Physiology and Pharmacology 28 (2024) 389-399
Experimental Research Article

The efficacy of Hypericum helianthemoides extraction with phenytoin in diabetic and non-diabetic wound healing: An experimental study in rats

Soheila Soltanbeigi[1], Zeinab Fahimi[1], Maryam Maleki[1], Aliashraf Mozafari[2], Mohammad Reza Kaffashian[1*]

1. Department of Physiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran
2. Non-Communicable Diseases Center, Ilam University of Medical Sciences, Ilam, Iran

ABSTRACT

Introduction: Traditional medicine recommends herbal medicines for metabolic disorders. The present study explores the skin wound healing potential of Hypericum helianthemoides (H. helianthemoides) extract in diabetic and non-diabetic rats.

Methods: Following wound induction in diabetic (n=50) (induced by a single dose of streptozotocin) and non-diabetic (n=50) rats, H. helianthemoides extract (5% and 10%) was administered versus standard drug phenytoin (1%) and Osrin to positive and sham control groups. Tropical ointment therapy was applied once a day until the end of the study period (20 days). A Vernier caliper (with a 0.1 mm accuracy) was used to measure the wound length at 1, 3, 7, 11, 15, and 20 days after induction. Furthermore, pathological examination categorized the wound healing process into five categories: poor, mild, moderate, fair, and excellent.

Results: On the study's first day, both diabetic and non-diabetic rats had the same wound area size. After 11 days, the wound area size significantly decreased in groups treated with 5% and 10% H. helianthemoides extract compared to the sham and control groups (P<0.001). Hence, based on the wound pathological evaluation scale, the most frequent phenytoin and H. helianthemoides extract-treated groups were classified as moderate to excellent (P<0.05).

Conclusion: H. helianthemoides extract accelerates full-thickness wound healing in diabetic and non-diabetic rats.

Keywords:
Hypericum helianthemoides
Wound healing
Herbal medicine
Streptozotocin
Diabetes mellitus

Introduction

A wound is defined as damage to the skin's two primary layers, the epidermis and dermis (Cañedo-Dorantes and Cañedo-Ayala 2019). The wound healing process is intricate but well-structured, with four stages: re-epithelialization, fibroblast migration for collagen synthesis, granulation tissue formation, and wound contraction (Fernández-Guarino et al., 2023). Untreated wounds can

* Corresponding author: Mohammad Reza Kaffashian, kaffashian-mr@medilam.ac.ir
Received 20 March 2024; Revised from 2 October 2024; Accepted 5 October 2024

Citation: Soltanbeigi S, Fahimi Z, Maleki M, Mozafari A, Kaffashian M.R. The efficacy of Hypericum helianthemoides extraction with phenytoin in diabetic and non-diabetic wound healing: An experimental study in rats Physiology and Pharmacology 2024; 28: 389-399. http://dx.doi.org/10.61186/phypha.28.4.389

continuously produce inflammatory mediators, causing pain and swelling (Sulakhiya et al., 2024). A variety of systemic and local factors influence healing, including age (Sorg and Sorg 2022), medication, nutrition, blood circulation, tissue oxygen levels (Attinger et al., 2006; Janis 2006), and topical treatments (khaksar et al., 2011).

Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia caused by insulin production or function defects (American Diabetes 2013). It leads to complications such as renal vascular disturbances, skin lesions, diabetic foot wounds, and impaired wound healing (American Diabetes 2013; World Health 1999). Elevated blood glucose causes chronic inflammation, inhibits cell proliferation, and elevates pro-inflammatory cytokines and matrix metalloproteinase levels, contributing to delayed wound healing (Singh and Kakkar 2009). Antibiotics are commonly used to prevent or treat infections, as well as advanced wound dressings and anti-glycemic agents in diabetics (Chakraborty et al., 2022). However, these treatments have limitations and side effects, like antibiotic resistance, and wound dressing skin irritation (Punjataewakupt et al., 2019). Diabetic wounds often necessitate more aggressive and prolonged treatment strategies, increasing the risk of complications such as chronic ulcers and infections (Bowers and Franco 2020). These challenges highlight the need for alternative therapies that improve wound healing while minimizing adverse effects.

Phenytoin, known for its anticonvulsant properties, promotes fibroblast proliferation, and collagen synthesis, accelerating wound healing (Anstead et al., 1996). It reduces wound exudate and edema, creating a conducive environment for wound healing (Anstead et al., 1996; Bhatia and Prakash 2004). However, applying phenytoin wound care is complicated by its dose-dependent efficacy and potential side effects (Scheinfeld 2003). Common side effects include gingival hyperplasia and hirsutism, while rare ones include drug-induced lupus and hypersensitivity syndrome (Scheinfeld 2003).

The challenges and side effects of medical treatments have paved the way for research into the efficacy of alternatives such as various herbs and natural ingredients for wound healing (Bedi and Shenefelt 2002). Sunny Hypericum (Zagros Hypericum) with the scientific name of Hypericum helianthemoides (H. helianthemoides) is a herbaceous perennial plant native to Ilam province in Iran. As illustrated in Figure 1, this plant contains a rich variety of bioactive compounds, such as phenolic acids, phloroglucinol, flavonoids, procyanidins, tannins, essential oils, amino acids, phenylpropanoids, and xanthonein (Heydari et al., 2015). It is also known by its botanical name, Hypericum helianthemoides (Spach) Boiss (Boissier 1867). These compounds exhibit various properties, including antibacterial, anti-inflammatory, and analgesic effects (Sánchez-Mateo et al., 2006; Schempp et al., 1999). Hypericum has been used to treat neurological disorders and has shown promise for burn healing and pain relief (Gangemi et al., 2015; Mirzaei et al., 2012).

Taken together, it appears that phenytoin and H. helianthemoides share some common wound-healing mechanisms, and thus it is argued that H. helianthemoides, as a natural plant, can be used as a wound-healing treatment. To the best of our knowledge, no study has compared the efficacy of H. helianthemoides in wound healing in healthy and diabetic patients to a first-line medical treatment like phenytoin. Moreover, there is still no consensus on the optimum amount of use for the most favorable outcome. To bridge these gaps, we tested the hypothesis that applying H. helianthemoides would improve wound healing in both diabetic and non-diabetic rat models compared to phenytoin, and if so, what is the optimal dosage?

