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Effect of 3-hydrazinylquinoxaline-2-thiol hydrogel on skin wound healing process in diabetic rats

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Impaired wound healing in diabetic individuals creates huge social and financial burdens for both diabetic patients and the health system. Unfortunately, the current treatment has not resulted in consistently lower amputation rates. Quinoxalines are heterocyclic compounds with multiple important pharmacological properties. Their effect on wound healing have not been closely studied. In the current work, the wound healing effect of 3-hydrazinylquinoxaline-2-thiol hydrogel is tested topically in a full-thickness excision wound in streptozotocin-induced type 1 diabetic rats. We examined the wound closure rate, expression of inflammatory factors, growth factors in addition to the histological analysis. The results revealed a significant acceleration in wound closure in the treated group compared with the control experimental animals. Histological data demonstrated enhanced re-epithelialization and collagen disposition. The healing effect was additionally evaluated by the inhibition of the inflammatory response of interleukin (IL)—1β interleukin (IL)—6, tumor necrosis factor (TNF-α) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) with a marked improvement of the transforming growth factor beta (TGFβ-1), antioxidant markers and collagen-1. In silico study indicated a favorable drug-like properties and toxicity profile. The present work showed that 3-hydrazinylquinoxaline-2-thiol holds great potential for the treatment of diabetic wounds.

Keywords Quinoxaline, Inflammation, Wound healing, Hydrogel, Collagen-1

Abbreviations

IL-1β	Interleukin 1β
IL-6	Interleukin 6
IL-10	Interleukin 10
TNF-α	Tumor necrosis factor and
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
Nrf2	Nuclear factor erythroid 2-related factor 2
TGFβ-1	Transforming growth factor beta one.
ECM	Extracellular matrix
QD	3-Hydrazinylquinoxaline-2-thiol
STZ	Streptozotocin
CD 68	Cluster of differentiation 68
SOD	Superoxide dismutase

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GSH	Glutathione
CAT	Catalase
HPMC	Hydroxypropyl methylcellulose
DMSO	Dimethyl sulfoxide

Diabetes mellitus (DM) is a worldwide healthcare issue that is causing significant health concerns[1,2]. Recent reports indicate a significant global rise in the prevalence of the disease during the past few decades. Diabetes impacts around 537 million adults globally, aged 20–79[3,4]**.** Around 25% of diabetic patients suffer from chronic ulcers, which typically take a long period to heal and have a recurrence probability of 65% with a higher rate of amputation[5]. Financially, the treatment of diabetic wounds consumes 12–15% of the total expenditure on diabetes care[6]. The significant burden that chronic diabetic wounds place on patients and the healthcare system highlights the immediate demand for more effective treatment options to enhance treatment outcomes.

Wound healing is a natural dynamic process that restores the structural integrity of the affected area; this is accomplished through the deposition of extracellular matrix (ECM), cell migration, and proliferation. Physiologically, it can be categorized into four distinct phases, including coagulation, inflammation, proliferation, and remodeling[7]. This vital process requires the coordinated activity of several cells, such as macrophages, platelets, neutrophils, monocytes, endothelial cells, keratinocytes, and fibroblasts. Nevertheless, in patients with diabetes, these mechanisms and healing phases have been demonstrated to be delayed, resulting in reduced healing of wounds. Consequently, patients are more susceptible to developing chronic, non-healing wounds, such as diabetic foot ulcers[8].

