

24-9-2025

Evaluation of oral administration of *Calendula officinalis* among the centesimal potencies in acute wound healing in male wistar rats

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George AV, Naik R, Rekha PD, et al. Evaluation of oral administration of *Calendula officinalis* among the centesimal potencies in acute wound healing in male wistar rats. *Indian J Res Homoeopathy*. 2025;19(3):291–303.

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Abstract

Background: *Calendula officinalis* (*Calendula*) is used in Homoeopathy to treat various wounds. The presence of bioactive compounds results in pharmacological properties like wound healing, antioxidants, and anti-inflammatory properties. Hence, clinicians use different centesimal potencies (6C, 30C, 200C) to heal wounds. **Objective:** To compare the wound-healing potential of *Calendula* dilutions (6C, 30C, 200C) through oral administration in a pre-clinical acute wound model. **Materials and Methods:** The study involved administering *Calendula* centesimal potencies (6C, 30C, 200C) orally. Male wistar rats aged eight to ten weeks were involved in the experiment. Every animal had full-thickness excisional wound created on its back and assigned into four groups: vehicle control (VC) group and three treatment groups receiving oral doses of dispensing alcohol, *Calendula* 6C, *Calendula* 30C, and *Calendula* 200C, respectively. Each animal received treatment twice daily for seven days, with six-hour interval between doses. Wound photographs, taken at regular intervals, were used to observe the rate of wound closure. Animals were sacrificed on 8th and 14th days to collect samples for histopathology and immunohistochemistry. **Results:** Analysis of variance assessed the statistical significance of the group differences. The study showed that wound healing was significantly higher in *Calendula-treated* groups than in VC. The results indicate that wound closure, epidermal thickness, collagen deposition, and the proliferative marker antigen Kiel-67 (Ki-67) expression levels were significantly higher ($p < 0.01$) in the *Calendula* 30C-treated group compared with other groups. **Conclusion:** Among the *Calendula* centesimal potencies tested for wound contraction, 30C proved to be the most effective for wound healing.

Acknowledgments and Source of Funding

The authors would like to acknowledge Dr. Shivaprasad K, Principal of Yenepoya Homoeopathic Medical College Hospital, and Dr. Lourdhuraj I, Principal of Yenepoya Physiotherapy College, for their support and guidance in this study. The authors also acknowledge Yenepoya Pharmacy College and Research Centre for the support provided for physicochemical analysis.

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ORIGINAL ARTICLE

Evaluation of oral administration of *Calendula officinalis* among the centesimal potencies in acute wound healing in male wistar rats

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ABSTRACT

Background: *Calendula officinalis* (*Calendula*) is used in Homoeopathy to treat various wounds. The presence of bioactive compounds results in pharmacological properties like wound healing, antioxidants, and anti-inflammatory properties. Hence, clinicians use different centesimal potencies (6C, 30C, 200C) to heal wounds. **Objective:** To compare the wound-healing potential of *Calendula* dilutions (6C, 30C, 200C) through oral administration in a pre-clinical acute wound model. **Materials and Methods:** The study involved administering *Calendula* centesimal potencies (6C, 30C, 200C) orally. Male wistar rats aged eight to ten weeks were involved in the experiment. Every animal had full-thickness excisional wound created on its back and assigned into four groups: vehicle control (VC) group and three treatment groups receiving oral doses of dispensing alcohol, *Calendula* 6C, *Calendula* 30C, and *Calendula* 200C, respectively. Each animal received treatment twice daily for seven days, with six-hour interval between doses. Wound photographs, taken at regular intervals, were used to observe the rate of wound closure. Animals were sacrificed on 8th and 14th days to collect samples for histopathology and immunohistochemistry. **Results:** Analysis of variance assessed the statistical significance of the group differences. The study showed that wound healing was significantly higher in *Calendula*-treated groups than in VC. The results indicate that wound closure, epidermal thickness, collagen deposition, and the proliferative marker antigen Kiel-67 (Ki-67) expression levels were significantly higher ($p < 0.01$) in the *Calendula* 30C-treated group compared with other groups. **Conclusion:** Among the *Calendula* centesimal potencies tested for wound contraction, 30C proved to be the most effective for wound healing.

Keywords: Albino wistar rats, *Calendula officinalis*, Centesimal potency, Homoeopathy, Pre-clinical model

Introduction

Cutaneous wounds represent one of the most universal healthcare problems, creating an economic burden worldwide. Healing wounds requires therapy and standard care to prevent infections, scars, and other complications. Based on the healing outcomes, acute and chronic wounds are classified.¹ An acute wound manifests abruptly, remains transitory, and quickly reestablishes functions.² Many sources provide a variety of wound-healing therapeutic agents.³ Complementary and Alternative Medicine (CAM) has

identified some wound care products with established clinical outcomes.⁴ In Homoeopathy, practitioners frequently manage wounds with medicines like *Calendula officinalis* (*Calendula*.), *Arnica montana*, and *Echinacea angustifolia*.⁵ The 5th edition of the *Organon of Medicine* includes Aphorism 290 by Dr. Christian Friedrich Samuel Hahnemann (1755–1843), which advocates for the internal administration of medicines through the mouth, tongue, and stomach.⁶

How to cite this article: George AV, Naik R, Rekha PD, et al. Evaluation of oral administration of *Calendula officinalis* among the centesimal potencies in acute wound healing in male wistar rats. *Indian J Res Homoeopathy*. 2025;19(3):291–303.

Received 16 April 2024; Accepted 25 August 2025.

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<https://doi.org/10.53945/2320-7094.2103>

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This holistic treatment method is natural, safe, cost-effective, and easily accessible.⁷ *Calendula* is an annual herb of the Asteraceae, commonly known as marigold. According to the *Homoeopathic Pharmacopoeia of India (HPI)*, *Calendula* mother tincture (θ) preparation follows the old method outlined in class I for juicy plants. It serves as a source for preparing *Calendula* θ from fresh flowers and leaves, with a drug strength of 1/10.⁸ Dr. Stuart Close's (1860–1929) philosophy states that the centesimal potencies accomplish both qualitative and quantitative drug action.⁹ These centesimal potencies are readily available, quickly acting, utilised as physiological doses, and primarily used in clinical practice.¹⁰ Hence, the study used centesimal potencies. From the *Calendula* θ centesimal potency (C) is prepared in the ratio of 1:99, so *Calendula* 6C drug strength is 1/10¹², *Calendula* 30C drug strength is 1/10⁶⁰, and *Calendula* 200C drug strength is 1/10⁴⁰⁰.⁸ *Calendula* θ chromatography analysis revealed active principles like saponins, triterpenoids, amino acids, sterols, and alkaloids associated with pharmacological action.¹¹ Dr. William Boericke (1849–1929) mentioned in the *Manual of Homoeopathic Materia Medica* that *Calendula* promotes granulation in the first intention, thereby hastening the healing process.¹² Various studies have reported several ethnopharmacological uses of *Calendula*, such as anti-inflammatory, anti-microbial, wound healing, angiogenic activity, anti-oxidant, anti-diabetic, and anti-hyperlipidaemic activities.¹³ *In vitro* studies, the potential wound-healing ability of *Calendula* 3C and *Calendula* 6C dilutions in human dermal fibroblasts, which have demonstrated their capacity to increase wound closure, cell viability, and cell proliferation.^{14,15} However, no systematic investigation has yet examined the efficacy of different dilutions of *Calendula* in wound healing using *in vivo* animal models.