Materials and methods

Animals

In this research, we utilized male Wistar rats weighing between 275 and 300 grams and aged 8 to 10 weeks. These rats were acquired from the Pasteur Institute in Karaj, Iran. Upon arrival, they were individually housed in cages maintained at 50-55% relative humidity and 23 ± 1°C, with a consistent 12-hour light/dark cycle. The rats had unrestricted access to standard rat chow and tap water. The Ilam University of Medical Sciences Ethics Committee pre-approved the experimental protocol (IR.MEDILAM.REC1396.76, date: October 10, 2015). The rats were randomly assigned into ten groups of ten individuals (n=10). These groups were categorized into two primary divisions: diabetic and non-diabetic wound groups. Each division was further segmented into five subgroups according to the type of wound treatment administered. The control group received no wound treatment. The sham group received Osrin (Kimia Gostar Co, Tehran, Iran). The Positive control group received

FIGURE 1. Sunny Hypericum (Hypericum helianthemoides) plant.

1% phenytoin (produced by Daru-Pakhsh Company, Iran) treatment; one group was treated with a 5% H. helianthemoides extract; and another with a 10% H. helianthemoides extract.

Induction of Experimental Diabetes

Animals (based on groups) received a single dose of streptozotocin (STZ, 60mg/kg IP. Merck, Germany) (Ramezani et al., 2019). STZ was dissolved in cold 0.9% saline immediately before use. Three days later, blood glucose levels were measured using a glucometer (One Touch Ultra; Life Scan, Milpitas, CA, USA) after collecting blood samples from the tail. Diabetic rats were defined as those with blood glucose levels greater than 300mg/dl and symptoms such as polydipsia, polyuria, and weight loss (Robertson et al., 1992).

Skin Wound

First, the animals were anesthetized in a glass container filled with chloroform-soaked cotton to induce wounds. After shaving off the dorsal region, a metal marker was placed on the stump, and shaved part of the animal's body to draw a circle approximately 19 mm in diameter. The skin in this area was then completely (full thickness) removed by sharp surgical scissors in a non-infectious setting (Dorsett-Martin 2004). The wound induction stage was carried out in diabetic groups after the animal's diabetic status was confirmed.

Plant Extract Preparation

The plant was collected from the Shirvan & Chardavol regions of the Zagros Mountain chain in Ilam province, Iran. All parts of the plant, including leaves, roots, and stems (50g), were shade-dried and powdered before being placed in a beaker containing 500 ml of olive oil and heated in an oven (Leica, Germany) at 47°C with daily stirring. After two weeks, the solution was filtered, yielding the oil extract (Süntar et al., 2010).

Wound Healing Evaluation

Following wound induction, each animal received a treatment (topical ointment therapy) based on their assigned group. Then wound's surface area was measured 1, 3, 7, 11, 15, and 20 days after wound induction, and the improvement in wound healing was calculated (Haji Zadeh et al., 1996). Vernier caliper (with a 0.1mm accuracy) was used to measure the wound's length.

Wound Sampling and Histological Procedures

The rats were anesthetized with diethyl ether on the twentieth day and immediately sacrificed. Then, a full-thickness strip (length: 3-4cm, width: 0.5-1cm) was removed from the wound's third midsection, revealing the central and marginal wound areas and the adjacent normal skin symmetrically (Haji Zadeh et al., 1996). The skin tissue samples were fixed in a 10% neutral formalin solution and then embedded in paraffin. Paraffin blocks were sliced into 4-μm-thick sections on a

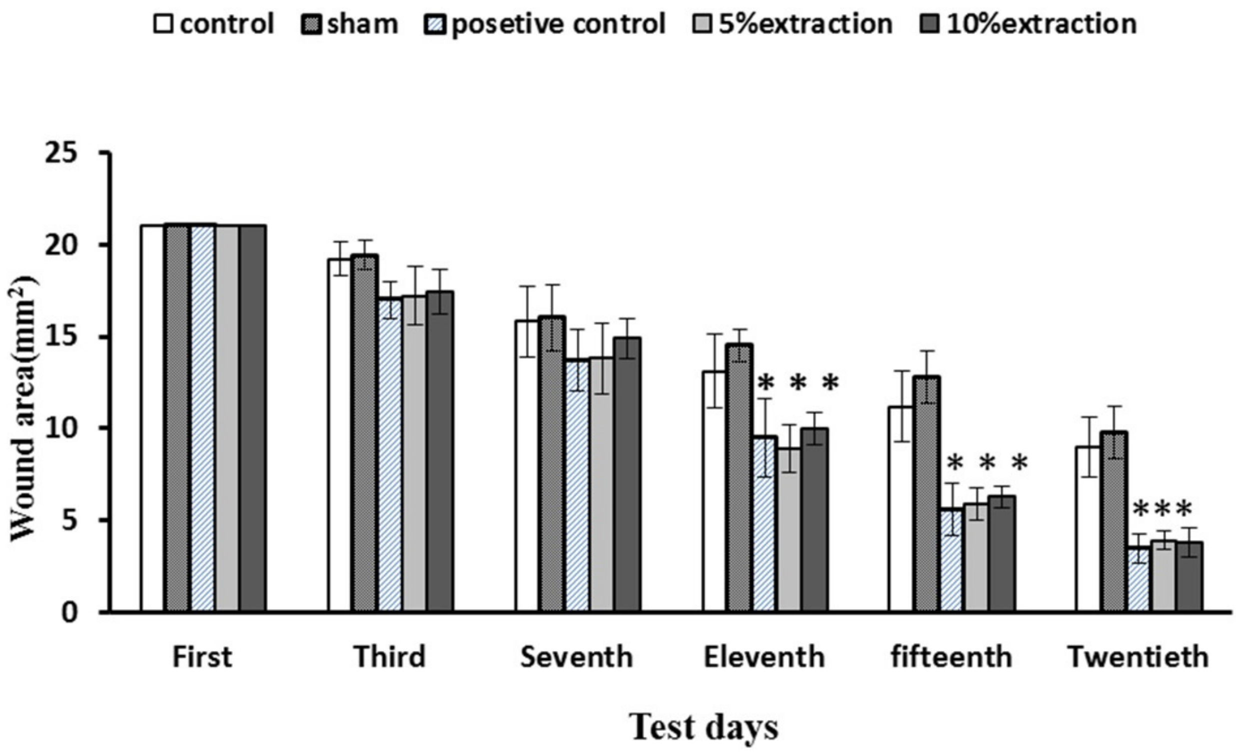

FIGURE 2. Comparison of the wound average size (wound healing) in control, sham, positive control and 5% and 10% H. helianthemoides extraction treated groups on different days of study in non-diabetic rats. (* indicates significant difference with control and sham groups, $P<0.05$ was considered to be significant).