Hydrogels have gained significant interest in managing diabetic foot ulcers (DFUs) due to their excellent moisture-retaining properties and biocompatibility. Their structural stability and adaptability make them particularly suitable for this application[9,10]. Hydrogels are made up of three-dimensional networks of water-absorbing polymers that can retain a large amount of water and sustain a moist wound environment. This moist environment aids in wound healing by supporting cell migration, promoting the formation of new blood vessels, and decreasing inflammation. They are non-adherent, allowing for gentle and painless removal, thereby minimizing the risk of trauma to the wound bed[11,12]. Moreover, hydrogels can be optimized and designed to fit the size and shape of the wound. In addition, they can be combined with other wound dressings to enhance their healing properties. While hydrogels provide numerous benefits, they also present certain limitations. For instance, they require frequent changes as they can become saturated with exudate. Moreover, there is a potential risk of maceration to the surrounding skin[13]. Nonetheless, several studies have demonstrated the effectiveness of hydrogels in treating diabetic wounds. According to a meta-analysis of 15 randomized controlled trials involving 872 patients, hydrogels are effective in promoting diabetic wound healing. They shorten the time to granulation and epithelial formation and reduce the rate of infection compared to conventional dressings[14].

Recently, the use of natural medicines has continued to expand rapidly due to their limited side effects and unique structural diversity, in addition to their rich source of valuable phytochemicals like flavonoids, terpenoids, saponins, and alkaloids[15,16]. Quinoxaline is an important skeleton of nitrogen-rich heterocycles that is found naturally and it provides a diverse range of potential biological activities. This class of compounds showed significant interest due to their broad spectrum of therapeutic properties, including antibacterial, antifungal, antiviral, antileishmanial, and antitubercular, antimalarial, anticancer, antidepressant, and neurological activities. Several heterocycles play essential roles in biological processes, including hemoglobin in blood, chlorophyll in photosynthesis, and the heterocyclic base pairs found in DNA and RNA. It is believed that the unique structural nucleus of quinoxaline is responsible for these varied activities. Some quinoxaline derivatives have been identified as potential agents for wound healing. Yet, these possibilities remain largely unexplored. In previously published studies, various antimicrobial, wound healing activities were assessed for quinoxaline-containing natural products or synthesized derivatives, including anti-inflammatory activity in rats using the 'carrageenan-induced paw edema technique' and analgesic activity in mice using the acetic acid-induced writhing method[17,18].

In the *Chromolaena odorata* plant, Charles and Minakiri have identified the natural occurrence of 2, 3-dimethylquinoxaline (DMQ)[16]. It was proposed that the antimicrobial properties of *Chromolaena odorata* might be due to the presence of DMQ. Alfadil and team verified DMQ's full spectrum antifungal capability[19]. Additionally, they highlighted DMQ's potential for skin wound healing, possibly because of its anti-inflammatory action[20]. Given this background, we aimed to investigate how 3-hydrazinylquinoxaline-2-thiol, which shares structural similarity with DMQ influences, wound healing in diabetic rats and facilitate the restoration of diabetic wounds. Offering insights into potential new therapeutic compounds for treating diabetic wounds.

Method

Animals

The in vivo wound healing experimental study involved the use of 42 healthy adult male Wister albino rats. These rats, weighing between 150 to 200g, were sourced from the animal house colony at the faculty of pharmacy in King Abdul-aziz University (KAU), Saudi Arabia, Jeddah. The rats were housed in clean cages with a regulated light–dark cycle and a chamber temperature maintained between 22 and 25 °C. The relative room humidity was set at 55%, and the rats had unrestricted access to water and food. "The study protocol was approved by the Committee of Research Ethics, Faculty of Pharmacy, KAU (King Abdul-aziz University) (Reference Number "PH-1443-76"), and all methods were carried out in accordance with animal research guidelines and ARRIVE guidelines.

Diabetes mellitus induction

To induce diabetes in the rats, a single intraperitoneal injection of streptozotocin (STZ) was administered. Freshly produced STZ with citrate buffer (pH 4.00) was given to the rats at a dosage of 55 mg/kg after an overnight fasting period[18,21]. After 72 h, the presence of type 1 diabetes mellitus (T2DM) was confirmed by measuring fasting glucose levels using an ACCU-CHEK® Active glucometer. The diabetic state was further confirmed by the presence of polyuria, polydipsia, polyphagia, and weight loss. Only rats with a blood glucose level exceeding 250 mg/dL were selected for subsequent experiments.

