BMC Veterinary Research

RESEARCH

Open Access

Histopathologic evaluation of skin wound healing due to local application of transdermal chitosan patch in combination with doxycycline, zinc nanoparticles, and selenium nanoparticles in mice

Pegah Khosravian[1], Negin Motamedi[2], Moosa Javdani[2*] and Elham Moghtadaei Khorasgani[3]

Abstract

Background The aim of this study is to compare the histopathological changes in experimental skin wound healing in mice treated with a topical application of chitosan combined with doxycycline, as well as zinc and selenium nanoparticles.

Methods Fifty mice with experimental skin wounds were divided into four equal groups: a control group with no therapeutic intervention, a CsD group receiving a transdermal patch containing doxycycline, a CsDZn group receiving a transdermal patch with chitosan-doxycycline-zinc nanoparticles, a CsZnS group receiving a transdermal patch with chitosan-zinc and selenium nanoparticles, and a CsDZnS group receiving a transdermal patch containing chitosan-doxycycline-zinc and selenium nanoparticles. After synthesizing and confirming the efficacy of the transdermal patches, we measured the wound area and histological indices of wound healing on the 7th and 14th days of the study.

Results The results showed that the use of selenium and zinc nanoparticles leads to a decrease in the wound surface in the CsDZnS group compared to the control group and remaining experimental groups. A significant reduction in inflammation and hemorrhage was recorded in the CsDZnS group compared to other groups. On the seventh day of the study, angiogenesis in the CsDZnS group was higher than in the other groups. In addition, on the 14th day of the study, orientation of collagen fibers and re-epithelialization were more observed in the CsDZnS group compared to the other groups.

Conclusion The chitosan skin patch containing doxycycline, zinc nanoparticles, and selenium nanoparticles (CsDZnS group) demonstrated the most effective results in skin healing, as evidenced by the highest control of inflammation on days 7 and 14, the greatest re-epithelialization on day 14, and the smallest wound area on both days compared to the other evaluated groups.

*Correspondence:
Moosa Javdani
javdani59@gmail.com

Full list of author information is available at the end of the article

Keywords Chitosan, Doxycycline, Zinc nanoparticles, Selenium nanoparticles

Introduction

Transdermal skin patches function as wound dressings that adhere to the skin, covering wounds and delivering specific doses of one or more drugs through the skin into the injured area and bloodstream. These pharmaceutical systems are synthesized using various scaffolds, including chitosan, which is favored for its unique properties, such as permeability, cross-linking ability, optimal membrane thickness, biodegradability, and non-toxicity [1]. Chitosan has been shown to be effective in the hemostasis, inflammation, and proliferation stages of wound healing. It aids in stopping bleeding by increasing the accumulation of platelets and red blood cells while inhibiting fibrin dissolution. Additionally, chitosan assists in bacterial removal from the wound site during the inflammatory phase and promotes the growth of granulation tissue [2]. Previously, the application of a chitosan skin patch loaded with selenium nanoparticles and doxycycline in skin wounds was evaluated in rats. It was found that the simultaneous presence of these two compounds in the chitosan scaffold promotes effective skin repair [3]. Additionally, in the study by Khosrovian et al. (2023), it was demonstrated that the combination of chitosan with doxycycline and mesoporous silica facilitates suitable skin repair in rats [4]. Chitosan biofilms containing selenium nanoparticles have been shown to enhance angiogenesis and facilitate the infiltration of multinuclear and mononuclear cells at the wound site [5] and further enhance skin wound healing by boosting collagen production and increasing the number of fibroblasts [6]. Selenium nanoparticles increase the levels of vascular endothelial growth factor (VEGF) and collagenase-1 while decreasing nitric oxide levels at the wound surface [7]. VEGF regulates angiogenesis by stimulating the proliferation, migration, differentiation, and survival of endothelial cells, facilitating the formation of new blood vessels [8]. In contrast, elevated nitric oxide levels can lead to increased nitro-oxidative stress [9]. Doxycycline, as a tetracycline, plays an important role in regulating inflammation, promoting new blood vessel formation, and supporting tissue regeneration by inhibiting metalloproteases. Additionally, it inhibits protein synthesis by binding to bacterial ribosomes [10].

Doxycycline inhibits reactive oxygen species, thereby preventing the conversion of pro-MMP into active matrix metalloproteinases [11, 12]. The use of zinc nanoparticles in wound healing has also been explored [13]. Zinc nanoparticles exhibit wound healing, anti-inflammatory, and bactericidal properties due to their capacity to generate reactive oxygen species [14]. Studies have shown that the local application of zinc nanoparticles enhances epithelialization, promotes wound closure, and supports skin regeneration [15]. Given that the efficacy of chitosan wound dressings containing doxycycline, selenium nanoparticles, and zinc nanoparticles has not yet been compared, this study aimed to evaluate the histopathological changes in experimental wound healing in mice following the local application of these dressings.

Materials and methods

Materials

Zinc nanoparticles was obtained from Sigma Aldrich (Seelze, Germany). Selenium dioxide, ascorbic acid, low molecular chitosan and propylene glycol were purchased from Merck (Darmstadt, Germany). Furthermore, doxycycline was obtained from Razak Pharma (Razak Pharma Co., Karaj, Iran). Other reagents and solvents were purchased from Merck (Darmstadt, Hesse, Germany).

Nanoparticles preparation and characterization

Selenium nanoparticles were synthesized according to the previous method [16]. At first, selenium dioxide solution (1 mM, 500 mL) was prepared and was left to stir at 800 rpm. Afterward, ascorbic acid solution (44 mM, 50 mL) was added gradually to the first solution and the final solution was left to stir for 24 h to form selenium nanoparticles. The prepared nanoparticles were washed by distilled water and centrifuged sometimes to obtain net Selenium nanoparticles.

Selenium and zinc nanoparticles were examined by dynamic light scattering (DLS) (DLS, Mastersizer 2000; Malvern Instruments, Malvern, Worcestershire, UK) and Field emission scanning electron microscope (FE-SEM) (FE-SEM, Tescan/Mira, Brno, Czech Republic) methods.

