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Original Research Article

Biological activity and mechanism of action of Gotu kola oil in skin wound repair

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Abstract

Purpose: *To investigate the biological activity and mechanism of action of Gotu Kola oil in skin wound repair.*
Methods: *In this study, a total of 40 Sprague-Dawley (SD) rats were meticulously chosen to establish a skin wound model. The rats were randomly allocated to a study group, which received topical application of Gotu kola oil, and control group, which served as the model control. Each group consisted of 20 rats to ensure an adequate sample size. The wound area was measured using precise digital imaging software to quantitatively determine the rate of wound healing. Expression levels of hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and transforming growth factor-beta 1 (TGF-β1) were determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) while the extent of microvassel regeneration at the wound site was determined by immunohistochemical method using CD34 as a marker.*
Results: *The wound healing rate in the study group was higher than in the control group. In the study group, the transcription levels of HGF and VEGF were higher than in the control group on days 3, 7, and 14, while the transcription level of TGF-β1 was higher on days 7 and 14. Microvessel density (MVD) in the study group was higher than in the control group on days 3 and 7 ($p < 0.05$).*
Conclusion: *Gotu kola oil facilitates wound healing by promoting the generation of capillaries at the wound site. Harnessing this potential of Gotu kola oil may lead to the development of more efficacious treatment strategies for skin wound repair, ultimately enhancing the quality of life of the patients.*

Keywords: *Gotu kola oil, Skin wound, Repair, Biological activity, Mechanism of action*

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INTRODUCTION

The skin is one of the largest organs in the human body and its integrity is crucial for maintaining physiological functions and defending against external environmental threats.

Skin injuries, resulting from various causes, are common and significant health concerns that profoundly affect patients' physical well-being and quality of life. Skin trauma refers to damage or harm inflicted upon the skin or underlying tissues due to physical trauma, irritation, or external factors. It encompasses a wide range of injuries, such as cuts, tears, abrasions, burns,

bruises, punctures, ulcers, and others. Different types of skin trauma can lead to varying degrees of damage, spanning from minor abrasions to severe burns or deep wounds [1,2]. Following trauma, the skin initiates a self-repair mechanism to expedite its restoration. Consequently, exploring effective approaches to promote skin wound repair and healing has been a paramount focus of clinical medicine and biomedical study.

In recent decades, extensive investigations have elucidated the molecular and cellular mechanisms underlying skin wound repair, aiming to identify potential therapeutic strategies. However, despite these efforts, an ideal treatment modality that simultaneously enhances healing speed, improves healing quality and reduces complications remains elusive. Consequently, the exploration of novel treatment approaches and efficacious medications has become a pivotal area of current study.

Gotu Kola oil, derived from natural sources, has garnered significant study interest in recent years. It has been widely utilized in traditional medical systems, especially in Asian regions, for the management of various skin conditions including burns, ulcers, trauma and inflammatory skin diseases [3,4]. Concurrently, modern scientific study has gradually unraveled the potential roles of Gotu Kola oil in skin wound repair. Therefore, the objective of this study is to elucidate the biological activity and mechanisms of action of Gotu Kola oil in the context of skin wound repair. Through comprehensive experimentation and molecular biology methodologies, this study comprehensively investigates the effects of Gotu Kola oil on wound sites, encompassing its potential impact on healing quality, vascular regeneration and other relevant factors. This in-depth exploration of these critical facets aims to provide scientific evidence for the development of more effective strategies for skin wound repair treatments, ultimately enhancing patients' quality of life and expediting their recovery process.

EXPERIMENTAL

Animals

Forty male Sprague-Dawley (SD) rats, weighing between 240 and 260 g were chosen for this study. These rats demonstrated normal body temperature, respiration and pulse rates. After the successful modeling process, they were randomly allocated into two groups: study group, consisting of 20 rats receiving Gotu Kola oil treatment, and control group, comprising 20 rats serving as the model control group.

Animal modeling

The experiment began after a one-week acclimatization period in a standard animal laboratory environment. To induce circular skin defects with a 10 mm diameter, a dermatological biopsy punch was employed. This standardized instrument allows for the precise and controlled removal of tissue, ensuring consistent dimensions in the circular wounds. The defects were created on both the left and right sides of the skin on the back of the rats' second lumbar vertebra. Subsequently, all rats were randomly assigned to two groups using a random number table method.