Model of excisional wound

Two weeks after confirming the presence of diabetes mellitus (DM), the rats were anesthetized with an intraperitoneal administration of ketamine (90 mg/kg) and xylazine (10 mg/kg). Before inflicting wounds, the dorsal fur of the rats was shaved using an electric clipper and disinfected with 70% ethanol. Using a sterile biopsy punch, circular pieces of full-thickness skin measuring 1 cm were excised from predetermined areas on the rats' backs. The animals were regularly monitored for signs of infection, and any rats displaying signs of infection were excluded from the experiment and replaced with new subjects.

Experimental protocol

After the wound excision procedure, the animals were placed into individual cages and then randomly assigned to three equal groups, with each group comprising 14 animals (n = 14). This number was determined for each group to ensure their aviliability until the end of the experiment and to minimize the risk of STZ toxicity. Group I: Non-diabetic control rats treated with daily topical application of white petrolatum jelly. Group II: Diabetic rats receiving vehicle alone daily. Group III: Diabetic animals treated with a 0.2% hydrogel containing 3-hydrazinylquinoxaline-2-thiol, specifically applied to the excision wounds. Key points represented in the graphical abstract (Fig. 1).

Estimation of wound contraction

The daily measurement of the gradual decrease in the wound area is conducted by delineating the wound region using clear sheets of graph paper. The process of wound contraction was quantified by expressing it as a proportion of the overall healing progress. A digital camera was used to capture an image of the wound in order to assess the progress of wound closure. The percentage of wound contraction was subsequently calculated using the following formula[22]:

$$\text{Percentage of wound contraction} = (\text{healed area} \times \text{initial wound area}) \times 100$$

$$*\text{Healed area} = \text{initial wound area} - \text{wound area on a given day}$$

Preparation of the test compound

The compound being tested, 3-hydrazinylquinoxaline-2-thiol was procured from Sigma Aldrich D184977 in Taufkirchen, Germany. To ensure sterility and prevent contamination, all procedures were performed in a sterile environment following a previously described protocol[20]. In brief, a 2% (w/w) gel of hydroxypropyl methylcellulose (HPMC) was prepared by dissolving 3 g of HPMC in distilled water until the total weight reached 100 g. The mixture was then covered, gently stirred using a magnetic stirrer, and left for 24 h to allow complete water

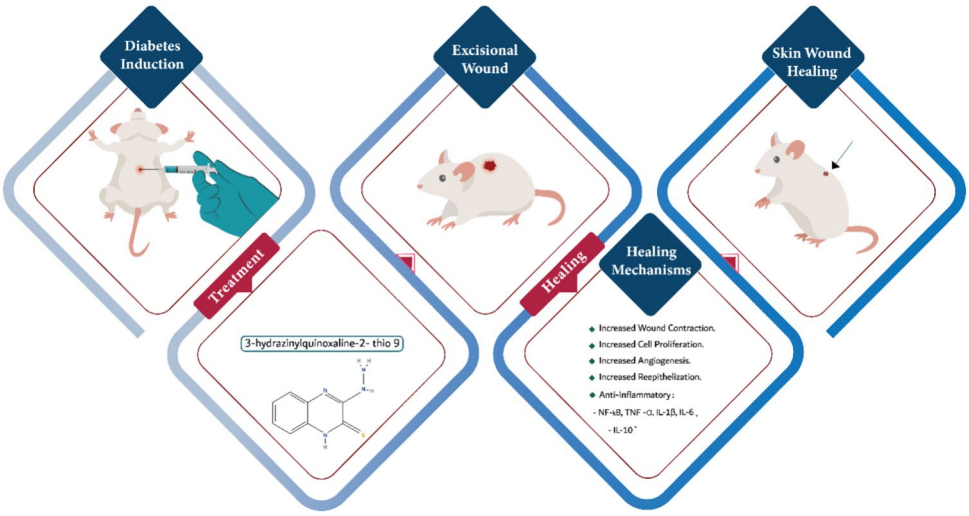


Fig. 1. Graphical abstract: Testing wound healing effect of 3-hydrazinylquinoxaline-2-thiol hydrogel topically in a full-thickness excision wound in streptozotocin-induced type-2 diabetic rats.