Patch preparation

Low molecular chitosan (100 mg) was dissolved in 10 ml of acetic acid (0.1 M) and 0.2 ml of propylene glycol. The solution was stirred for 2 h to get completely homogeneous. Afterward, for preparation of CsD, CsDZn, CsZnS and CsDZnS patches, the mentioned amount in Table 1 were added to chitosan solution. Afterward, the prepared solution for each patch was stirred for 2 h and then divided to ten molds and let to dry at 24 °C and dark condition. The dried patches were checked and select the flat and bubble free one. They were characterized by FE-SEM method and stored at room temperature in a glass container for the next procedures.

Study design and animals

Fifty male mice, aged 9 weeks, were purchased and randomly assigned to five equal groups: (a) a control group

Table 1 Amount of Chitosan, Doxycycline, zinc nanoparticles, selenium nanoparticles in different prepared patches

Amount in each patch (mg)	chitosan	doxycycline	zinc nanoparticles	Selenium nanoparticles
CsD	10	0.03	0	0
CsDZn	10	0.03	1.8	0
CsZnS	10	0	1.8	0.03
CsDZnS	10	0.03	1.8	0.03

CsD (chitosan containing doxycycline patch); CsDZn (chitosan containing doxycycline and zinc nanoparticles patch): CsZnS (chitosan containing doxycycline and selenium nanoparticles patch); CsDZnS (chitosan containing doxycycline and zinc nanoparticles, and selenium nanoparticles patch)

with a skin wound that did not receive any therapeutic intervention; (b) the CsD group, in which a chitosan transdermal patch containing doxycycline was applied to the skin wound; (c) the CsDZn group, where the skin wound was covered with a chitosan transdermal patch containing doxycycline and zinc nanoparticles; (d) the CsZnS group, in which the skin wound was covered with a chitosan transdermal patch containing zinc and selenium nanoparticles; and (e) the CsDZnS group, in which a transdermal patch of chitosan-doxycycline-zinc-selenium nanoparticles was placed on the skin wound.

Before the study commenced, the mice were housed in the rodent facility of the Veterinary Faculty at Shahrekord University for one week to de-stress and adapt to the new environmental conditions, and they were fed using standard methods.

Creating experimental skin wounds

Mice were anesthetized via intraperitoneal injection of ketamine (60 mg/kg) and xylazine (10 mg/kg). They were positioned prone, their back hair was clipped, and the skin was surgically prepared in the usual manner. To create an experimental wound, a circular area of full-thickness skin measuring 10 mm in diameter was excised from the backs of the mice using a sharp cut. In accordance with the different experimental groups, a skin patch was implanted, and the mice were then transferred to small cages. Daily individual care, including wound management, feeding, access to water, and environmental maintenance (such as bedding changes and ensuring appropriate temperature and humidity), was provided until the end of the study.

Histopathological examination

In addition to measuring the skin wound area (mm^2) on days 7 and 14 post-surgery, tissue samples including the wound site and 2 mm of surrounding intact skin—were collected from 5 mice in each group following induction of anesthesia via injection. The samples were washed with PBS, fixed in a container containing 10% buffered formalin, and then transferred to the histology laboratory. Three parallel slices were prepared from each sample and stained with hematoxylin and eosin. Skin regeneration parameters, including inflammation, hemorrhage, angiogenesis, orientation of collagen fibers, and epithelial tissue regeneration, were assessed using a light microscope. The scoring system mentioned in the study of Görgülü et al. (2015) was used to rank the histological parameters [17].

Statistical analysis

The median histological parameters and wound surface area among different groups at the same time were compared after scoring the evaluated parameters using the Kruskal-Wallis statistical test, with a significance level set at $P < 0.05$.

Results

Characterization of prepared nanoparticles

The DLS analysis diagram for selenium nanoparticles was obtained, showing a dispersion distribution index (PDI) of 0.178, which indicates a uniform and satisfactory distribution. The hydrodynamic diameter of the selenium nanoparticles measured by DLS was 192.5 nm (Fig. 1A), closely matching the size observed in FE-SEM imaging. The zeta potential of the selenium nanoparticles was calculated at -19.91 mV, reflecting the negative charge due to free metal electrons on the nanoparticle surface, in agreement with findings from other studies (Fig. 1B). The morphological and surface characteristics of the synthesized nanoparticles were further assessed using FE-SEM, with images confirming a spherical shape and uniform distribution of the particles, which ranged in size from approximately 100 to 200 nm (Fig. 1C).

The DLS analysis diagram of zinc nanoparticles was examined. The PDI for these nanoparticles was 0.606, indicating a uniform and satisfactory distribution. Additionally, the hydrodynamic diameter measured by DLS for the zinc nanoparticles was reported to be 176.2 nm (Fig. 2A), which is consistent with the size obtained from scanning electron microscopy. The zeta potential of the zinc nanoparticles was measured at -13.57 mV, reflecting the negative charge due to the presence of free metal electrons on the nanoparticle surfaces, consistent with findings from other studies (Fig. 2B). Moreover, according to Fig. 2C, FE-SEM image of zinc nanoparticles shows 100 to 200 nm almost spherical particles with uneven surface and uniform particle size distribution.

Examining the appearance of chitosan patch with nanoparticles

The final patch containing zinc and selenium nanoparticles was visually inspected. The prepared patch exhibited a uniform, soft, smooth, and bubble-free surface, with a red coloration (Fig. 3A). Additionally, a FESEM image of