Treatment method

Both groups underwent wound treatment twice daily at scheduled intervals. Study group received Gotu Kola oil administered as follows: 480 milligrams dissolved in 200 microliters of DMSO, thoroughly mixed to achieve a uniform and stable solution. Subsequently, 20 microliters of the solution were extracted and combined with 16 grams of white Vaseline. The mixture was heated until melted, then cooled to produce a blue, semi-transparent, viscous paste. The resulting ointment, containing 3 milligrams of blue oil per gram, was meticulously applied to cover the wound, ensuring complete coverage of the wound site. In contrast, control group was treated with Vaseline ointment containing DMSO applied to the wound.

Evaluation of parameters/indices

Wound healing parameters

Photographs and samples were obtained at various time points, including the day of successful modeling and at days 3, 7, 14 and 21. ImageJ software was employed to measure the wound area (WA). The healing rate (HR) was calculated using Eq 1.

$$HR = (WA_p/WA_i)100 \quad \text{..... (1)}$$

where WA_p: wound area at the time of photography; WA_i : wound area (initial). This method allowed for an accurate assessment of the progression of wound healing over the specified time intervals.

Gene expression analysis

Transcription levels of HGF, VEGF and TGF-β1 were determined using quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-

PCR). Biological samples, comprising RNA extracted from tissue specimens, underwent a systematic processing method for gene expression analysis. Following RNA extraction and purification, the RNA was reverse transcribed into complementary DNA (cDNA). The resulting cDNA served as a template for quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

Immunohistochemical assessment of CD34-negative marked blood vessels

Tissue samples were dewaxed, dehydrated, incubated using specific antibodies, stained and clarified. Dewaxing involved the removal of paraffin from the tissue sections using a xylene, while dehydration ensured the elimination of residual water content. Paraffin sections were prepared accordingly. At least, three random 200x magnification fields were selected from each group of sections for photography. The average number of blood vessels in these fields was calculated as the Microvessel Density (MVD) value, representing the number of blood vessels per high-power field (typically expressed as number/field). This method facilitated the quantitative analysis of blood vessel density within the tissue samples.

Statistical analysis

Graphs in this study were processed using GraphPad Prism 8, while data organization and analysis were conducted with SPSS 21.0 software. Quantitative data was presented as mean ± standard deviation and intergroup comparisons were performed using the *t*-test. Count data was expressed as n (%) and intergroup comparisons were analyzed using the χ^2 test. Differences between groups were considered statistically significant when the *p*-value was less than 0.05.

RESULTS

Healing rate

At the 7th and 14th days, the wound healing rate of study group rats was higher than that of control group ($p < 0.05$) as presented in Figure 1.

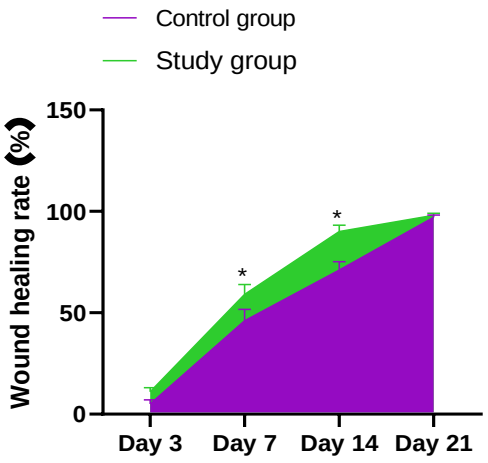

Figure 1: Comparison of wound healing rates between the two groups. *$P < 0.05$ vs. control group

Transcription levels of relevant growth factors

In study group rats, the transcription levels of HGF and VEGF were higher than those in control group on the 3rd day, transcription level of TGF-β1 was higher than that in control group on the 7th day ($p < 0.05$) as shown in Figure 2.