absorption and gel formation. For the preparation of the hydrogel (0.2%), 0.2 g of the drug was dissolved in 2 g of dimethyl sulfoxide (DMSO). The solution was then gently mixed with 3% HPMC gel (98 g) while continuously stirring to ensure uniform dispersion of the drug within the gel matrix. A similar procedure was followed to prepare the control sample. The gel was transferred to collapsible tubes and stored in a cool, dry place. The pH value of the hydrogel was determined using a digital pH meter. One gram of the prepared hydrogel was dissolved in 25 mL of distilled water, and the pH was measured at room temperature using a Thermo Scientific Expert pH meter. The pH measurement was conducted in triplicate, and the average value was calculated. The viscosity of the samples at 25 °C was measured using a Brookfield digital viscometer. The hydrogel was gradually administered starting from the outer edges and moving toward the core, ensuring comprehensive treatment of all affected areas.

Histological study

Before sacrificing the animals and harvesting the sample, the animals were euthanized via intraperitoneal injection of sodium pentobarbitone (800 mg/kg)[23]. Histological examinations were conducted using histological evaluations at 0, 14, and 21 days post-wounding. The collected representative tissue of the wound areas was fixed in a 10% buffered formalin solution. Skin samples were then subjected to the standard dehydration process in a series of increasing ethanol concentrations for 24 h. Then, the tissue was embedded in paraffin and sectioned at a 5-µm thickness. The sections were stained with hematoxylin and eosin (H&E) and examined using 100 × magnification light microscopy. Wound tissue sections were graded using the healing grade of fibroblast proliferation, angiogenesis, and the presence of inflammatory cells. All images were captured using an Olympus microscope (BX-51; Olympus, Tokyo, Japan).

Determination of pro-inflammatory cytokines and NF-κB

Skin tissue homogenate was tested for inflammation markers (TNF- α, IL-6, IL-1β) and NF-κB, Cluster of Differentiation 68 (CD68), and collagen-1 using specific ELISA kits and the manufacturer's instructions on days 3, 7, 14 and day 21 post-wounding.

Biochemical analyses

The following biochemical parameters (oxidative stress) were assessed in skin tissue homogenate: Glutathione (GSH), Superoxide dismutase (SOD), and Catalase (CAT), using biochemical ELISA kits as per the manufacturer's instructions on days 3, 7, 14 and day 21 post-wounding.

Preparation of tissue homogenate

The tissue was carefully washed with ice-cold normal saline, followed by gentle pressing in between filter papers. Samples were then homogenized in a phosphate-buffered saline (PBS) solution to obtain 10% w/v tissue homogenates. The prepared homogenate was then centrifuged at 3000 rpm for 20 min at 4 °C. Later, the supernatants were analyzed for the estimation of each specific parameter.

In silico activity

Predicting ADME properties, molecular targets, and CYP P450 enzyme inhibition profile for 3-hydrazinoquinoxaline-2-thiol

In this study, we utilized multiple online computational tools to predict various properties of 3-Hydrazinoquinoxaline-2-thiol. We used the Simplified Molecular Input Line Entry System (SMILES) as input (Table 1) to predict properties based on the chemical structure. The Swiss ADME webserver helped us predict pharmacokinetic parameters such as molecular weight, lipophilicity, solubility, blood brain barrier (BBB) permeability, oral (gastrointestinal/GI) absorption, and skin penetration. To further analyze the molecule, we generated a pharmacokinetic radar chart and a molecular targets pie chart using Swiss ADME and Swiss Target Prediction. We also evaluated the inhibition profile of the CYP isoenzymes including CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4[24–26].