Therefore, this study aims to investigate the *in vivo* wound-healing potential of various potencies of *Calendula* on the centesimal potencies (*Calendula* 6C, *Calendula* 30C, *Calendula* 200C) through oral administration. It also seeks to identify the most effective potency among them.

Materials and methods

Source of medicines and physicochemical study

Calendula dilutions were obtained from Dr. Willmar Schwabe India Pvt. Ltd. in India, and dispensing alcohol was supplied by Mangalore Homoeopathic Pharmacy in India.

The physicochemical analysis of different *Calendula* dilutions, such as *Calendula* 6C, *Calendula* 30C, *Calendula* 200C, and dispensing alcohol, was studied using

standard protocols.¹⁶ The optical properties of *Calendula* dilutions were analysed using UV-visible spectroscopy over a wavelength range of 200–800 nm. Origin software, version 2025, was used to evaluate the highest absorption wavelength of *Calendula* dilutions and dispensing alcohol. The pH of the *Calendula* dilutions and dispensing alcohol was determined using a digital pH meter (Labtronics, India). The sample density was measured using a specific gravity bottle. For this, the bottle was rinsed with distilled water and dried in a hot air oven. The initial weight (W1) and the weight of the bottle with all the samples (W2) were recorded separately. The bottle was rewashed and weighed (W3) using a digital analytical balance. The sample density was calculated using the formula;

$$\text{Density of sample} = \frac{w_2 - w_1}{w_3 - w_1} \times \text{Density of water}^{16}$$

Animal models

Twenty four male wistar rats were procured from the university's animal husbandry department. The study used eight to ten weeks of healthy male wistar rats weighing approximately 250–350 g.¹⁷ All the animals were caged separately and maintained on a 12-hour light-dark cycle, kept at a temperature of 20 to 25°C and a humidity of 30 to 70% throughout the study.¹⁸ Animal care, feeding, and procedures followed the guidelines of the Committee for the Conduction and Supervision of Experiments on Animals (CCSEA).¹⁹ The university's Institutional Animal Ethics Committee (IAEC) approved the experimental procedures (Approval No. 347/PO/ReBi-S/Rc-L/01/CPCSEA).²⁰

Design of animal experiments

Excisional wound creation

An intraperitoneal injection of *ketamine hydrochloride* 100 mg/kg was administered to the rats to induce anaesthesia. The dorsal neck region of each rat was shaved and cleaned with antiseptic solutions before creating a full-thickness excision wound on its back, measuring 3 × 2.5 cm.¹⁷ Six rats were assigned into four groups: (1) the vehicle control (VC) group, which received dispensing alcohol, and (2–4) the treatment groups, which received *Calendula* 6C, *Calendula* 30C, and *Calendula* 200C, respectively.²¹

Medicine preparation and administration

The *Homoeopathic Pharmacopoeia of India (HPI)* Volume 1 prescribes the standard protocol for preparing medication for treatment groups. The medicated globule was dissolved in 10 mL of purified water and

administered orally to the animals in a dose of 1 mL of medicine using an oral feeding needle. Likewise, dispensing alcohol (90% ethanol/10% distilled water) was prepared and administered.⁸ The animals received their respective medications twice daily at 6-hour intervals for seven days. The animals were monitored throughout the study for any signs of pain, bleeding, wound discharge, or crust formation.¹⁸

Evaluation of wound healing

Wound photographs were taken on the 4th, 8th, and 14th post-treatment days using *Image J software (version 1.53t)* to measure the wound area.²² Blood was collected from the tail veins at the same three intervals. Blood cell analysis was conducted on the same day using the obtained blood. On the 8th and 14th days, three animals from each group were given an excess dose of ketamine hydrochloride (300 mg/kg) intraperitoneally for sacrifice, and the wound tissue was collected. Those samples were washed with sterile saline and then fixed in a 10% formalin solution in separate sterile containers after labelling. The histological examination was performed by staining samples with hematoxylin and

eosin (H&E), measuring collagen deposition using Masson-trichrome (MT) staining, and assessing cell proliferation using immunohistochemistry with the Ki-67 marker. Images of H&E slides were obtained using a microscope and analysed using ImageJ software to determine epidermal thickness.²³

Statistical analysis

The study evaluated data on wound contraction, epidermal thickness, and the Ki67 proliferative marker for significant differences between the groups and days using two-way analysis of variance (ANOVA) in GraphPad Prism v10. A *p*-value of < 0.01 determined the statistical significance between and within groups.²⁴

Results

Physicochemical characteristics of *Calendula* dilutions

The physicochemical analysis is briefly outlined in Table 1, which presents the results, and Fig. 1