microtome (Leica, Germany). Then, hematoxylin-eosin (H&E) staining was performed to examine the alterations in the skin tissue structures. Inflammatory cells, fibroblasts, and vascular sections were detected using a qualitative method (Haji Zadeh et al., 1996). Pathological examination categorized the wound healing process into five levels: poor, mild, moderate, fair, and excellent (Ashrafi et al., 2010; Yu and Lee 2017).

Statistical Analysis

The data are expressed as mean and SEM. The wound area size (mm2) was compared using one-way ANOVA between diabetic and non-diabetic groups. Data were analyzed using SPSS 20 software, with a significance level of $P<0.05$.

Results

Wound Size in Non-Diabetic Groups

The results revealed that the wound area size decreased significantly in groups treated with 5% or 10% H. helianthemoides extract compared to the sham and control groups. On the first day of the study, the wound area size was consistent across all groups ($p>0.05$). However, from day 11 to the end of the study, the wound area size of the control and sham groups differed significantly from that of the other groups. The wound area was largest on the final day of the study ($P<0.001$). Yet, there was no significant difference between the phenytoin and H. helianthemoides extract treatment groups (Figure 2).

Wound Size in Diabetic Groups

During the early phase of the study, no significant differences in wound area size were found between the groups treated with 5% or 10% H. helianthemoides extract and the control group. However, after 11 days, significant differences emerged between the control group and the groups treated with concentrations of H. helianthemoides extract and phenytoin. These differences became more pronounced towards the end of the study ($P<0.05$) (Figure 3).

In both diabetic and non-diabetic rats, statistical analysis showed no significant difference in wound size between the phenytoin groups (positive control) and the groups treated with 5% and 10% H. helianthemoides extract on

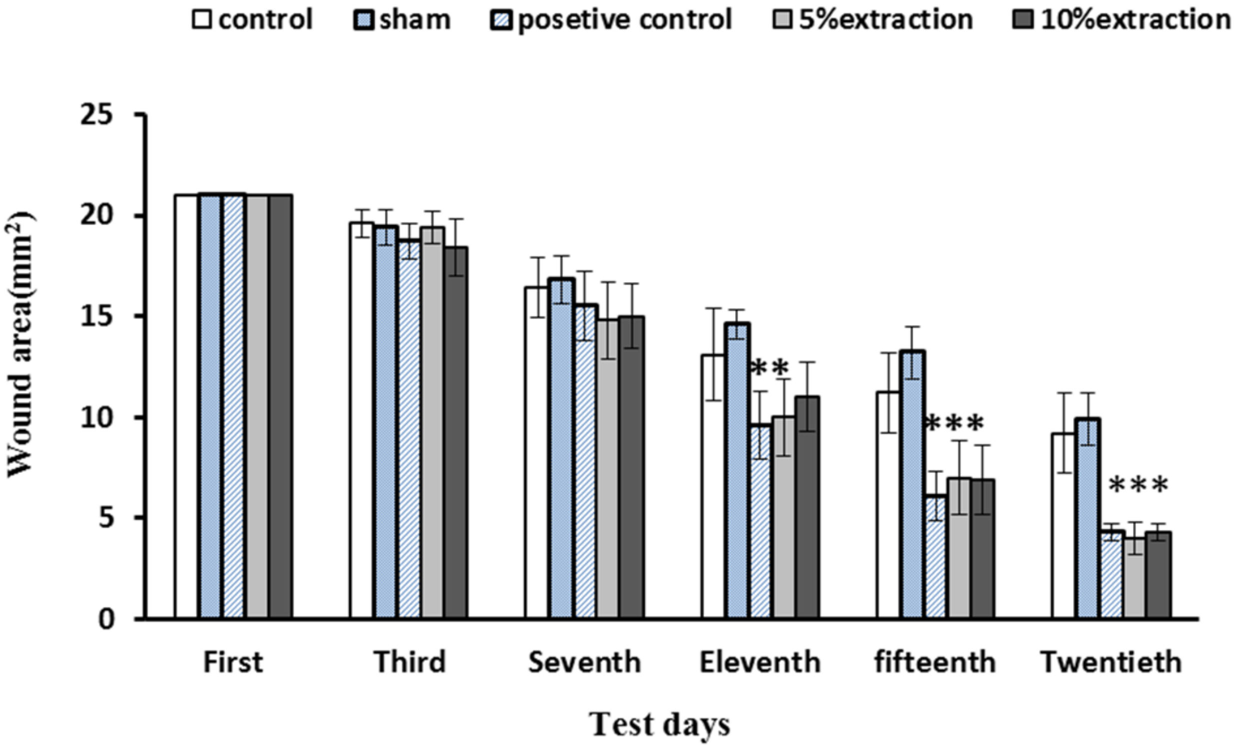

FIGURE 3. Comparison of the wound average size (wound healing) in control, sham, positive control and 5% and 10% H. helianthemoides extraction treated groups on different days of study in diabetic rats. (* indicates significant difference with control and sham groups, P< 0.05 was considered to be significant).