Predictions of organ and endpoint toxicity profile for 3-hydrazinoquinoxaline-2-thiol chemical structure

In order for a chemical scaffold to reach the drug market, it is crucial to evaluate its toxicity and safety. To achieve this, we utilized two web servers, Protox II and predherg. These tools provide us with a comprehensive overview of various toxicity parameters, such as organ-specific toxicities like hepatotoxicity and cardiac toxicity, as well as endpoints like immunotoxicity, cytotoxicity, carcinogenicity, and mutagenicity. By employing these web servers, we gained valuable insights into the toxicity profile of small molecules, facilitating drug development[27,28].

Acute dermal irritation test

For evaluating the skin tolerance of the prepared hydrogel, six Wister rats with unblemished skin were selected. The backs of these rats were properly shaved with care, and 200 mg of the prepared hydrogel was applied to

Name	SMILES
3-Hydrazinoquinoxaline-2-thiol	C1=CC=C2C(=C1)NC(=S)C(=N2)NN

Table 1. Simplified molecular input line entry system (SMILES) for 3-hydrazinoquinoxaline-2-thiol chemical structure.

the shaved area. Subsequently, the treated region was covered with a cotton bandage for a duration of 24 h. A quantity of 500 mg of the test samples was applied to the selected test site. Observations for signs of morbidity and toxicity along with adverse skin reactions, including pruritus, edema, eschar formation and erythema were conducted initially within the first 24 h and then over a period of 21 days[29].

Statistical analysis

Data were analyzed using Graph Pad Prism 10.1.1, and statistical significance values were set at $P \leq 0.05$. Biomarkers were computed for each group and subsequently compared using either One-way or Two-way ANOVA with multiple comparisons. The data, representing three or more biological replicates, is presented as means ± SD. Statistical significance is denoted as follows: (*) $P \leq 0.05$, (**) $P \leq 0.01$, (***) $P \leq 0.001$, (****) $P \leq 0.0001$.

Results

Impact of 3-hydrazinylquinoxaline-2-thiol hydrogel on wound contraction in experimental rat model

The results depicted in Fig. 2 demonstrate the progressive changes in wound contraction size (cm) over a 3-week period in the rat groups under study. It is noteworthy that all groups exhibited an increase in wound contraction, with treated diabetic rats and non-diabetic control rats showing a more substantial increase compared to untreated diabetic control rats throughout the study period. Importantly, on day 3 post-operation, the control group and untreated diabetic rats displayed similar levels of wound contraction. However, by day 14, the group treated with 0.2% QD hydrogel exhibited significantly higher wound contraction than the untreated group, with a notable statistical significance (P-value < 0.0001), indicating the potential efficacy of the treatment. Additionally, the treated diabetic rats with 0.2% QD hydrogel consistently demonstrated significantly better wound contraction on all days compared to the untreated diabetic control group. These compelling findings suggest that the 0.2% QD hydrogel treatment holds promise for promoting wound healing, especially in diabetic rats, and therefore merits further investigation. Figure 3 displays representative images of the wound contraction size among different groups.

Effect of 3-hydrazinylquinoxaline-2-thiol hydrogel on inflammatory markers in an experimental rat model

In order to elucidate the potential mechanism underlying the hydrogel's impact on the inflammatory response, we quantified various inflammatory markers, including NF-κB, TNF-α, IL-1β, IL-6, and IL-10. The results depicted in Fig. 4 revealed a notable increase in the activity of inflammatory markers in diabetic control rats compared to normal control rats. Conversely, the diabetic rats treated with 0.2% QD hydrogel exhibited a significant (P-value < 0.0001) reduction of 71.45%, 65.7%, 63.1%, 72.3%, and 64.63% in the activity of NF-κB, TNF-α, IL-1β, IL-6, and IL-10, respectively, as compared to the untreated diabetic group.