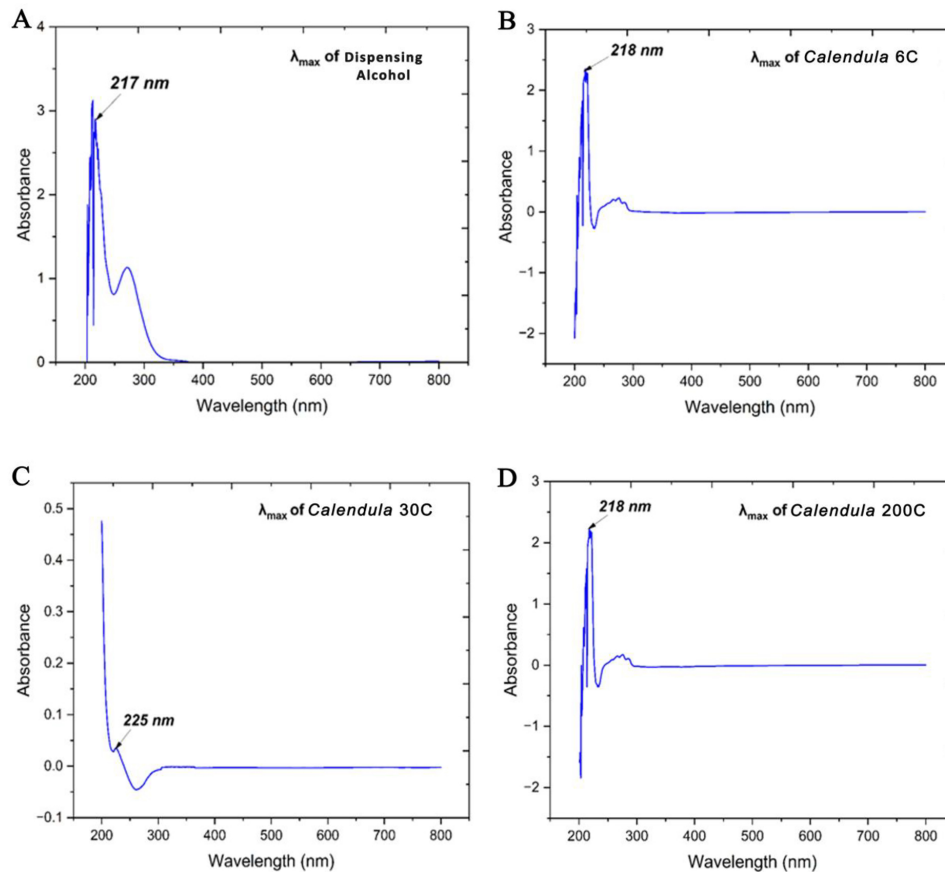


Fig. 1. UV absorption spectra (λ_{\max}) of *Calendula* dilutions and dispensing alcohol (A) Dispensing alcohol, (B) *Calendula* 6C, (C) *Calendula* 30C, (D) *Calendula* 200C

Table 1. Physicochemical analysis of *Calendula* dilutions and Dispensing alcohol

| Parameters/Samples | Dispensing alcohol | <i>Calendula</i> 6C | <i>Calendula</i> 30C | <i>Calendula</i> 200C |
|---------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Wavelength (λ_{\max}) | 217 nm | 218 nm | 225 nm | 218 nm |
| pH | 7.04 | 8.80 | 8.94 | 9.24 |
| Density (kg/m ³) | 830.80 kg/m ³ | 827.14 kg/m ³ | 822.83 kg/m ³ | 906.25 kg/m ³ |

illustrates the wavelength (λ_{\max}) of *Calendula* dilutions and dispensing alcohol as measured by a UV-visible spectrometer.

Effects of *Calendula* dilutions on wound contraction

All animals in the *Calendula*-treated groups showed significantly higher wound contraction ($p < 0.0001$) from the fourth day compared to the VC [Fig. 2A]. The VC demonstrated wound contraction, ranging from 10% to 95% between the 4th and 14th days. *Calendula* 30C showed a significantly higher wound contraction ($p < 0.0001$) than the other groups on the fourth day, achieving the highest contraction at 61% [Fig. 2B]. On the eighth day post-treatment, a significant increase in wound contraction was observed in all groups, including VC, with the *Calendula* 6C group showed the highest wound contraction, followed by the *Calendula* 200C and *Calendula* 30C groups. However, by day 14, the wounds in all groups had contracted almost entirely without significant difference ($p > 0.05$) [Fig. 2B]. Wound contraction exceeded 95% in all groups by the 14th day [Fig. 2A].

Effects of *Calendula* dilutions on epidermal thickness

All groups showed a significant increase in epidermal thickness from the 8th to the 14th day. All *Calendula* dilutions (6C, 30C, 200C) significantly affected epidermal thickness at both time points compared to the VC [Fig. 3A]. On the 8th and 14th day, VC epidermal thickness ranged from 1.16 ± 0.04 to $1.48 \pm 0.10 \mu\text{m}$. However, *Calendula* 30C-treated wounds showed significantly higher epidermal thickness than the other two *Calendula* dosages (*Calendula* 6C and *Calendula* 200C) [Fig. 3B]. *Calendula* 30C reached the highest epidermal thickness, increasing from 3.00 ± 0.21 to $4.6 \pm 1.12 \mu\text{m}$ between the 8th and 14th day, respectively [Fig. 3B]. Among the other treatment groups, *Calendula* 200C showed higher epidermal thickness, ranging from 2.02 to 0.20 to $2.21 \pm 0.38 \mu\text{m}$, followed by *Calendula* 6C, which ranged from 1.53 ± 0.1 to $1.6 \pm 0.15 \mu\text{m}$, respectively, from the 4th day to 14th days [Fig. 3B]. The *Calendula* 30C epidermal thickness was three times higher than that of VC.

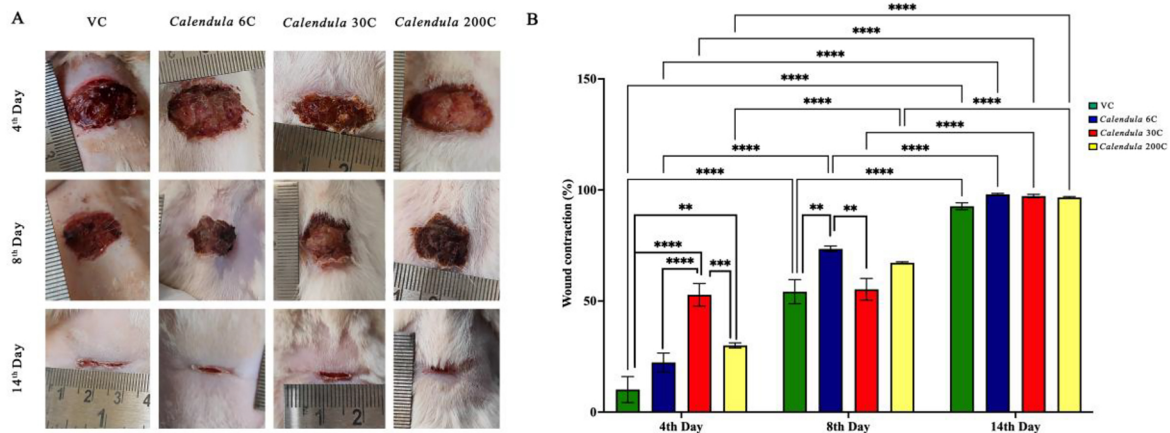


Fig. 2. (A) Photomicrographs of the wounds after treatment with VC, *Calendula* 6C, *Calendula* 30C, and *Calendula* 200C. (B) Graph representing the rate of wound closure calculated for VC, *Calendula* 6C, *Calendula* 30C, and *Calendula* 200C. Results are expressed as mean \pm SD ($n = 6$); **** indicates statistical significance ($p < 0.0001$), *** indicates significance ($p < 0.001$), and ** indicates statistical significance ($p < 0.01$)

VC – Vehicle control, *Calendula* – *Calendula officinalis*, C – Centesimal potency .