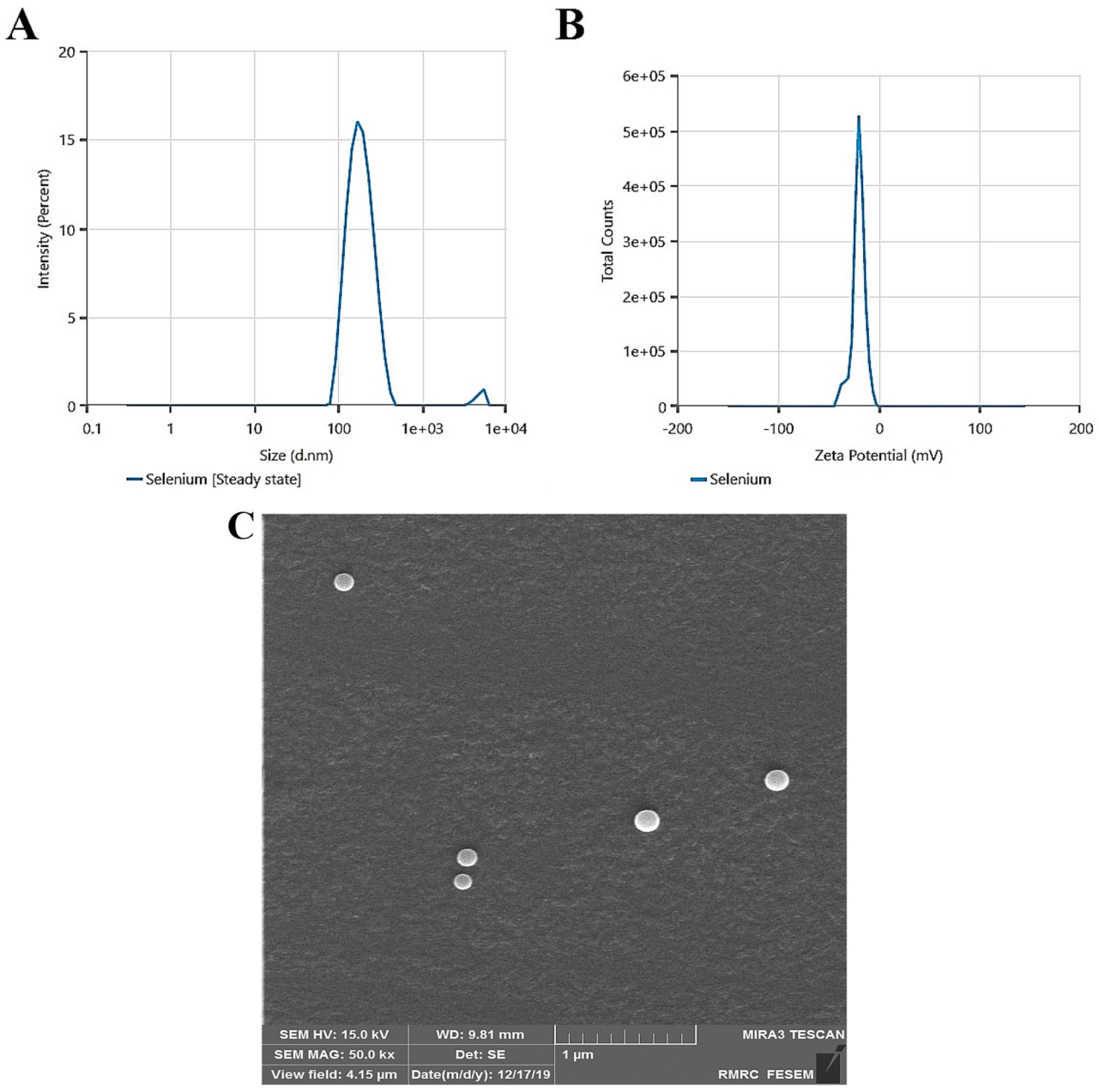

Fig. 1 Size and zeta surface potential of selenium nanoparticles (**A**&**B**); FE-SEM image of selenium nanoparticles (**C**)

the final patch was obtained, demonstrating the uniform distribution of nanoparticles within it (Fig. 3B).

Comparison of the wound area in different groups

The areas of wound surfaces in different groups at two time points, days 7 and 14 of the study, are compared in Table 2. On the 7th day, the lowest wound level was observed in the CsDZnS group, with no significant differences among the other treatment groups; the largest wound area was found in the control group. Additionally, the comparison of the median wound surface on the 14th day revealed that the CsDZnS group again had the lowest wound area, while the other groups showed no significant differences, with the highest wound level belonging to the control group.

The comparison of histological parameters in the different studied groups on day 7 is presented in Table 3; Fig. 4. The histopathological results indicated that inflammation in the control group was significantly higher than in the other groups ($P \leq 0.05$), while the CsDZnS group exhibited significantly lower inflammation compared to the others. However, no significant differences were observed among the other treatment groups ($P \geq 0.05$).