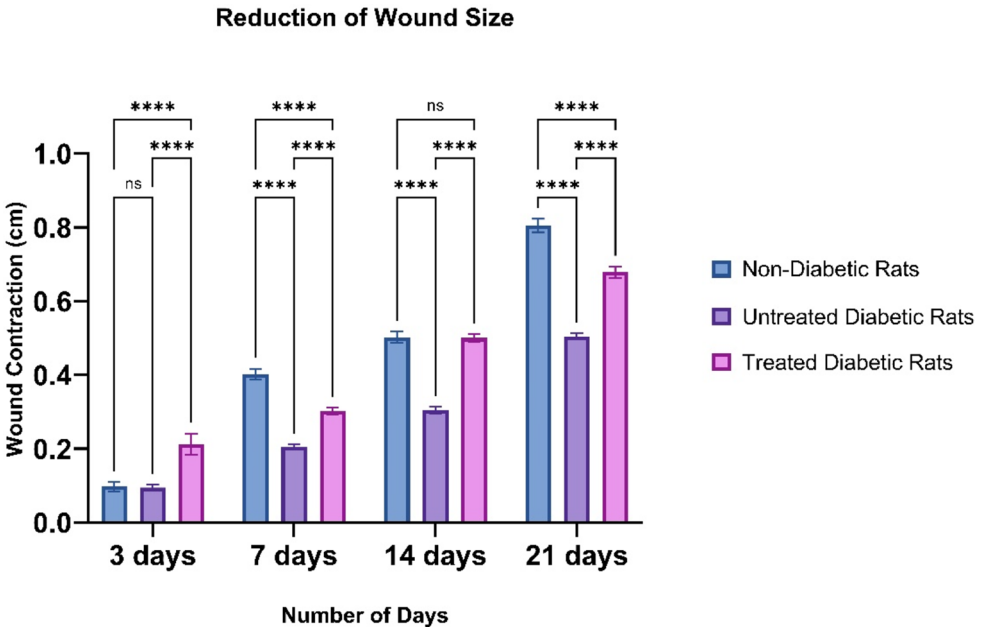


Fig. 2. Variation in wound contraction size among different rat groups. The statistical significance of **** indicates a P-value of less than 0.0001. The reported values (mean ± SEM) were derived from each group consisting of 10 animals.