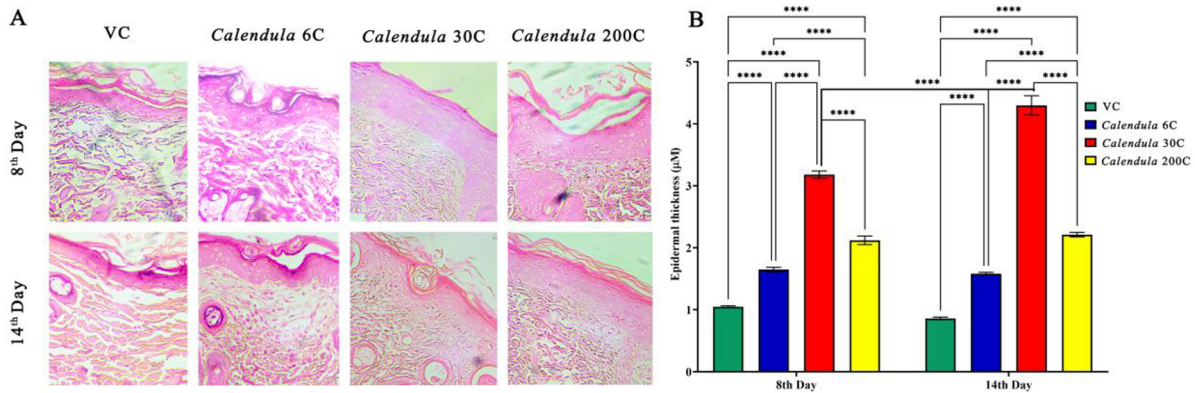


Fig. 3. (A) H&E staining of wound tissues of VC, *Calendula* 6C, *Calendula* 30C, and *Calendula* 200C (scale bar 10 μ m). (B) Graph describing the epidermal thickness calculated from the H&E-stained sections of VC, *Calendula* 6C, *Calendula* 30C, and *Calendula* 200C. Results are expressed as mean \pm SD (n = 6); **** indicates statistical significance ($p < 0.0001$) VC – Vehicle control, *Calendula* – *Calendula officinalis*, C – Centesimal potency .

Effects of *Calendula* dilutions on collagen deposition

MT staining assessed collagen synthesis in wound tissues, a component of wound contraction. On day 8, VC exhibited loosely deposited collagen and sparsely generated fibroblasts [Fig. 4]. Dense and compact formation of granulation tissue, keratin, and collagen in the *Calendula* centesimal potencies was observed. *Calendula* 30C-treated wounds showed higher collagen than the other centesimal potencies of *Calendula* [Fig. 4]. Examination of collagen deposition revealed that collagen was present loosely and sparsely in the VC group, whereas in the *Calendula*-treated groups, it was dense and compact.

Effects of *Calendula* dilutions on Ki-67 expression levels in the wound tissue

Immunohistochemistry staining of Ki-67 was used to investigate cell proliferation mediated by *Calendula* centesimal potencies at different intervals. *Calendula*-treated groups showed significantly higher expression of Ki-67 ($p < 0.0001$) [Fig. 5] compared to the VC on days 8 and 14. The VC expression of Ki-67 with positive cells ranged from 79 to 84 on the 8th and 14th days, respectively. *Calendula* 30C showed significantly higher cell proliferation ($p < 0.001$) on days 8 and 14 compared to *Calendula* 6C, *Calendula* 200C, and VC, as illustrated in Fig. 5. In *Calendula* 30C, the number of Ki-67-positive cells increased from 288 to 306 cells between the 8th and 14th day, indicating improved wound healing [Fig. 5]. *Calendula* 200C showed higher expression of Ki-67 cells than *Calendula* 6C. The expression of Ki-67 in *Calendula* 30C was 5 times higher than the VC. Treatment group was compared with the VC group in the study. VC

group was not required to compare with the treatment group.

Effects of *Calendula* dilutions on haematology

All treatment groups showed significant variation in White blood cell (WBC) counts. *Calendula* 6C showed a higher WBC count on the 8th day, which decreased by the 14th day ($p < 0.05$) [Fig. 6]. The WBC count in the *Calendula* 200C group steadily increased from the 4th day to the 14th day ($p < 0.01$). Interestingly, the *Calendula* 30C group showed an increase in the WBC count from the 4th day to the 14th day, though this increase was not significant ($p > 0.05$).

Discussion

The findings of this study indicate that *Calendula* 30C stands out as the most effective oral potency compared to *Calendula* 6C, *Calendula* 200C, and VC in the treatment of cutaneous wounds. The potentiation procedure involves preparing dilutions of homeopathic medicines through a process that combines succussion and dilution.⁶

Dr. Hahnemann's concept of homeopathic dynamisation aligns with nano-pharmacology, contrasting with conventional materialistic pharmacology.²⁵ Electron microscopy, spectroscopy, and nanoparticle tracking studies have demonstrated the existence of nanoparticles in homeopathic dilutions, surpassing Avogadro's number. These particles in dilutions exhibit greater structural complexity, size, quantity, and distribution, and may have pharmacological action.^{26,27} Hence, the possible presence of nanoparticles in *Calendula* 6C, 30C, and 200C may show the pharmacological action of *Calendula*, as noted in this