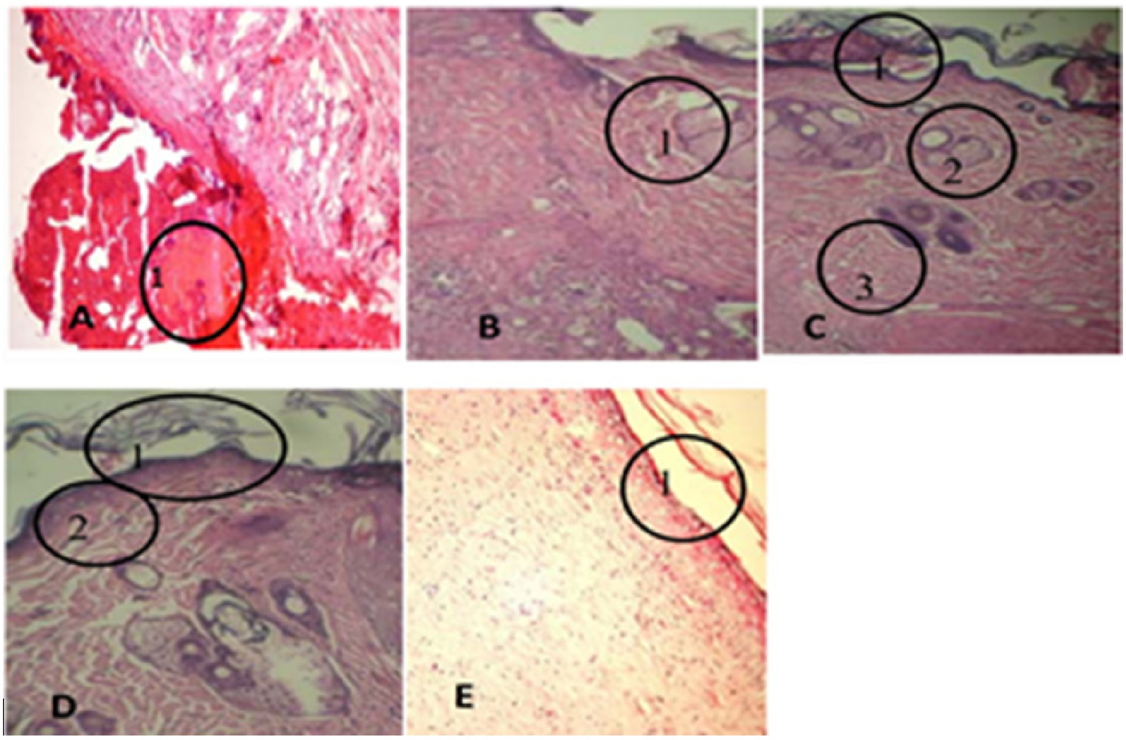

FIGURE 4. A-E: Sample images of the wound on the twentieth day, in non-diabetic rats. A: control group, B: sham, C: positive control group, D and E: 5% and 10% H. helianthemoides extraction treated groups respectively. (A,1: inflamed and bleeding area. B, 1: inflamed area. C, 1: Epidermis, 2: Scar tissue, 3: Collagen tissue. D, 1: Epidermis, 2: Collagen tissue. E, 1: Dermis and epidermis).

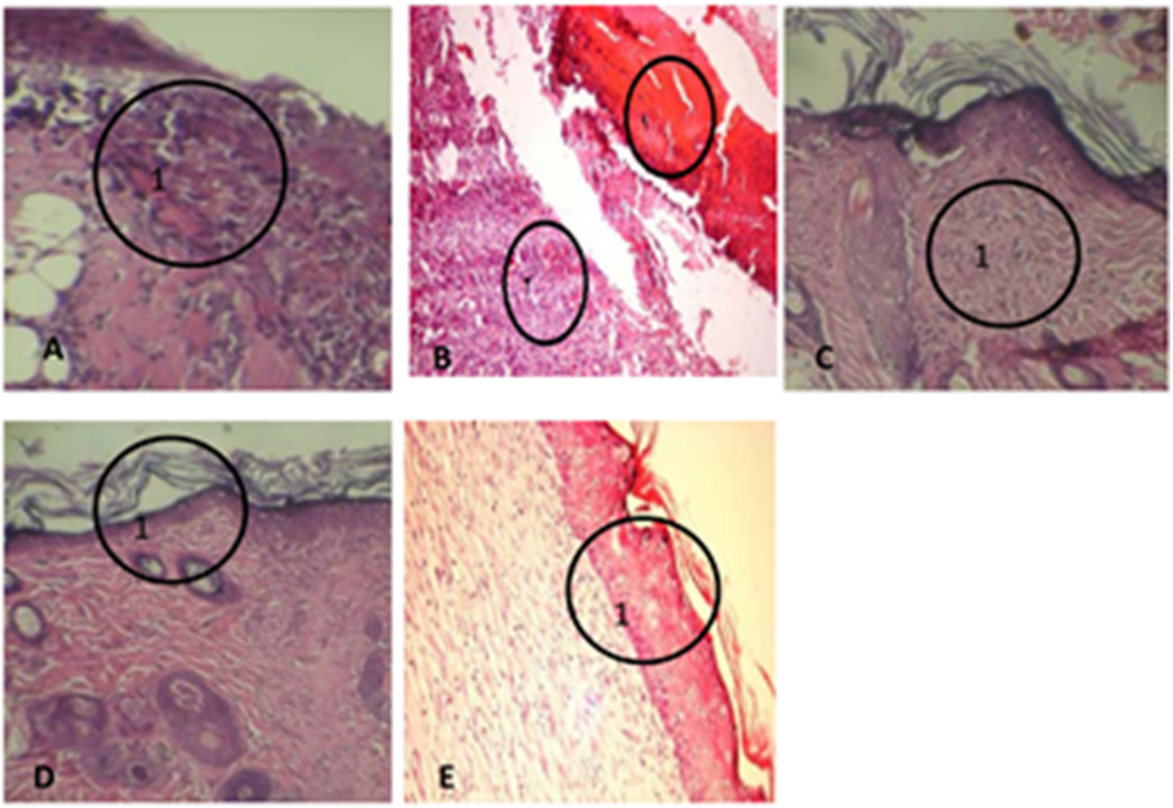

FIGURE 5. A-E: sample images of the wound on the twentieth day, in diabetic rats. A: control group, B: sham, C: positive control group, D and E: 5% and 10% H. helianthemoides extraction treated groups, respectively. (A, 1: inflamed area. B, 1: inflamed and bleeding area. C, 1: Collagen tissue. D, 1: Epidermis. E, 1: Dermis and epidermis).

various examination days (P>0.05) (Figures 2 and 3).