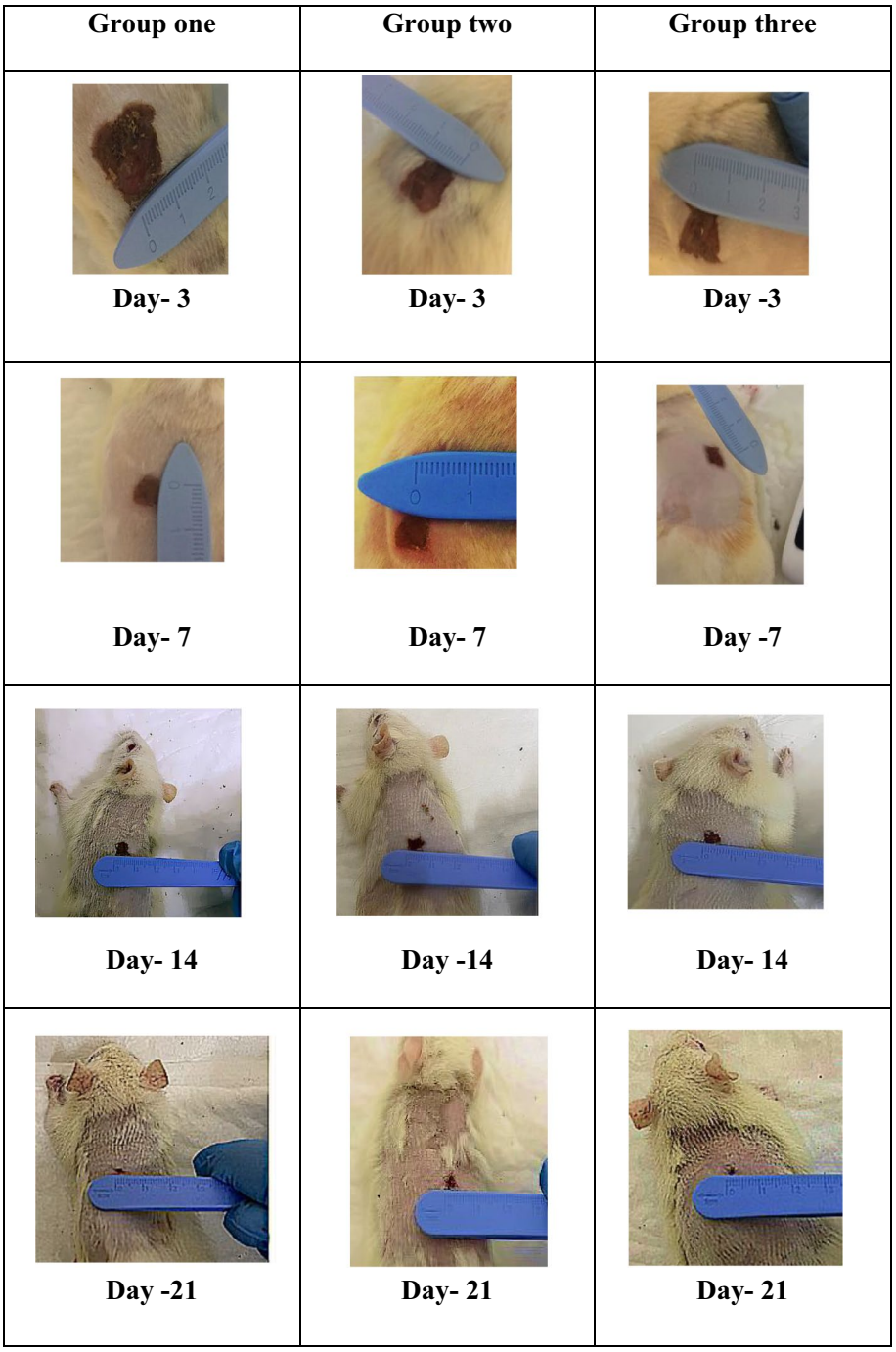


Fig. 3. Representative images of full-thickness skin wound after cleaning the residual hydrogels in a group 1: non-diabetic control rats; group 2: diabetic control rats, group 3: diabetic rats treated with 0.2% QD hydrogel at day 3, 7, 14 and 21 days post operation.

Modulation of antioxidant parameters by 3-hydrazinylquinoxaline-2-thiol hydrogel

In the present study, the progression of oxidative stress was evaluated through the assessment of pivotal antioxidant parameters, including SOD, GSH, and CAT in the skin tissue of distinct treatment groups, as outlined in Fig. 5. Notably, SOD, CAT, and GSH levels exhibited a significant decrease in diabetic control rats compared to those in the normal control group ($P < 0.0001$). Conversely, the topical application of 0.2% QD hydrogel led to a substantial ($P < 0.0001$) increase in SOD, GSH, and CAT levels by 100.9%, 351%, and 58.9%, respectively.