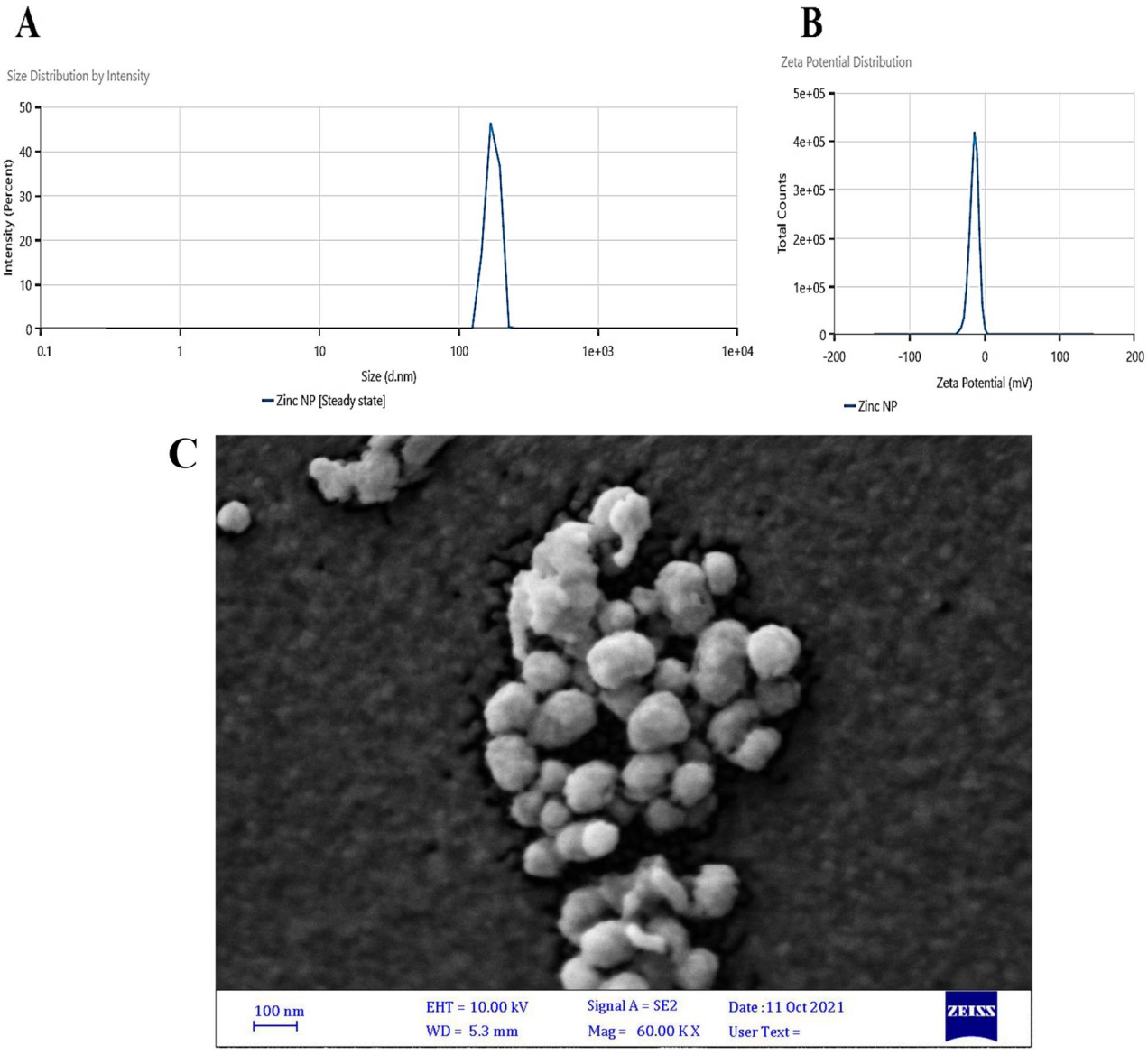

Fig. 2 Size and zeta surface potential of zinc nanoparticles (**A**&**B**); FE-SEM image of zinc nanoparticles (**C**)

The results also showed that hemorrhage in the CsDZnS treatment group was less than in the other groups, whereas the control group exhibited more hemorrhage compared to the others ($P \leq 0.05$). The remaining three groups did not show significant differences. Angiogenesis in the CsDZnS group was greater than in the other groups, with no significant differences observed among the latter.

Additionally, the orientation of collagen fibers in the control group on the 7th day was significantly lower than in the other groups ($P \leq 0.05$), while no significant differences were noted among the other groups. Re-epithelialization was not observed in any of the groups. Tissue parameters on the 14th day of the study are compared in Table 4; Fig. 5. The histopathological evaluation on day 14 revealed that inflammation in the control group was significantly higher than in the other groups, while the CsDZnS group exhibited lower inflammation compared to the others ($P \leq 0.05$). However, no significant differences were found among the other treatment groups. Hemorrhage in the CsDZnS group was significantly less than in the other groups, whereas the control group had more hemorrhage compared to the others ($P \leq 0.05$). Angiogenesis was not observed in any of the groups on day 14. The orientation of collagen fibers in the control group was lower than in the other groups ($P \leq 0.05$). The density of collagen fibers in the CsDZnS group was significantly higher than in the other groups, but no significant differences were noted among the remaining groups. Additionally, re-epithelialization in the CsDZnS

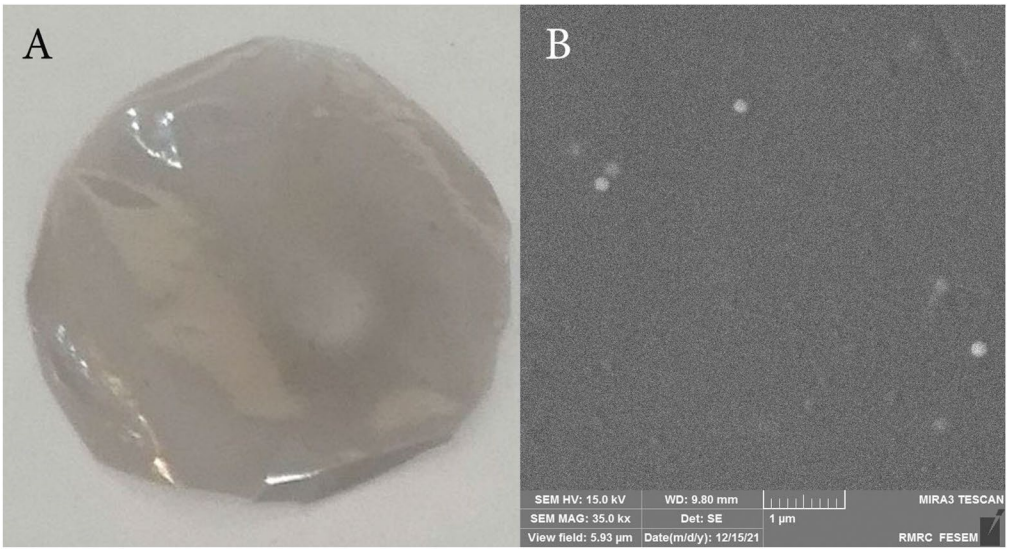

Fig. 3 Gross image (**A**) and FE-SEM image (**B**) of CsDZnS (chitosan containing doxycycline and zinc nanoparticles) patch

Table 2 Comparison of wound area surface in different groups in 7 and 14 days of study

Group	Day 7	Day 14
Control	9 (8–10)[a]	7 (5–8)[a]
CsD	7 (6–8)[b]	5 (3–6)[b]
CsDZn	6 (5–8)[b]	4 (4–6)[b]
CsZnS	6 (5–7)[b]	5 (3–5)[b]
CsDZnS	4 (4–6)[c]	3 (2–4)[c]

Different letters indicate significant differences in each column (P<0.05); CsD (chitosan containing doxycycline patch); CsDZn (chitosan containing doxycycline and zinc nanoparticles patch): CsZnS (chitosan containing doxycycline and selenium nanoparticles patch); CsDZnS (chitosan containing doxycycline and zinc nanoparticles, and selenium nanoparticles patch)

group was significantly higher than in the other groups ($P \leq 0.05$), while no significant differences were observed among the other groups.