Microscopic Study

On the twentieth day, wound samples were evaluated for signs of healing, including skin re-epithelialization, fibroplasia, and the presence of inflammatory cells. In the non-diabetic groups, the epidermis was not visible in the control and sham groups, but inflammation was evident in the dermis. The positive control group showed epidermis formation and visible collagen fiber. Also, fibroblasts in the dermis were noted. The epidermis had formed, granulation was complete, and collagen fiber deposition was observed in the group treated with 5% H. helianthemoides. The 10% H. helianthemoides extract-treated group had a fully formed epidermis and dermis with apparent collagen formation and horny layers (Figure 4).

In diabetic animals, the control and sham groups had no epidermis or collagen fibers, with apparent inflammation and bleeding. The positive control and 5% H. helianthemoides extract-treated groups had epidermis, dermis, and collagen formation in the dermis. The epidermis and dermis were fully formed in the 10% H. helianthemoides extract-treated group, as was collagen formation, but no hair follicles were observed in the skin tissue (Figure 5).

The healing process in the phenytoin and H. helianthemoides extract-treated groups differed significantly from the control groups (P<0.05) (Figure 6). The most frequent wound healing stages in the positive control and H. helianthemoides treated groups ranged from moderate to excellent, whereas the control groups were typically poor to mild.

Discussion

This study examined the efficacy of H. helianthemoides extracts (5 and 10%) in wound healing in diabetic and non-diabetic rats, comparing its effects with 1% topical phenytoin. Our findings revealed that, in both healthy and diabetic conditions, wound size was significantly smaller after the 11th day in the H. helianthemoides extraction at

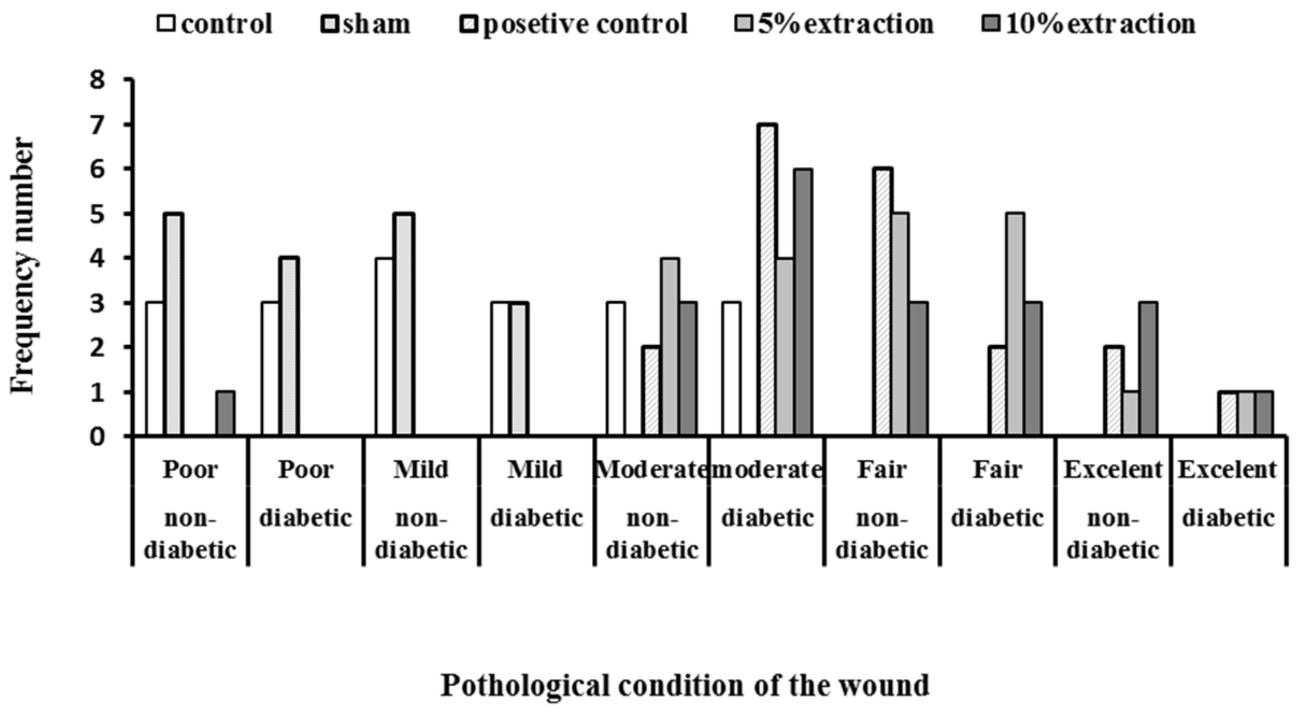

FIGURE 6. Frequency of pathological conditions in control, sham, positive control and 5% and 10% H. helianthemoides extraction treated groups on the twentieth day of study in normal and diabetic rats. In pathological examination, wound healing process divided to the 5 categories, poor, mild, moderate, fair, and excellent.

concentrations of 5% and 10% compared to the control and sham groups. Interestingly, wound size did not differ significantly between H. helianthemoides extract at 5% and 10% concentrations and the 1% topical phenytoin group.

Other studies have found that Hypericum perforatum treats deep skin burns due to its anti-inflammatory properties, which is consistent with our findings (Hoffmann et al., 2020; Prisăcaru et al., 2013). Farsak et al. recommended that Hypericum perforatum could be effective on dorsal dermo-epidermal wound healing in diabetic rats (Farsak et al., 2017). They reported that the topical application of Hypericum perforatum made no difference on the third day (Farsak et al., 2017). However, it positively affected wound healing on the seventh and tenth days in diabetic rats with mucosal defects in the palatal area (Altan et al., 2018), consistent with our findings in the present study. This efficacy might be attributed to the inhibition of Cyclooxygenase (COX), prostaglandin synthesis, protein kinase, or arachidonic acid release (Prisăcaru et al., 2013). To provide more support for this notion, Menegazzi et al., (2006) have reported that anti-inflammatory effects of the Hypericum are likely due to inhibition of nuclear factor kappa B and STAT-3 activation (Menegazzi et al., 2006). There is also evidence that topical application of Hypericum oil significantly affects wound healing, provides local anesthesia, and spares pain and discomfort (M. Mahmoudi et al., 2006). It also has antiseptic properties and can treat burns in the short term (Brondz et al., 1982). A major part of the anti-inflammatory effect of Hypericum has been attributed to flavonoids, hypericin, and hyperforin, which inhibit and reduce the production of inflammatory mediators (Bork et al., 1999; Novelli et al., 2020). The plant's antibacterial and antiviral properties may also contribute to wound healing (Cinci et al., 2017; Kucukboyaci et al., 2020).