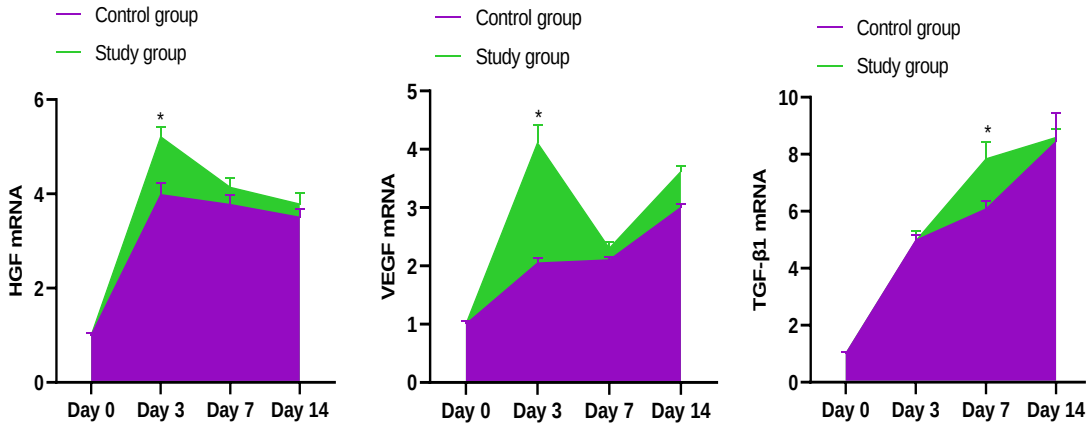

Figure 2: Comparison of transcription levels of HGF, VEGF, and TGF-β1 between the two groups. *$P < 0.05$ vs. control group

Wound microvascular density

In study group rats, the microvascular density (MVD) was higher than in control group on the 3rd and 7th day and lower on the 14th day, while the differences were insignificant ($p > 0.05$) as depicted in Figure 3.

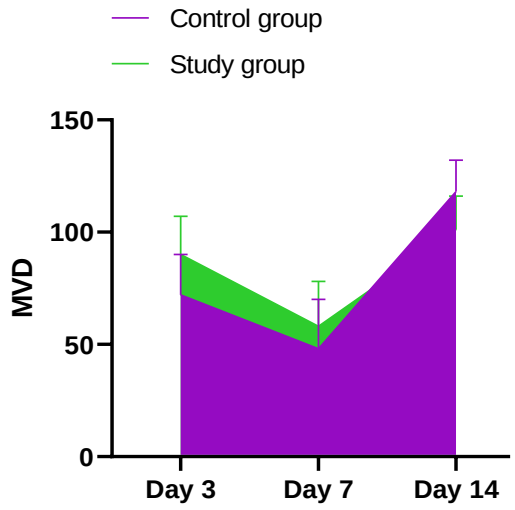

Figure 3: Comparison of wound microvascular density between the two groups

DISCUSSION

Gotu Kola oil (*Centella asiatica*), also known as Asiatic pennywort or Indian pennywort, is a common herbaceous plant widely distributed in Asia, Africa and Latin America. It has been used in traditional herbal medicine to treat various diseases and symptoms, and has gained extensive study interest in recent years, particularly in the context of skin health and wound healing [5,6]. Gotu kola oil, at room temperature, is a dark blue viscous liquid, also known as Gotu kola extract. Its chemical name is madecassoside, with a molecular formula of $C_{15}H_{18}$. It is a widely used topical medication commonly employed for treating burns and frostbite in everyday life. Gotu kola oil contains various bioactive compounds, including triterpenoids and polysaccharides, which exhibit significant anti-inflammatory properties. These compounds mitigate inflammatory responses by inhibiting the production of inflammatory mediators and the activation of inflammatory signaling pathways, helping to alleviate skin inflammation and discomfort [7]. Gotu kola oil contains polyphenolic compounds such as catechins and anthocyanins, which possess potent antioxidant properties. These antioxidants help neutralize free radicals, reduce oxidative stress, and consequently decrease cellular and tissue damage. Gotu kola oil has been shown to promote the healing of skin wounds. It stimulates the synthesis of collagen, and accelerates the repair and regeneration of damaged tissues. Additionally, Gotu kola oil aids in increasing cell proliferation, thus facilitating the speedy recovery of wounds [8]. Some studies suggest that Gotu Kola oil has antibacterial and antifungal activity, which helps prevent or treat wound infections.

The antioxidant properties of Gotu Kola oil and its promotion of collagen make it a potentially anti-aging compound. It helps maintain skin elasticity and a youthful appearance. Gotu Kola oil thus exhibits multiple biological activities, including anti-inflammatory, antioxidant, wound-healing promotion, antibacterial and anti-aging effects. These characteristics have garnered significant attention in the fields of skin health and wound healing, and Gotu kola oil is widely used in skincare products and medical treatments [5,9].

The results indicate that *Gotu kola oil* possesses significant biological activity and contributes to the promotion of skin wound healing. It was observed that in groups treated with *Gotu kola oil*, wound healing rate was significantly higher than in control group. This result is consistent with previous study, demonstrating the positive role of *Gotu kola oil* in wound healing. This may be attributed to the polysaccharide components of *Gotu kola oil* [8,10], which promote the proliferation and repair of skin cells, as well as its anti-inflammatory and antioxidant properties that alleviate inflammation at the wound site.