Discussion

Activation of the inflammatory response initiates tissue regeneration and repair following an injury. Well-controlled inflammation can promote wound healing; thus, wound dressings that modulate immune cells and cytokines at the wound site can effectively reduce inflammation [18]. Conversely, oxidative stress has been found to regulate wound healing through various pathways. Therefore, both topical and systemic use of antioxidants can mitigate stress-induced damage and enhance wound healing [19]. Chitosan is a functional and versatile biological substance that serves as a suitable option for preparing wound dressings due to its proven antioxidant and anti-inflammatory effects [20]. Furthermore, the addition of any substance that has synergistic effects with chitosan can significantly enhance wound healing [20]. In the present study, doxycycline, zinc, and selenium nanoparticles were incorporated into the chitosan scaffold, resulting in a significant reduction in wound surface area and inflammation compared to the control group. Notably, a marked reduction in these evaluated parameters was observed with the simultaneous presence of zinc and selenium nanoparticles in the chitosan scaffold. The anti-inflammatory effect of doxycycline is mediated through the suppression of TNF-α, IL-1β, and IL-6, and it has been shown to inhibit IgE-mediated degranulation and histamine-induced vascular permeability [21]. These cytokines play a crucial role in initiating and maintaining the immune and inflammatory response. Doxycycline also reduces the recruitment of inflammatory cells, such

Table 3 Comparison of histological parameters in different groups on day 7 of the study

Group	Inflammation	Hemorrhage	Angiogenesis	orientation of collagen fibers	Re-epithelialization
Control	2 (2–3)[a]	2 (1–3)[a]	1 (0–2)[a]	0 (0–1)[a]	0 (0–0)[a]
CsD	1 (1–2)[b]	0 (0–1)[b]	1 (1–2)[a]	1 (1–2)[b]	0 (0–0)[a]
CsDZn	1 (1–2)[b]	1 (1–2)[b]	1 (1–2)[a]	1 (0–2)[b]	0 (0–0)[a]
CsZnS	1 (1–2)[b]	1 (1–2)[b]	1 (1–2)[a]	1 (1–2)[b]	0 (0–0)[a]
CsDZnS	0 (0–1)[c]	0 (0–1)[c]	2 (2–2)[b]	1 (1–2)[b]	0 (0–0)[a]

Different letters indicate significant differences in each column (P<0.05); CsD (chitosan containing doxycycline patch); CsDZn (chitosan containing doxycycline and zinc nanoparticles patch): CsZnS (chitosan containing doxycycline and selenium nanoparticles patch); CsDZnS (chitosan containing doxycycline and zinc nanoparticles, and selenium nanoparticles patch)