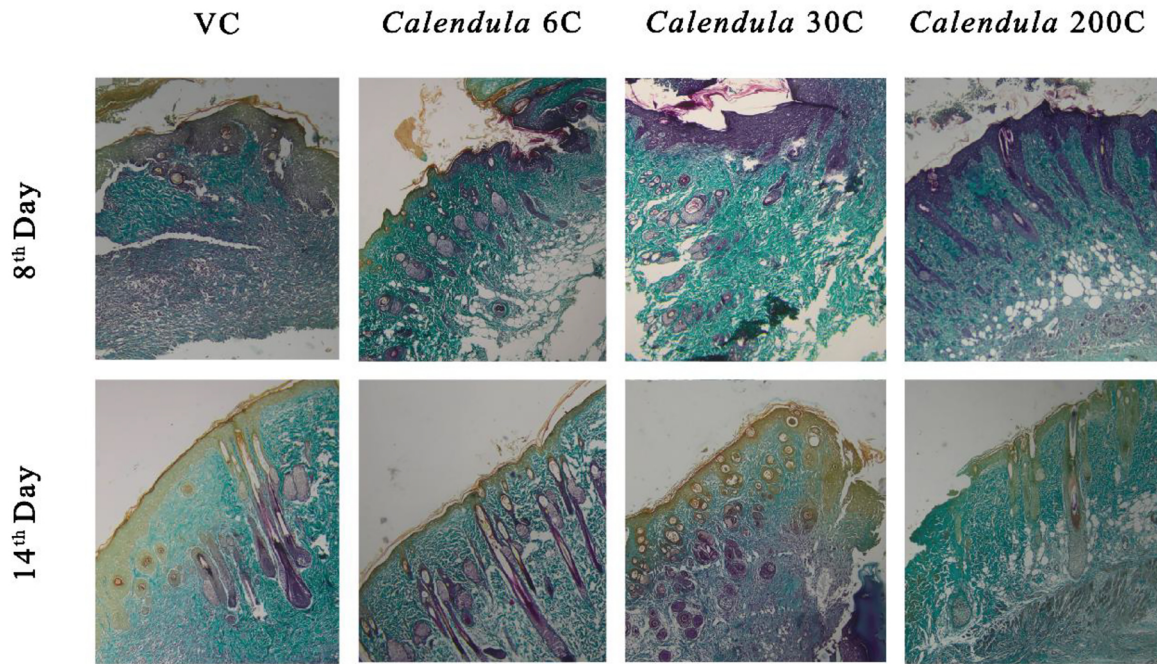


Fig. 4. Collagen deposition in wound tissue at different time points treated with *Calendula* 6C, *Calendula* 30C, and *Calendula* 200C compared with VC. Photomicrographs of MT-stained wound tissues from the VC, *Calendula* 6C, *Calendula* 30C, and *Calendula* 200C treated groups on the 8th and 14th days (scale bar, 10 μ m); the blue-green colour indicates stained collagen fibers VC – Vehicle control, *Calendula* – *Calendula officinalis*, C – Centesimal potency.

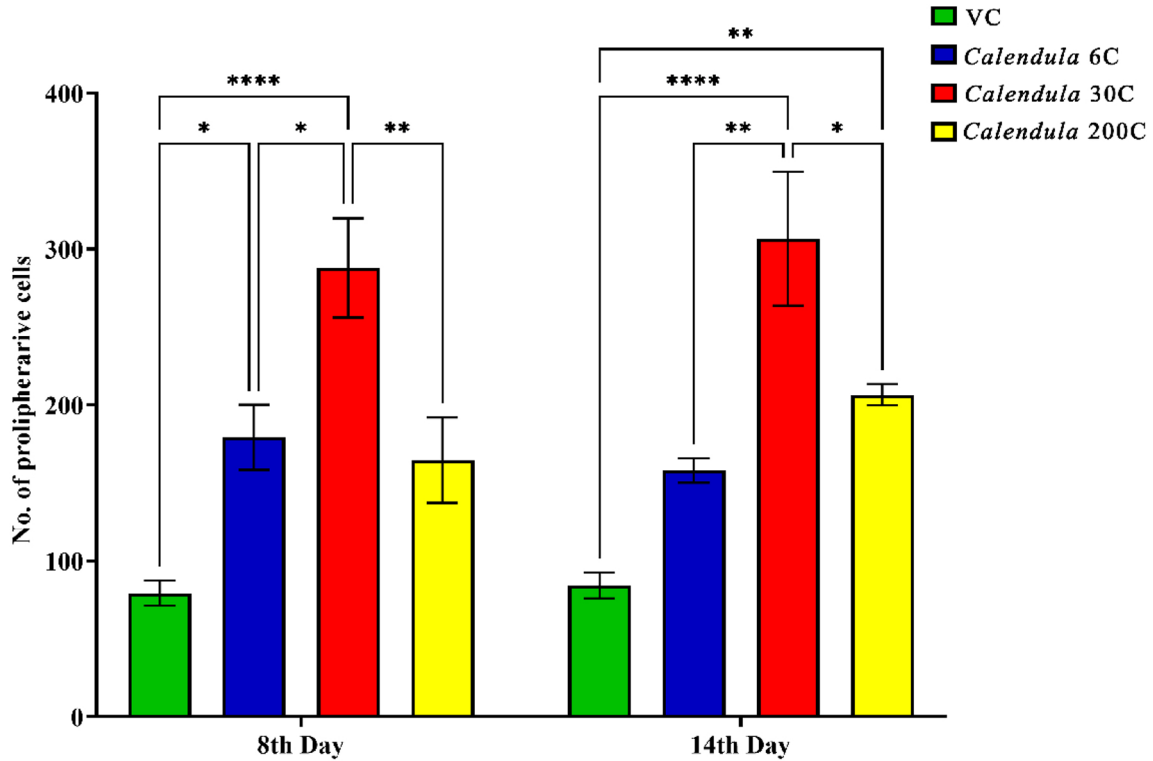


Fig. 5. Immunohistochemistry staining of Ki-67 in wound tissue at different time points after treatment with *Calendula* 6C, *Calendula* 30C, and *Calendula* 200C compared with VC. Graph describing the expression of Ki-67 cell proliferation of VC, *Calendula* 6C, *Calendula* 30C, and *Calendula* 200C orally treated groups on the 8th and 14th day. Results are expressed as mean \pm SD (n = 6); **** indicates statistical significance ($p < 0.0001$); ** indicates statistical significance ($p < 0.01$); * indicates statistical significance ($p < 0.05$) compared to the VC VC – Vehicle control, *Calendula* – *Calendula officinalis*, C – Centesimal potency.