Additionally, this study delves into the mechanisms of action of *Gotu kola oil*. First, *Gotu kola oil* accelerates the repair of damaged tissue by promoting cell proliferation and collagen synthesis at the wound site [8,11]. Growth factors are pivotal in orchestrating the intricate process of wound healing. Among them, TGF-β1 plays a crucial role and exerts its influence throughout the various stages of wound healing via the smad2/3 signaling pathway [12,13]. In addition, TGF-β1 not only governs the proliferation of keratinocytes, facilitating epidermal regeneration, but also regulates fibroblast proliferation, thereby promoting collagen synthesis and the formation of granulation tissue. These mechanisms are believed to contribute to the wound healing-promoting effects observed in the context of *Gotu kola oil*. Furthermore, the anti-inflammatory effect of *Gotu kola oil* reduces inflammation at the wound site by inhibiting the release of inflammatory mediators, contributing to the healing process. It also reduces the adverse effects of oxidative stress on wound healing through antioxidant mechanisms [14,15]. These

mechanisms interact synergistically to accelerate the rapid healing of skin wounds.

Furthermore, it is widely acknowledged that vascular regeneration plays a pivotal role in the process of wound repair [12]. Vascular regeneration not only facilitates the establishment of new tissue structures but also provides essential materials and nutrients for repair while aiding in waste removal. Notably, growth factors such as HGF and VEGF assume crucial roles in promoting the formation of blood vessels, significantly augmenting the population of endothelial progenitor cells, facilitating their mobilization and differentiation, and thereby facilitating vascular regeneration. Additionally, VEGF also exerts a significant impact on vascular permeability and anti-apoptotic mechanisms, thus promoting the formation of new blood vessels [12,13].

In the present study, immunohistochemical labeling utilizing CD34 antibodies was employed to identify newly formed blood vessels, characterized by brown circular or strip-like structures, followed by subsequent counting and comparative analysis. On the 3rd and 7th day, study group exhibited higher levels of VEGF expression compared to control group, which aligns with the observed trend of microvessel density changes. Although the differences were not significant, this may be due to the uneven distribution of identified vascular markers.

Gotu kola oil may promote the generation of capillaries at the wound site. However, on the 14th day, the microvessel density in the study group was lower than that in the control group. This may be because the wound healing rate in the study group was faster, entering the stage of granulation tissue filling the wound earlier, at which point capillaries cease to regenerate. In contrast, the wound healing rate in control group was slower and was still in the phase of rapid new blood vessel formation. The disparity in microvessel density could potentially be attributed to varying stages of the healing process. *Gotu kola oil* which comprises a diverse range of bioactive constituents such as polysaccharides, alkaloids, and polyphenols, has demonstrated multiple bioactivities in laboratory investigations. These activities encompass anti-inflammatory, antioxidant, antimicrobial, and cell proliferation-stimulating properties [16,17].

Study limitations

Although this study provides evidence to support the potential application of *Gotu kola oil* in skin wound repair, there are still some limitations. Firstly, it is important to note that this study primarily focused on a laboratory mouse model and further studies should be conducted in settings that closely resemble clinical conditions. Secondly, additional investigations are required to determine the optimal usage and dosage of *Gotu kola oil* for the most effective treatment regimen. Lastly, a more profound elucidation of the molecular mechanisms underlying the effect of *Gotu kola oil* is necessary to identify additional pathways and potential targets.

Previous studies suggested that its notable anti-inflammatory effect is achieved through the inhibition of inflammatory factor release and the regulation of immune cell activity, which contributes to reducing inflammation at the wound site and expediting the healing process. However, this particular study did not specifically address this aspect. Therefore, future study could consider expanding the sample size, conducting large-scale multicenter clinical studies to validate the findings of this study, and comprehensively evaluate the practical application value of *Gotu kola oil*.

CONCLUSION

Gotu kola oil shows great potentials for skin wound repair. A comprehensive understanding of the healing bioactivity and mechanisms of action of *Gotu kola oil* has been achieved, providing evidence for the advancement of more efficacious strategies in skin wound repair. Subsequent investigations will continue to broaden this field, aiming to ultimate advance to achieve patient recovery and improved overall quality of life.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

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

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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