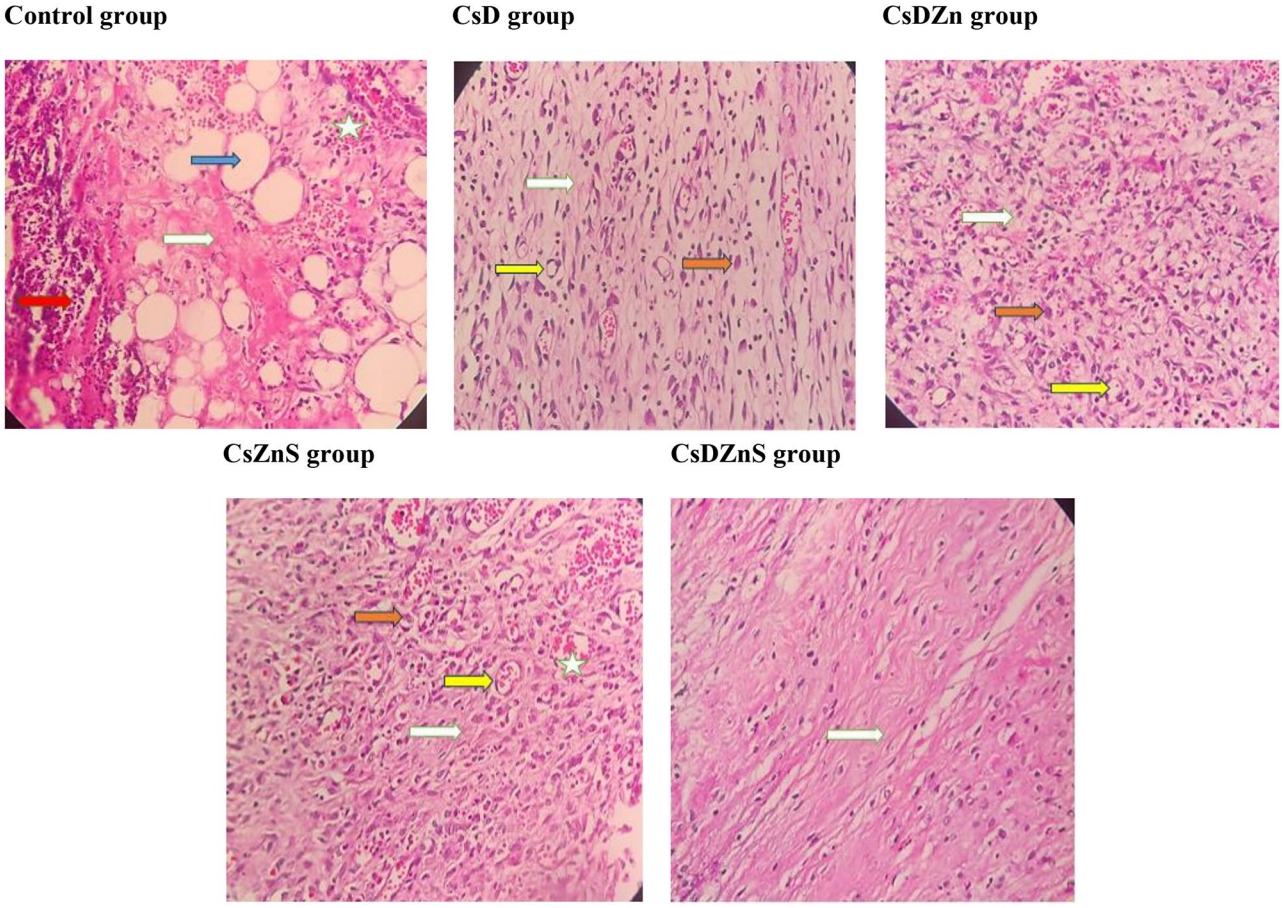

Fig. 4 Histopathological sections on day 7 in different groups (10x magnification); Control; (Edema (blue arrow), inflammation (red arrow), hemorrhage (white star), very fine collagen fibers (white arrow); CsD (chitosan containing doxycycline; fine collagen fibers (white arrow), newly formed vessels (yellow arrow), fibroblast cells (orange arrow); CsDZn (chitosan containing doxycycline and zinc nanoparticles; newly formed vessels (yellow arrow), fine collagen fibers (white arrow), presence of fibroblasts (orange arrow); CsZnS (chitosan containing doxycycline and selenium nanoparticles; hematoma (white star), fine connective tissue (white arrow), fibroblasts (orange arrow), newly formed vessels (yellow arrow); CsDZnS (chitosan containing doxycycline and zinc nanoparticles, and selenium nanoparticles; regular and relatively dense collagen fibers (white arrow), very little edema, no inflammation)

Table 4 Comparison of histological parameters in different groups on day 14 of the study

Group	Inflammation	Hemorrhage	Angiogenesis	orientation of collagen fibers	Re-epithelialization
Control	2 (2–3)[a]	1 (1–2)[a]	0 (0–1)[a]	0 (0–1)[a]	1 (1–2)[a]
CsD	1 (1–2)[b]	0 (0–1)[b]	0 (0–1)[a]	1 (1–2)[a]	1 (1–2)[a]
CsDZn	1 (0–1)[b]	0 (0–1)[b]	0 (0–1)[a]	1 (0–2)[a]	2 (1–2)[a]
CsZnS	1 (1–2)[b]	0 (0–1)[b]	0 (0–1)[a]	1 (1–2)[a]	2 (1–2)[a]
CsDZnS	0 (0–1)[c]	0 (0–1)[b]	0 (0–1)[a]	1 (1–2)[b]	2 (2–3)[b]

Different letters indicate significant differences in each column ($P<0.05$); CsD (chitosan containing doxycycline patch); CsDZn (chitosan containing doxycycline and zinc nanoparticles patch): CsZnS (chitosan containing doxycycline and selenium nanoparticles patch); CsDZnS (chitosan containing doxycycline and zinc nanoparticles, and selenium nanoparticles patch)

as macrophages and neutrophils, to the wound site, ultimately decreasing scar formation [22]. TNF-α acts by binding to two cell surface receptors, TNFR1 and TNFR2. Upon binding to TNFR1, a signaling cascade is initiated that activates nuclear factor kappa B (NF-κB). The activation of NF-κB results in a decrease in the expression of several pro-inflammatory mediators, including adhesion molecules, cytokines, chemokines, and enzymes such as cyclooxygenase-2 (COX-2), ultimately reducing the inflammatory response and the activation of immune cells [23]. Conversely, oxidative stress can disrupt wound healing due to its role in promoting inflammation. Doxycycline exhibits antioxidant properties by removing reactive oxygen species (ROS), leading to anti-inflammatory effects and creating a more favorable environment for wound healing [24]. Additionally, doxycycline has several other beneficial effects, including antibacterial activity, stimulation of angiogenesis, inhibition of matrix