This study also evaluated the effects of H. helianthemoides on diabetic wound healing and found that it was effective in both normal and diabetic wound conditions. Diabetes can lead to impaired wound healing due to chronic inflammation, microvascular changes, endothelial proliferation disturbances, and capillary basement membrane thickening (Graves and Donaghue 2020; LoGerfo and Coffman 1984), creating a vulnerable environment for infection (Jeffcoate and Harding

2003; Rodríguez-Rodríguez et al., 2022), reduced collagen formation, and increased free oxygen radicals and blood sugar levels, which may inhibit macrophage phagocytosis (Swoboda and Held 2022). Hypericum's healing effects on pressure-induced wounds are attributed to increased capillary blood circulation (Torra i Bou et al., 2003). Its full-thickness wound-healing potential could be attributed to anti-inflammatory compounds such as flavonoids, hyperforin, and pseudohypericin (Hammer et al., 2007). Because of its anti-inflammatory and antibacterial properties, Hypericum extract promotes diabetic wound healing. In-vitro evidence suggests flavonoids, hyperforin, and hypericin are possibly the primary anti-inflammatory compounds (Gruenwald J. 2007). Moreover, tannins are antioxidants that increase fibroblast proliferation and can secrete considerable amounts of TNF (Nikrooze et al., 2013). Fibroblasts induce nitric oxide and vascular endothelial growth factor, which regulate granulation tissue formation and reinforce angiogenesis (Momeni et al., 2014). Fibroblasts are also involved in collagen fiber production, and the tannins in Hypericum increase the collagen rate in the skin's second layer by affecting fibroblasts' activity. Eventually, collagen fibers, nucleic acids, and protein synthesis improve wound healing (Jafari Barmak 2014; Oryan et al., 2010).

While our findings highlight the potential effects of H. helianthemoides extraction, a more comprehensive comparison with other standard wound healing treatments, including various herbal remedies, remains essential. This comparison would provide a clearer perspective on where H. helianthemoides fits into the available spectrum of wound healing modalities, offering clinicians more comprehensive treatment options. Besides, a more in-depth exploration at the cellular and molecular levels could provide deeper insights into how H. helianthemoides facilitates wound healing. Such information is crucial for understanding how bioactive compounds interact with cellular pathways involved in wound repair and inflammation. Our study did not investigate the long-term effects and safety profile of H. helianthemoides treatment, particularly in wound healing. Given the complexities of diabetic wound management and potential underlying health issues, it is critical to understand any long-term implications, side effects, or contraindications of H. helianthemoides use. Future research should focus on the chronic impacts of H. helianthemoides application and its safety in diverse patient populations, bridging the gap between laboratory findings and clinical practice. Furthermore, a critical aspect that requires attention is the dosage optimization and standardization of H. helianthemoides extract for effective wound healing. The concentrations used in this study serve as a baseline, but further research is needed to determine the optimal dosages for human application and standardize these dosages for clinical use. It is also critical to delve into the specific challenges that diabetes poses in wound healing, such as impaired microvascular function and chronic inflammation, and to understand how H. helianthemoides may address these challenges. This study would help us better understand the therapeutic potential of H. helianthemoides in diabetic wound care.

According to the study, olive oil had no significant impact on the outcomes that were examined. These results were not reported because the study's thorough analysis demonstrated that olive oil was not a confounding factor, enabling us to attribute the observed effects to the primary factors. Finally, acknowledging the study's limitations would provide a more balanced perspective. Mechanically created wounds in research models may not accurately represent the complexities of diabetic wounds. Diabetic wounds frequently involve chronic factors such as impaired circulation, neuropathy, and persistent inflammation, which have a different effect on healing than controlled, mechanically induced wounds. Consequently, the healing responses observed in experimental models may not accurately reflect the challenges and mechanisms present in diabetic wounds, potentially limiting the applicability of research findings to real-world diabetic wound care. To minimize these differences, we induced diabetic conditions before creating wounds. Other limitations may include the constraints of the animal model used, the duration of the study, or the range of parameters measured.

Conclusion

In conclusion, the current study found that H. helianthemoides, at two different dosages (5 and 10%), has a strong potential in full-thickness wound healing and could accelerate its process in diabetic and non-diabetic rats, which is most likely related to anti-inflammatory combinations such as flavonoids (i.e. quercetin and Amentoflavone), hyperforin, and pseudohypericin in this plant. While our findings provide promising insights into the efficacy of H. helianthemoides in wound healing, particularly in diabetic patients, they also pave the way for more comprehensive research. Future studies should build upon these findings by exploring the broader potential of H. helianthemoides in wound management across various patient demographics and clinical settings.

Acknowledgments

This study was supported by School of Medicine, Ilam University of Medical Sciences, Ilam, Iran (Grant number; 941031-1).

Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics statement

All experimental protocols in this study adhered to the animal study guidelines set forth by the National Institutes of Health (NIH) Guide (1978) and received approval from the Ethics Committee of Ilam University of Medical Sciences (approval date: October 10, 2015, Ethical code: IR.MEDILAM.REC1396.76). Additionally, the authors fully complied with ethical standards concerning plagiarism, data fabrication, and duplicate publication.

References

Altan A, Aras M H, Damlar İ, Gökçe H, Özcan O, Alpaslan C. The effect of Hypericum Perforatum on wound healing of oral mucosa in diabetic rats. Eur Oral Res 2018; 52: 143-149. https://doi.org/10.26650/eor.2018.505

American Diabetes A. Diagnosis and classification of diabetes mellitus. Diabetes Care 2013; 37: S81-S90. https://doi.org/10.2337/dc14-S081

Anstead G M, Hart L M, Sunahara J F, Liter M E. Phenytoin in wound healing. Ann Pharmacother 1996; 30: 768-775. https://doi.org/10.1177/106002809603000712

Ashrafi A, Rezaii A A, Haghdost I S, Mehdineghad B, Ashrafi I, Asmariyan S H. Histometric and histophatologic evaluation of the effects of equistum arvense herbal extract versus zinc oxide in rabbit skin wound healing model. Veterinary Medicine Faculty 2010; 4: 843-853.