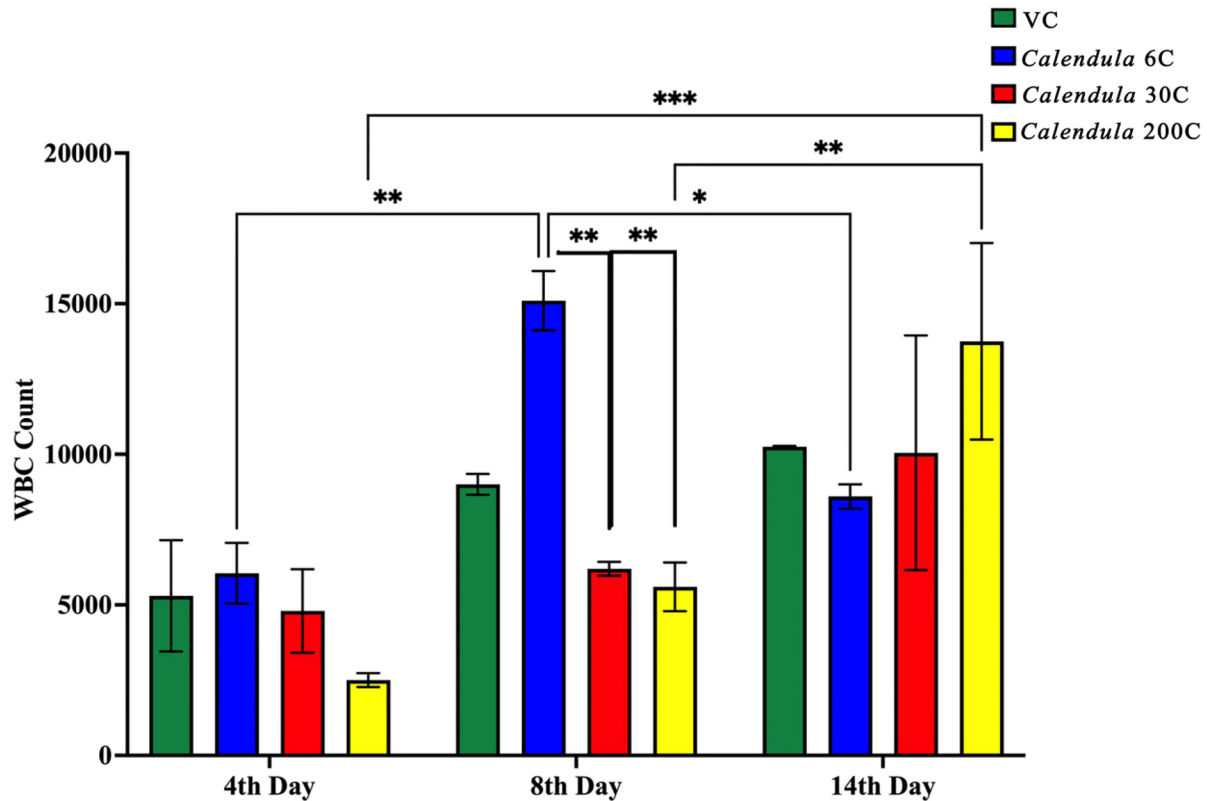


Fig. 6. Changes in WBC counts at different time points treated with *Calendula* 6C, *Calendula* 30C, and *Calendula* 200C compared with VC. Graph describing the WBC counts of VC, *Calendula* 6C, *Calendula* 30C, and *Calendula* 200C orally treated groups on the 8th and 14th day. Results are expressed as mean \pm SD (n = 6); *** indicates statistical significance ($p < 0.001$); ** indicates statistical significance ($p < 0.01$); * indicates statistical significance ($p < 0.05$) compared to the VC
VC – Vehicle control, *Calendula* – *Calendula officinalis*, C – Centesimal potency.

study, for wound healing. The differences between potencies remain unclear and are debated in the scientific literature, underscoring the need for future research to establish a solid scientific basis for the variations in homeopathic potencies.

Dispensing alcohol is the liquid vehicle for preparing all dilutions of homeopathic medicine. It consists of 90% ethyl alcohol and 10% distilled water. There is a lack of nanoparticles, and it does not provide any therapeutic effect.²⁸ Therefore, dispensing alcohol served as the VC for this study and was compared with *Calendula* dilutions. Additional research on *Calendula* extracts containing a high concentration of flavonoids like quercetin and rutin. This substance can promote blood flow to the injured area, providing the nutrients and oxygen required for tissue repair, reducing inflammation, and enhancing wound healing.²⁹ These bioactive substances have pharmacological effects such as antioxidants, wound healing, antibacterial, and anti-inflammatory agents.^{10,30} Compared with the VC, all *Calendula*-treated groups showed effectiveness in wound healing, as evidenced by wound contraction, epidermal thickness, collagen deposition, Ki-67 expression, and white blood cell

(WBC) counts. This was due to the absence of medicinal properties in the VC.

A toxicity study of *Calendula* flower extract at 2000 mg/kg administered to wistar rats showed no acute toxicity, indicating its suitability for oral use.³¹ A study in Brazil examined the effects of *Calendula* flowers on wound healing and inflammation in wistar rats. The wounds showed no purulent exudate, only serous exudate, and crust formation started earlier in the *Calendula*-treated group.³² This study also noted similar effects in serous exudate and crust formation. Increased wound closure and collagen deposition were observed in the *Calendula* groups on the 8th and 14th day, indicating that *Calendula* contributed positively to wound healing. This chemotaxis effect of *Calendula* may minimise inflammation and promote angiogenesis, enhancing healing by reducing wound size and fibroplasia. Similar changes may occur in both studies as they used *Calendula* flower extract.²⁹

A prior study investigated the effects of a topical *Calendula* gel containing different concentrations (5%, 7%, and 10%) on excisional wounds in male rats. The results indicated that the 7% *Calendula* gel significantly promoted healing compared to the other

concentrations. By the 14th day, all groups exhibited a reduction in wound contraction.³³ In the present study, three centesimal potencies of *Calendula* (6C, 30C, and 200C) were administered orally to treat excisional wounds in male wistar rats. The results showed that *Calendula* 30C significantly enhanced wound healing, as evidenced by increased wound closure, greater epidermal thickness, improved collagen deposition, and elevated Ki67 protein expression, compared to the other groups. All groups exhibited an increased rate of wound closure by day 14. The presence of active compounds like terpenoids, carotenoids, flavonoids, carbohydrates, and coumarins in *Calendula* plays a crucial role in its anti-inflammatory properties.³⁰ Additionally, inflammation is managed by forming new blood vessels and promoting the proliferation of fibroblasts, which contribute to the formation of granulation tissue.²⁹

A clinical trial investigating second-degree burn wounds showed significantly greater wound healing on the 7th and 15th day after administering 2 grams of *Calendula* extract orally than in the control group.³⁴ Wound contraction is a normal healing process, and *Calendula* may enhance this process due to its antioxidant properties.²⁹ The spectrophotometric analysis of *Calendula* flowers identifies and quantifies the presence of flavonoids, pigments (such as chlorophyll), and polyphenols, which are essential for their antioxidant activity.³⁵ Furthermore, the formation of new blood vessels and the promotion of fibroblast proliferation contribute to the development of granulation tissue. Through this process, *Calendula* aids in the formation of granulation tissue with the primary intention of restoring continuity by minimal scarring.^{12,29} This study found that the *Calendula* 30C group showed significantly greater wound contraction from the 4th to the 14th day than the *Calendula* 6C, *Calendula* 200C, and VC.

Clinically, oral *Calendula* 30C is a notable option, offering superior benefits in wound care. The maximum effect of *Calendula* 30C may be attributed to the increased concentration of active molecules and the optimal size of the nanoparticles present, which facilitate wound healing.^{36,37}

Additionally, *Calendula* is used to treat various wounds such as abrasions, burn sores, fissures, diabetic and non-healing ulcers, and to stop bleeding following tooth extraction. Clinically, *Calendula* θ is also applied externally to treat wounds as a healing agent.^{12,38}

This study focuses on acute wounds and the oral administration of *Calendula* at specific centesimal potencies (6C, 30C, 200C), comparing it with a vehicle control group only, without including positive

or untreated control groups. Future research should investigate acute and chronic wounds to assess the healing potential of *Calendula* by including those control groups. Additionally, exploring other administration methods, such as the topical application of *Calendula*, is advisable. Studies should also be conducted on other homoeopathic medicines for wound healing using this excisional wound model.

