuscript for important intellectual content: A.Z, M.M.
Statistical analysis: A.G, E.Z.
Administrative, technical, and material support: A.Z, M.M.

Ethics

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

Conflict of Interest

The authors declare that they have no conflict of interest.

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Data Availability

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

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