Attinger C E, Janis J E, Steinberg J, Schwartz J, Al-Attar A, Couch K. Clinical approach to wounds: débridement and wound bed preparation including the use of dressings and wound-healing adjuvants. Plast Reconstr Surg 2006; 117: 72s-109s. https://doi.org/10.1097/01.prs.0000225470.42514.8f

Bedi M K, Shenefelt P D. Herbal therapy in dermatology. Arch Dermatol 2002; 138: 232-242. https://doi.org/10.1001/archderm.138.2.232

Bhatia A, Prakash S. Topical phenytoin for wound healing. Dermatol Online J 2004; 10: 5. https://doi.org/10.5070/D30Z3612W1

Boissier E. Flora orientalis, sive enumeratio plantarum in Oriente a Graecia et Aegypto ad Indiae fines hucusque observatarum. Georg; 1879. https://doi.org/10.5962/bhl.title.20323

Bork P M, Bacher S, Schmitz M L, Kaspers U, Heinrich M. Hypericin as a non-antioxidant inhibitor of NF-kappa B. Planta Med 1999; 65: 297-300. https://doi.org/10.1055/s-1999-13989

Bowers S, Franco E. Chronic Wounds: Evaluation and Management. Am Fam Physician 2020; 101: 159-166.

Brondz I, Greibrokk T, Groth P A, Aasen A J. The relative stereochemistry of hyperforin - an antibiotic from hypericum perforatum L. Tetrahedron Letters 1982; 23: 1299-1300. https://doi.org/10.1016/S0040-4039(00)87088-4

Cañedo-Dorantes L, Cañedo-Ayala M. Skin acute wound healing: A comprehensive review. Int J Inflam 2019; 2019: 3706315. https://doi.org/10.1155/2019/3706315

Chakraborty R, Borah P, Dutta P P, Sen S. Evolving spectrum of diabetic wound: Mechanistic insights and therapeutic targets. World J Diabetes 2022; 13: 696-716. https://doi.org/10.4239/wjd.v13.i9.696

Cinci L, Di Cesare Mannelli L, Maidecchi A, Mattoli L, Ghelardini C. Effects of Hypericum perforatum extract on oxaliplatin-induced neurotoxicity: in vitro evaluations. Z Naturforsch C J Biosci 2017; 72: 219-226. https://doi.org/10.1515/znc-2016-0194

Dorsett-Martin W A. Rat models of skin wound healing: a review. Wound Repair Regen 2004; 12: 591-599. https://doi.org/10.1111/j.1067-1927.2004.12601.x

Farsak M, Özdağli G, Özmüş D, Çömelekoğlu Ü, Yalın S, Bozdoğan Arpacı R, et al. Effects of hypericum perforatum on an experimentally induced diabetic wound in a rat model. Wounds 2017; 29: 10-17.

Fernández-Guarino M, Hernández-Bule M L, Bacci S. Cellular and molecular processes in wound healing. Biomedicines 2023; 11: 2526. https://doi.org/10.3390/biomedicines11092526

Gangemi S, Minciullo P L, Miroddi M, Chinou I, Calapai G, Schmidt R J. Contact dermatitis as an adverse reaction to some topically used European herbal medicinal products - part 2: Echinacea purpurea-Lavandula angustifolia. Contact Dermatitis 2015; 72: 193-205. https://doi.org/10.1111/cod.12328

Graves L E, Donaghue K C. Vascular complication in adolescents with diabetes mellitus. Frontiers in Endocrinology 2020; 11. https://doi.org/10.3389/fendo.2020.00370

Gruenwald J. B T. PDR for herbal medicines. Rutgers: Fourth edition. Montvale, NJ : Thomson, [2007] ©2007, 2007.

Haji Zadeh S, Khoshbaten A L I, Asgari A, Khaksari M. Low voltage electrical stimulation and wound healing in rabbits: effect of altering frequency. MJIRI 1996; 10: 225-228.

Hammer K D, Hillwig M L, Solco A K, Dixon P M, Delate K, Murphy P A, et al. Inhibition of prostaglandin E(2) production by anti-inflammatory hypericum perforatum extracts and constituents in RAW264.7 Mouse Macrophage Cells. J Agric Food Chem 2007; 55: 7323-7331. https://doi.org/10.1021/jf0710074

Heydari M, Poorbabaei H, Esmaaelzade O. Indicator plant species in monitoring forest soil conditions using logistic regression model in Zagros Oak (Quercus brantii var.persica) forest ecosystems, Ilam city. Journal of Plant Research (Iranian Journal of Biology) 2015; 27: 811-828.

Hoffmann J, Gendrisch F, Schempp C M, Wölfle U. New herbal biomedicines for the topical treatment of dermatological disorders. Biomedicines 2020; 8. https://doi.org/10.3390/biomedicines8020027

Jafari Barmak M. Compare Oak extract, Teucrium Polium, Hyper Perforatum and silver sulfadiazine1% on a second grade burn wounds in rats. Nautilus 2014; 128: 28-32.

Janis J. Clinical approach to wounds: debridement and wound bed preparation including the use of dressings and wound-healing adjuvants. American Society of Plastic Surgeons 2006; 117. https://doi.org/10.1097/01.prs.0000225470.42514.8f

Jeffcoate W J, Harding K G. Diabetic foot ulcers. Lancet 2003; 361: 1545-1551. https://doi.org/10.1016/S0140-6736(03)13169-8

khaksar s, kesmati m, rezaie a, Rasekh A. Topical estrogen accelerates wound healing in diabetic rats. IJEM 2011; 12: 544-551.

Kucukboyaci N, Tamer U, Karasu Ç, Torul H. Evaluation of phenolic compounds and protective effects of olive (Olea europaea L.) leaf extracts on endothelial cells against hydrogen peroxide-induced toxicity. Journal of Research in Pharmacy 2020; 24: 497-507. https://doi.org/10.35333/jrp.2020.198

LoGerfo F W, Coffman J D. Vascular and microvascular disease of the foot in diabetes. New England Journal of Medicine 1984; 311: 1615-1619. https://doi.org/10.1056/NEJM198412203112506

M. Mahmoudi M, Javanmardi A, Morteza Semnani K, Saeedi M. Anti-inflammatory, analgesic activity, acute toxicity and hypericins content of iranian hypericum perforatum. Babol-Jbums 2006; 8: 7-14.