Due to the preliminary nature of the study, vehicle control was used as a negative control and a normal control was not used in this study. This was the limitation of this study. Hence, in the next detailed mechanistic study, normal control and positive control may be used to find out the mechanistic evaluation.

Conclusion

The study focused on assessing the wound-healing potential of *Calendula* through oral administration among the centesimal potencies (6C, 30C, 200C). Evaluation of wound contraction, epidermal thickness, collagen deposition, and proliferative marker Ki67 indicated the efficacy of *Calendula* dilutions. The findings conclude that *Calendula* 30C is the most effective centesimal potency for treating acute wounds among all the centesimal potencies used in this study. The *in vivo* study further enhances the understanding of the basic mechanisms of *Calendula* wound healing.

Acknowledgement

The authors would like to acknowledge Dr. Shivaprasad K, Principal of Yenepoya Homoeopathic Medical College Hospital, and Dr. Lourdhuraj I, Principal of Yenepoya Physiotherapy College, for their support and guidance in this study. The authors also acknowledge Yenepoya Pharmacy College and Research Centre for the support provided for physicochemical analysis.

Funding statement

Yenepoya (Deemed to be University), Mangalore, India, supported this study through a university seed grant (YU/Seed grant/098/2021).

Conflict of interest

None declared.

Author contribution

A. Vincer George: Concepts, Design, Definition of intellectual content, Literature search, Experimental studies, Data acquisition, Data analysis, Manuscript preparation, Manuscript editing, Manuscript review, and Guarantor.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Évaluation de l'administration orale de *Calendula officinalis* parmi les dilutions centésimales pour la cicatrisation des plaies aiguës chez le rat Wistar mâle

Contexte : *Calendula officinalis* (*Calend.*) est utilisé en homéopathie pour traiter diverses plaies. La présence de composés bioactifs lui confère des propriétés pharmacologiques telles que la cicatrisation, les antioxydants et les propriétés anti-inflammatoires. Par conséquent, les cliniciens utilisent différentes dilutions centésimales (6 CH, 30 CH, 200 CH) pour cicatriser les plaies. **Objectif :** Comparer le potentiel cicatrisant des dilutions de *Calendula* (6 CH, 30 CH, 200 CH) par administration orale dans un modèle préclinique de plaie aiguë. **Matériel et méthodes :** L'étude consistait à administrer des doses centésimales de *Calendula* (6 CH, 30 CH, 200 CH) par voie orale. Des rats Wistar mâles âgés de huit à dix semaines ont participé à l'expérience. Chaque animal a subi une excision complète sur le dos et a été réparti en quatre groupes : un groupe témoin (VC) et trois groupes de traitement recevant respectivement des doses orales d'alcool, de *Calendula* 6 CH, de *Calendula* 30 CH et de *Calendula* 200 CH. Chaque animal a reçu le traitement deux fois par jour pendant sept jours, à six heures d'intervalle. Français entre les doses. Des photographies de plaies, prises à intervalles réguliers, ont été utilisées pour observer le taux de fermeture des plaies. Les animaux ont été sacrifiés les 8^e et 14^e jours afin de recueillir des échantillons pour histopathologie et immunohistochimie. **Résultats :** L'analyse de la variance a évalué la significativité statistique des différences entre les groupes. L'étude a montré que la cicatrisation des plaies était significativement plus élevée dans les groupes traités au *Calendula* que dans ceux traités par CV. Les résultats indiquent que la fermeture des plaies, l'épaisseur de l'épiderme, le dépôt de collagène et les niveaux d'expression de l'antigène marqueur prolifératif Kiel-67 (Ki-67) étaient significativement plus élevés ($p < 0,01$) dans le groupe traité au *Calendula* 30C par rapport aux autres groupes. **Conclusion :** Parmi les puissances centésimales de *Calendula* testées pour la contraction des plaies, le 30C s'est avéré le plus efficace pour la cicatrisation des plaies.

Bewertung der oralen Verabreichung von *Calendula officinalis* in verschiedenen Centesimalpotenzen zur akuten Wundheilung bei männlichen Wistar-Ratten

Hintergrund: *Calendula officinalis* (*Calend.*) wird in der Homöopathie zur Behandlung verschiedener Wunden eingesetzt. Die enthaltenen bioaktiven Verbindungen führen zu pharmakologischen Eigenschaften wie Wundheilung, Antioxidantien und Entzündungshemmung. Daher verwenden Ärzte verschiedene Centesimalpotenzen (C6, C30, C200) zur Wundheilung. **Ziel:** Vergleich des Wundheilungspotenzials von *Calendula*-Verdünnungen (C6, C30, C200) bei oraler Verabreichung in einem präklinischen Modell für akute Wunden. **Material und Methoden:** Die Studie umfasste die orale Verabreichung von *Calendula*-Centesimalpotenzen (6C, 30C, 200C). Männliche Wistar-Ratten im Alter von acht bis zehn Wochen wurden an dem Experiment beteiligt. Jedes Tier erhielt eine vollflächige Exzisionswunde am Rücken und wurde in vier Gruppen eingeteilt: eine Kontrollgruppe (VC) und drei Behandlungsgruppen, die orale Dosen von Alkohol, *Calendula* 6C, *Calendula* 30C und *Calendula* 200C erhielten. Jedes Tier wurde sieben Tage lang zweimal täglich im Abstand von sechs Stunden behandelt. zwischen den Dosen. In regelmäßigen Abständen aufgenommene Wundfotos wurden verwendet, um die Wundverschlussrate zu beobachten. Die Tiere wurden am 8. und 14. Tag getötet, um Proben für die Histopathologie und Immunhistochemie zu sammeln. **Ergebnisse:** Die Varianzanalyse bewertete die statistische Signifikanz der Gruppenunterschiede. Die Studie zeigte, dass die Wundheilung in den mit *Calendula* behandelten Gruppen signifikant schneller verlief als in

der VC-Gruppe. Die Ergebnisse deuten darauf hin, dass Wundverschluss, Epidermisdicke, Kollagenablagerung und die Expression des proliferativen Markerantigens Kiel-67 (Ki-67) in der mit *Calendula* 30C behandelten Gruppe im Vergleich zu anderen Gruppen signifikant höher waren ($p < 0,01$). **Schlussfolgerung:** Unter den auf Wundkontraktion getesteten *Calendula*-Centesimalpotenzen erwies sich 30C als die wirksamste für die Wundheilung.