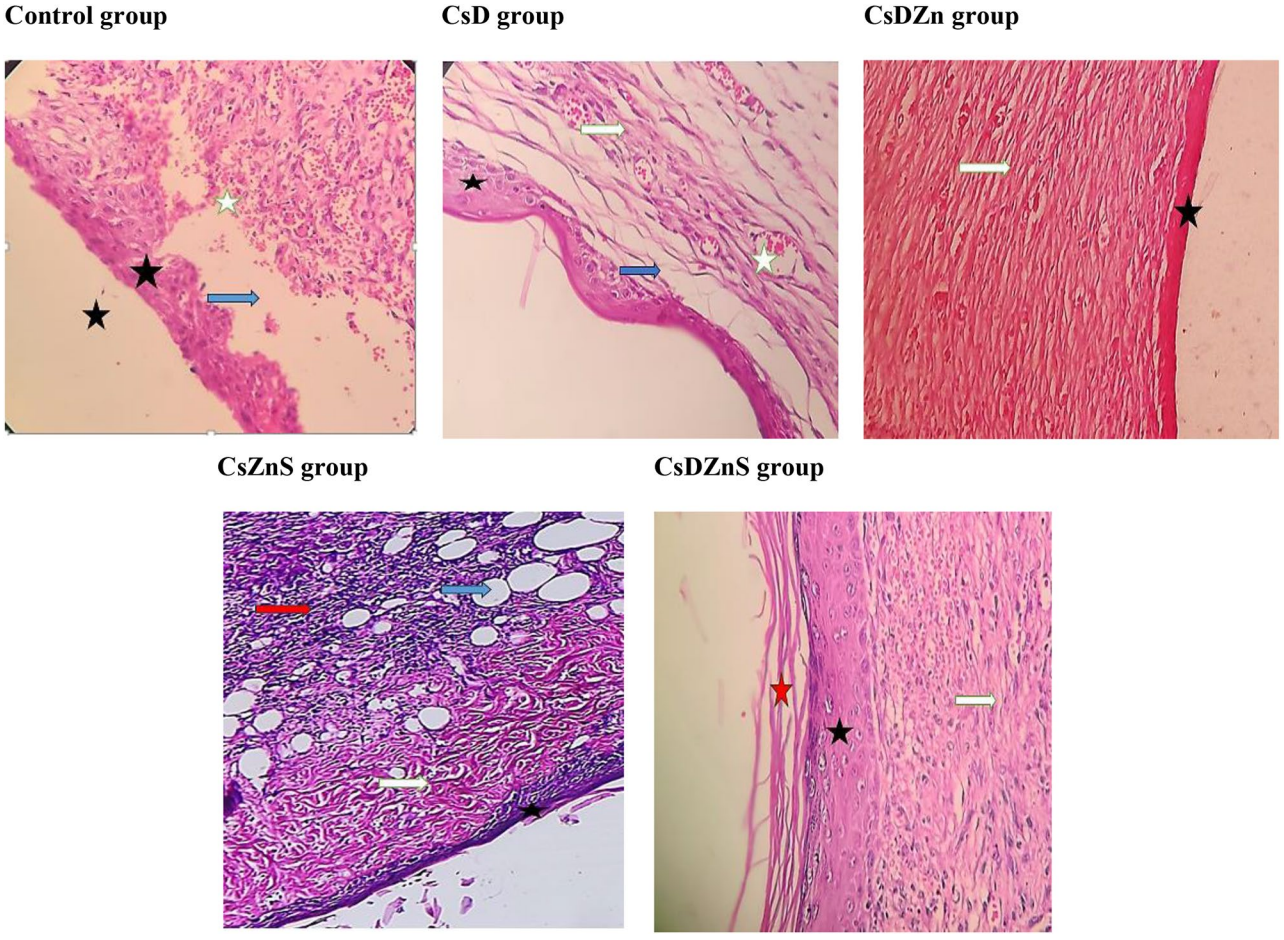

Fig. 5 Histopathological sections on day 14 in different groups (10x magnification); control (lack of proper epithelium formation (black star), edema (blue arrow) and hemorrhage (white star); CsD (chitosan containing doxycycline; formation of thin epithelium (black star), edema (blue arrow) and the absence of dense and regular collagen fibers (white arrow), hyperemia (white star); CsDZn (chitosan containing doxycycline and zinc nanoparticles; formation of thin epithelium (black star), fine collagen fibers (white arrow); CsZnS (chitosan containing doxycycline and selenium nanoparticles; formation of delicate epithelium (black star), edema (blue arrow), absence of regular collagen fibers (white arrow), inflammation (red arrow); CsDZnS (chitosan containing doxycycline and zinc nanoparticles, and selenium nanoparticles; formation of thick and unified epithelium (black star) and surface keratin (red star), relatively dense connective fibers in the dermis (white arrow)

metalloproteinases (MMPs), and modulation of fibroblast activity, all of which contribute to wound healing. Zinc nanoparticles have also been reported to possess anti-inflammatory, antibacterial, and antioxidant properties, along with stimulation of cell migration, angiogenesis, and re-epithelialization [25]. Various mechanisms of action have been attributed to zinc nanoparticles, including inhibiting the expression of pro-inflammatory cytokines, blocking inducible nitric oxide synthase (iNOS), preventing mast cell degranulation, inhibiting the myeloperoxidase enzyme, and blocking the NF-κB pathway [26]. These nanoparticles are therefore effective in promoting wound healing. Selenium nanoparticles, due to their small size and large surface area, have a high potential for scavenging free radicals and improving wound healing. Research has shown that selenium nanoparticles enhance wound healing by reducing the inflammatory response and promoting wound closure, granulation tissue formation, collagen deposition, and angiogenesis [27]. The anti-inflammatory effects of selenium nanoparticles are mediated by downregulating the mRNA synthesis of pro-inflammatory cytokines, including IL-1, TNF-α, and inducible nitric oxide synthase. Selenium nanoparticles also reduce the production of pro-inflammatory mediators through the nuclear translocation of NF-κB [28]. The antimicrobial effect of selenium nanoparticles, attributed to their ability to disrupt bacterial cell walls, is one of the key benefits of these nanoparticles in enhancing wound healing. It has been reported that an ointment containing 5% selenium nanoparticles was highly effective in repairing the skin of wistar rats [29]. Similarly, in the study by Abbaszadeh et al. (2019), the combination of chitosan and selenium nanoparticles was found to be effective in healing skin wounds in rats [5]. Based on the results of

the present study, the incorporation of selenium and zinc nanoparticles into the chitosan scaffold accelerated the wound healing process compared to the control group. Notably, the wound area was smaller with zinc compared to selenium when loaded onto chitosan. However, the simultaneous incorporation of both zinc and selenium nanoparticles on the chitosan scaffold exhibited a synergistic and positive effect on the acceleration of skin wound healing.

Conclusion

The highest control of inflammation observed on the 7th and 14th days of the study, along with the greatest re-epithelialization on the 14th day and the smallest wound area on both the 7th and 14th days, indicates that the chitosan skin patch containing doxycycline, zinc, and selenium nanoparticles (CsDZnS group) is more effective for skin healing compared to the other evaluated groups.

Acknowledgements
The authors wish to acknowledge the valuable assistance of the staff in the Veterinary Surgery Section at Shahrekord University, particularly Mr. Jamshid Kabiri and Mohammad Salimi Beni.

Author contributions
PK: Design and synthesis of transdermal patches, NM: Investigation, Methodology, MJ: Designing and conducting study and data analysis and paper writing, EMK: Histological examination of the study.

Funding
This research has not received any specific funding from any funding organization.

Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
The study plan and scientific principles of the present study were confirmed by the Scientific and research Council of the Faculty of Veterinary Medicine of Shahrekord University, and the ethics committee in research of Shahrekord University approved the ethical principles of the present study (IR.SKU.REC.1402.060).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Medical Plants Research Center, Basic Health Science Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
[3]Pathobiology Department, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

Received: 22 November 2024 / Accepted: 14 March 2025
Published online: 31 March 2025