Menegazzi M, Di Paola R, Mazzon E, Muià C, Genovese T, Crisafulli C, et al. Hypericum perforatum attenuates the development of carrageenan-induced lung injury in mice. Free Radic Biol Med 2006; 40: 740-753. https://doi.org/10.1016/j.freeradbiomed.2005.08.034

Mirzaei M, Sewell R D E, Kheiri S, Rafieian-kopaei M. A clinical trial of the effect of St. John's wort on migraine headaches in patients receiving sodium valproate. Journal of Medicinal Plants Research 2012; 6: 1524-1531. https://doi.org/10.5897/JMPR11.431

Momeni E, Aroi N, Aroi M, Jafari Barmak M, Mahmoudi R, Malekzadeh J M, et al. the effect of hypericum perforatum extract and 1 % silver sulfadiazine on second degree burn wound healing in male rats. yums-armaghan 2014; 19: 625-632.

Nikrooze L, Jafari Barmak M, Naghmachi M, Ghafarian shirazi H, Dehghani N. Study of Jaft Aqueous Extract and Silver Sulfadiazine on Burn Healing in Male Rat. yums-armaghan 2013; 18: 107-114.

Novelli M, Masiello P, Beffy P, Menegazzi M. Protective role of St. John's Wort and its components hyperforin and hypericin against diabetes through inhibition of inflammatory signaling: evidence from in vitro and in vivo studies. Int J Mol Sci 2020; 21. https://doi.org/10.3390/ijms21218108

Oryan A, Naeini A, B N, Gorjlan E. Effect of aqueous extract of Aloe vera on experimental cutaneous wound healing in rat. Veterinarski Arhiv 2010; 80: 509-522.

Prisăcaru A I, Andriţoiu C V, Andriescu C, Hăvârneanu E C, Popa M, Motoc A G, et al. Evaluation of the wound-healing effect of a novel Hypericum perforatum ointment in skin injury. Rom J Morphol Embryol 2013; 54: 1053-9.

Punjataewakupt A, Napavichayanun S, Aramwit P. The downside of antimicrobial agents for wound healing. Eur J Clin Microbiol Infect Dis 2019; 38: 39-54. https://doi.org/10.1007/s10096-018-3393-5

Ramezani J, Azarbayjani M A, Peeri M. Simultaneous effects of aerobic training and berberine chloride on plasma glucose, IL-6 and TNF-α in type 1 diabetic male wistar rats. Nutrition and Food Sciences Research 2019; 6: 9-16. https://doi.org/10.29252/nfsr.6.1.9

Robertson S, Cameron N E, Cotter M A. The effect of the calcium antagonist nifedipine on peripheral nerve function in streptozotocin-diabetic rats. Diabetologia 1992; 35: 1113-1117. https://doi.org/10.1007/BF00401363

Rodríguez-Rodríguez N, Martínez-Jiménez I, García-Ojalvo A, Mendoza-Mari Y, Guillén-Nieto G, Armstrong D G, et al. Wound chronicity, impaired immunity and infection in diabetic patients. MEDICC Rev 2022; 24: 44-58. https://doi.org/10.37757/MR2021.V23.N3.8

Sánchez-Mateo C C, Bonkanka C X, Hernández-Pérez M, Rabanal R M. Evaluation of the analgesic and topical anti-inflammatory effects of Hypericum reflexum L. fil. J Ethnopharmacol 2006; 107: 1-6. https://doi.org/10.1016/j.jep.2006.01.032

Scheinfeld N. Phenytoin in cutaneous medicine: its uses, mechanisms and side effects. Dermatol Online J 2003; 9: 6. https://doi.org/10.5070/D32197W4T4

Schempp C M, Pelz K, Wittmer A, Schöpf E, Simon J C. Antibacterial activity of hyperforin from St John's wort, against multiresistant Staphylococcus aureus and gram-positive bacteria. Lancet 1999; 353: 2129. https://doi.org/10.1016/S0140-6736(99)00214-7

Singh J, Kakkar P. Antihyperglycemic and antioxidant effect of Berberis aristata root extract and its role in regulating carbohydrate metabolism in diabetic rats. J Ethnopharmacol 2009; 123: 22-26. https://doi.org/10.1016/j.jep.2009.02.038

Sorg H, Sorg C G G. Skin wound healing: of players, patterns, and processes. European Surgical Research 2022; 64: 141-157. https://doi.org/10.1159/000528271

Sulakhiya K, Soni P, Tembhre M K, Kungumaraj H J, Paliwal R, Kumar S. 2 - Physiology and pharmacology of wounds. In: Solanki PR, Kumar A, Pratap Singh R, Singh J and Rb Singh K, editors. Nanotechnological aspects for next-generation wound management: Academic press, 2024: 21-54. https://doi.org/10.1016/B978-0-323-99165-0.00011-3

Süntar I P, Akkol E K, Yilmazer D, Baykal T, Kirmizibekmez H, Alper M, et al. Investigations on the in vivo wound healing potential of Hypericum perforatum L. J Ethnopharmacol 2010; 127: 468-77. https://doi.org/10.1016/j.jep.2009.10.011

Swoboda L, Held J. Impaired wound healing in diabetes. J Wound Care 2022; 31: 882-885. https://doi.org/10.12968/jowc.2022.31.10.882

Torra i Bou J-E, Rueda López J, Segovia Gómez T, Bermejo Martínez M. [Topical administration of an hyperoxygenated fatty acid compound. Preventive and curative effects on pressure ulcer]. Revista de enfermeria (Barcelona, Spain) 2003; 26: 54-61.

World Health O. Definition, diagnosis and classification of diabetes mellitus and its complications : report of a WHO consultation. Part 1, Diagnosis and classification of diabetes mellitus. Journal 1999.

Yu M, Lee J Y. Polydeoxyribonucleotide improves wound healing of fractional laser resurfacing in rat model. Journal of Cosmetic and Laser Therapy 2017; 19: 43-48. https://doi.org/10.1080/14764172.2016.1247966