नर विस्तार चूहों में तीव्र घाव भरने में कैलेंडुला ऑफिसिनेलिस की विभिन्न सेंटिसिमल पोटेंसी के मौखिक प्रशासन का मूल्यांकन

पृष्ठभूमि: होम्योपैथी में कैलेंडुला ऑफिसिनेलिस (कैलेंडुला) विभिन्न प्रकार के घावों के उपचार में किया जाता है। जैवसक्रिय यौगिक घाव भरने, एंटीऑक्सीडेंट तथा सूजन-रोधी जैसे औषधीय गुण प्रदान करते हैं। इसी कारण, चिकित्सक घाव भरने हेतु इसकी विभिन्न सेंटिसिमल पोटेंसी (6C, 30C, 200C) का प्रयोग करते हैं। **उद्देश्य:** पूर्व-नैदानिक तीव्र घाव मॉडल में मौखिक प्रशासन द्वारा कैलेंडुला की सेंटिसिमल पोटेंसी (6C, 30C, 200C) की घाव भरने की क्षमता की तुलना करना। **सामग्री और विधियाँ:** इस अध्ययन में 8-10 सप्ताह आयु के नर विस्तार चूहों को शामिल किया गया। प्रत्येक जानवर की पीठ पर पूरी मोटाई का एक घाव बनाया गया और उन्हें चार समूहों में विभाजित किया गया: वाहन नियंत्रण (वीसी) समूह तथा तीन उपचार समूह जिन्हें क्रमशः डिस्पेंसिंग अल्कोहल, कैलेंडुला 6C, कैलेंडुला 30C, और कैलेंडुला 200C की मौखिक खुराक दी गई। प्रत्येक पशु को सात दिनों तक प्रतिदिन दो बार, छह घंटे के अंतराल पर उपचार दिया गया। घाव की तस्वीरें, नियमित अंतराल पर ली गईं, जिनका प्रयोग घाव के बंद होने की दर के आकलन हेतु किया गया। 8वें और 14वें दिन हिस्टोपैथोलॉजी और इम्यूनोहिस्टोकेमिस्ट्री परीक्षण हेतु नमूने एकत्र किए गए। **परिणाम:** विचरण के विश्लेषण से समूह के बीच अंतर का सांख्यिकीय मूल्यांकन किया गया। परिणामों से स्पष्ट हुआ कि वाहन नियंत्रण (वीसी) की तुलना में कैलेंडुला-उपचारित समूहों में घाव भरने की दर उल्लेखनीय रूप से अधिक थी। परिणामों से स्पष्ट हुआ कि कैलेंडुला 30सी-उपचारित समूह में घाव का बंद होना, एपिडर्मल मोटाई, कोलेजन जमाव और प्रोलिफेरेटिव मार्कर एंटीजन कील-67 (की-67) अभिव्यक्ति का स्तर अन्य समूहों की तुलना में काफी अधिक ($p < 0.01$) था। **निष्कर्ष:** परीक्षण की गई सेंटिसिमल पोटेंसी में कैलेंडुला 30 C तीव्र घाव भरने के लिए सबसे प्रभावी सिद्ध हुई।

Evaluación de la administración oral de *Calendula officinalis* entre las potencias centesimales en la cicatrización de heridas agudas en ratas Wistar macho

Antecedentes: La *Calendula officinalis* (Calend.) se utiliza en homeopatía para tratar diversas heridas. La presencia de compuestos bioactivos le confiere propiedades farmacológicas como cicatrización de heridas, antioxidantes y antiinflamatorias. Por lo tanto, los médicos utilizan diferentes potencias centesimales (6C, 30C, 200C) para la cicatrización de heridas. **Objetivo:** Comparar el potencial de cicatrización de las diluciones de *caléndula* (6C, 30C, 200C) mediante administración oral en un modelo preclínico de herida aguda. **Materiales y métodos:** El estudio consistió en la administración oral de potencias centesimales de *caléndula*

(6C, 30C, 200C). Se utilizaron ratas Wistar macho de ocho a diez semanas de edad. A cada animal se le realizó una herida escisional de espesor completo en el dorso y se asignó a cuatro grupos: grupo control con vehículo (VC) y tres grupos de tratamiento que recibieron dosis orales de alcohol de dispensación, *caléndula* 6C, *caléndula* 30C y *caléndula* 200C, respectivamente. Cada animal recibió el tratamiento dos veces al día durante siete días, con un intervalo de seis horas. Entre dosis. Se utilizaron fotografías de la herida, tomadas a intervalos regulares, para observar la tasa de cierre de la misma. Los animales fueron sacrificados los días 8 y 14 para recolectar muestras para histopatología e inmunohistoquímica. **Resultados:** El análisis de varianza evaluó la significancia estadística de las diferencias entre los grupos. El estudio mostró que la cicatrización de la herida fue significativamente mayor en los grupos tratados con *caléndula* que en el grupo con VC. Los resultados indican que el cierre de la herida, el grosor epidérmico, la deposición de colágeno y los niveles de expresión del antígeno marcador proliferativo Kiel-67 (Ki-67) fueron significativamente mayores ($p < 0,01$) en el grupo tratado con *caléndula* 30C en comparación con los otros grupos. **Conclusión:** Entre las potencias centesimales de *caléndula* analizadas para la contracción de la herida, la 30C demostró ser la más eficaz para la cicatrización de la herida.

评估口服不同浓度金盏花对雄性Wistar大鼠急性伤口愈合的影响

背景: 金盏花 (学名: *Calend.*) 在顺势疗法中用于治疗各种伤口。其生物活性化合物具有促进伤口愈合、抗氧化和抗炎等药理特性。因此, 临床医生使用不同浓度 (6°C、30°C、200°C) 的金盏花来愈合伤口。**目的:** 比较金盏花不同稀释度 (6°C、30°C、200°C) 在临床前急性伤口模型中口服给药的伤口愈合潜力。**材料与方法:** 本研究采用金盏花百倍效价 (6C、30C、200C) 口服给药。实验对象为8至10周龄雄性Wistar大鼠。每只大鼠背部均制作全层切除伤口, 并被随机分为四组: 载体对照组 (VC) 和三个治疗组, 分别口服配制酒精、金盏花6C、金盏花30C和金盏花200C。每只大鼠每天接受两次治疗, 持续七天, 每次间隔六小时。剂量之间。定期拍摄伤口照片, 以观察伤口愈合率。在第8天和第14天处死动物, 收集样本进行组织病理学和免疫组织化学分析。**结果:** 方差分析评估了组间差异的统计学意义。研究表明, 金盏花治疗组的伤口愈合率明显高于VC组。结果表明, 与其他组相比, 金盏花30C治疗组的伤口愈合、表皮厚度、胶原沉积和增殖标志抗原Kiel-67 (Ki-67) 的表达水平明显较高 ($p < 0.01$)。**结论:** 在用于伤口收缩的百倍效力测试中, 30C被证明对伤口愈合最有效。