References

1. Che X, Zhao T, Hu J, Yang K, Ma N, Li A, Sun Q, Ding C, Ding Q. Application of Chitosan-Based hydrogel in promoting wound healing: A review. Polymers. 2024;16(3):344.
2. Feng P, Luo Y, Ke C, Qiu H, Wang W, Zhu Y, Hou R, Xu L, Wu S. Chitosan-Based functional materials for skin wound repair: mechanisms and applications. Front Bioeng Biotechnol. 2021;18(9):650598.
3. Altememy D, Javdani M, Khosravian P, Khosravi A, Moghtadaei Khorasgani E. Preparation of transdermal patch containing selenium nanoparticles loaded with Doxycycline and evaluation of skin wound healing in a rat model. Pharmaceuticals. 2022;15(11):1381.
4. Khosravian P, Javdani M, Noorbakhnia R, Moghtadaei-Khorasgani E, Barzegar A. Preparation and evaluation of Chitosan skin patches containing mesoporous silica nanoparticles loaded by Doxycycline on skin wound healing. Arch Dermatol Res. 2023;315:1333–45.
5. Abbaszadeh A, Tehmasebi-Foolad A, Rajabzadeh A, Beigi-Brojeni N, Zarei L. Effects of Chitosan/nano selenium biofilm on infected wound healing in rats; an experimental study. Bull Emerg Trauma. 2019;7:284–91.
6. Rostami H, Mohammadi R, Asri-Rezaei S, Tehrani AA. Evaluation of application of Chitosan/nano selenium biodegradable film on full thickness excisional wound healing in rats. Iran J Vet Surg. 2018;13:14–22.
7. At K, Ya A. Effect of selenium nanoparticles in wound healing. Biochem Lett. 2020;16:160–68.
8. Johnson KE, Wilgus TA. Vascular endothelial growth factor and angiogenesis in the regulation of cutaneous wound repair. Adv Wound Care. 2014;3:647–61.
9. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev. 2002;82:47–95.
10. Gabriele S, Buchanan B, Kundu A, Dwyer HC, Gabriele JP, Mayer P, Baranowski DC. Stability, activity, and application of topical Doxycycline formulations in a diabetic wound case study. Wounds. 2019;31:49–54.
11. Antoniou S, Antoniou G, Granderath F, Simopoulos C. The role of matrix metalloproteinases in the pathogenesis of abdominal wall hernias. Eur J Clin Investig. 2009;39:953–59.
12. Wilcox JR, Covington DS, Paez N. Doxycycline as a modulator of inflammation in chronic wounds. Wounds. 2012;24:339–49.
13. Han B, Fang WH, Zhao S, Yang Z, Hoang BX. Zinc sulfide nanoparticles improve skin regeneration. Nanomedicine. 2020;29:102263.
14. Melnikova N, Balakireva A, Orekhov D, Kamorin D, Didenko N, Malygina D, Knyazev A, Novopoltsev D, Solovyeva A. Zinc oxide nanoparticles protected with terpenoids as a substance in redox imbalance normalization in burns. Pharmaceuticals. 2021;14:492.
15. Foroutan A, Abbas Zadeh Haji Abadi M, Kianinia Y, Ghadiri M. Critical importance of PH and collector type on the flotation of sphalerite and Galena from a Low-Grade Lead–Zinc. Ore Sci Rep. 2021;11:3103.
16. Altememy D, Javdani M, Khosravian P, Khosravi A, Moghtadaei Khorasgani E. Preparation of Transdermal Patch Containing Selenium Nanoparticles Loaded with Doxycycline and Evaluation of Skin Wound Healing in a Rat Model. Pharmaceuticals (Basel). 2022 10;15(11):1381.
17. Görgülü T, Olgun A, Torun M, Kargi E. Application of n-butyl cyanoacrylate to split-thickness skin grafts in rats: an experimental study. Dermatol Surg. 2015;41:1024–9.
18. Hong YK, Chang YH, Lin YC, Chen B, Guevara BEK, Hsu CK. Inflammation in wound healing and pathological scarring. Adv Wound Care. 2023;12:288–300.
19. Deng L, Du C, Song P, Chen T, Rui S, Armstrong DG, Deng W. The role of oxidative stress and antioxidants in diabetic wound healing. Oxid Med Cell Longev. 2021;4:8852759.
20. Maita KC, Avila FR, Torres-Guzman RA, Garcia JP, Eldaly AS, Palmieri L, Emam OS, Ho O, Forte AJ. Local anti-inflammatory effect and Immunomodulatory activity of chitosan-based dressing in skin wound healing: A systematic review. J Clin Transl Res. 2022;8(6):488–98.
21. Navarro-Triviño FJ, Pérez-López I, Ruiz-Villaverde R. Doxycycline, an antibiotic or an anti-inflammatory agent? The most common uses in dermatology. Actas Dermo-Sifiliográficas (English Edition). 2020;111(7):561–66.
22. Samartzis EP, Fink D, Stucki M, Imesch P. Doxycycline reduces MMP-2 activity and inhibits invasion of 12Z epithelial endometriotic cells as well as MMP-2 and– 9 activity in primary endometriotic stromal cells in vitro. Reproduct Biol Endocrinol. 2019;17:38.
23. Dos Santos Pereira M, do Nascimento GC, Bortolanza M, Michel PP, Raisman-Vozari R, Del Bel E. Doxycycline attenuates L-DOPA-induced dyskinesia through an anti-inflammatory effect in a Hemiparkinsonian mouse model. Front Pharmacol. 2022;13:1045465.

24. Clemens DL, Duryee MJ, Sarmiento C, Chiou A, McGowan JD, Hunter CD, Schlichte SL, Tian J, Klassen LW, O'Dell JR, Thiele GM, Mikuls TR, Zimmerman MC, Anderson DR. Novel antioxidant properties of Doxycycline. Int J Mol Sci. 2018;19(12):4078.
25. Khan AR, Huang K, Jinzhong Z, Zhu T, Morsi Y, Aldalbahi A, El-Newehy M, Yan X, Mo X. Exploration of the antibacterial and wound healing potential of a PLGA/silk fibroin based electrospun membrane loaded with zinc oxide nanoparticles. J Mater Chem B. 2021;5(9):1452–65.
26. Agarwal H, Shanmugam V. A review on anti-inflammatory activity of green synthesized zinc oxide nanoparticle: Mechanism-based approach. Bioorg Chem. 2020;94:103423.
27. Fang M, Zhang H, Wang Y, Zhang H, Zhang D, Xu P. Biomimetic selenium nanosystems for infectious wound healing. Eng Regen. 2023;4(2):152–60.
28. Ansari JA, Malik JA, Ahmed S, Manzoor M, Ahemad N, Anwar S. Recent advances in the therapeutic applications of selenium nanoparticles. Mol Biol Rep. 2024;51:688.
29. Ramya S, Shanmugasundaram T, Balagurunathan R. Biomedical potential of actinobacterially synthesized selenium nanoparticles with special reference to anti-biofilm, anti-oxidant, wound healing, cytotoxic and anti-viral activities. J Trace Elem Med Biol. 2015;32:30–9.

Publisher's note