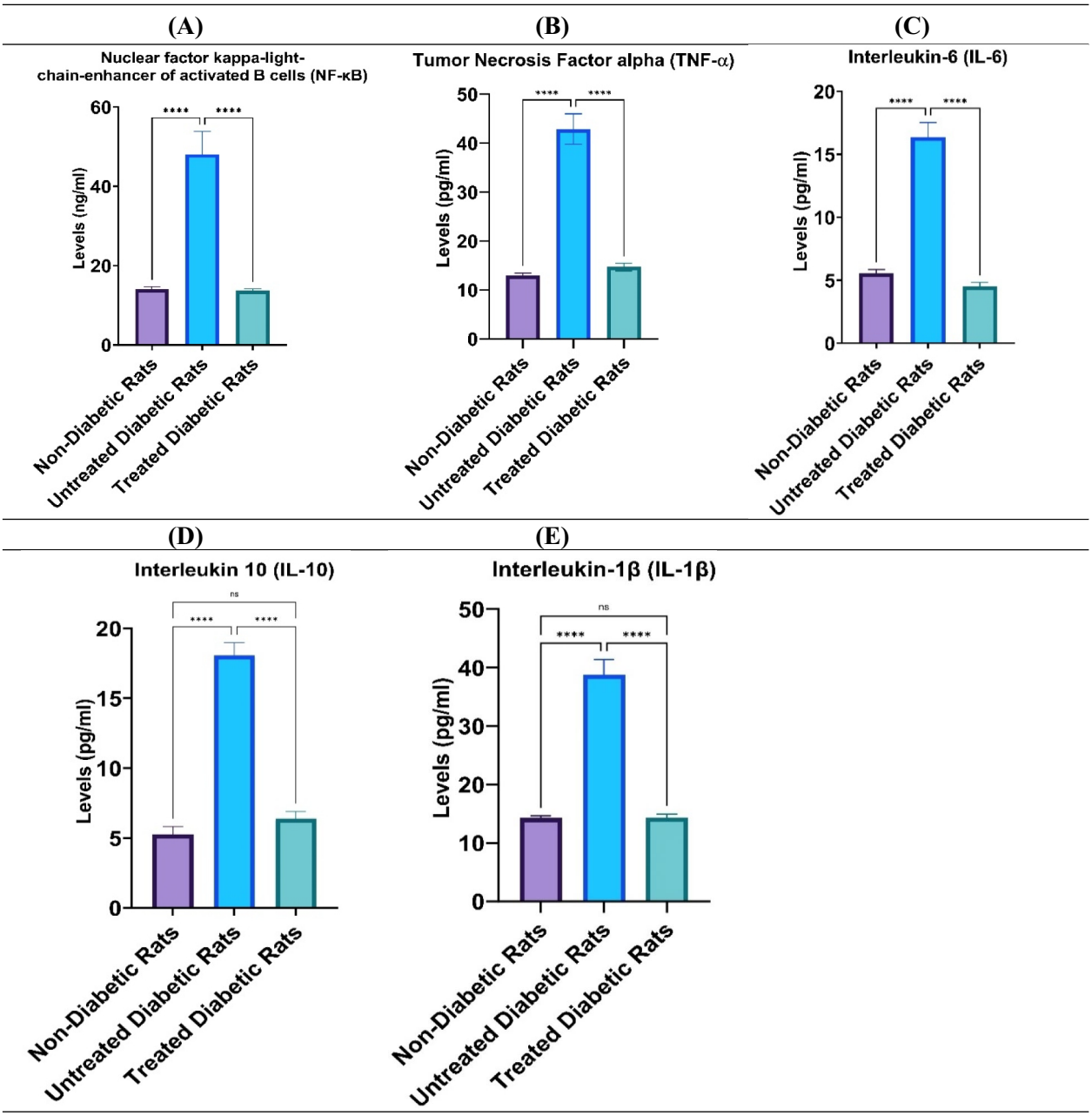


Fig. 4. The impact of 0.2% QD hydrogel on the inflammatory markers (**A**) NF-κB, (**B**) TNF-α, (**C**) IL-6, (**D**) IL-10, and (**E**) IL-1β in control and topically treated diabetic rats. The reported values (mean ± SEM) were derived from each group consisting of 10 animals. Notably, the statistical significance of **** indicates a P-value of less than 0.0001.

Influence of 3-hydrazinylquinoxaline-2-thiol hydrogel on CD68 (cluster of differentiation 68) expression in experimental rats

The impact of topically administered 0.2% QD hydrogel on the glycoprotein CD68 level in various experimental groups is depicted in Fig. 6. Our findings from the study demonstrate a noteworthy ($P < 0.0001$) increase in CD68 levels in diabetic control rats. However, treatment with 0.2% QD hydrogel resulted in a normalization of CD68 levels in treated diabetic rats, aligning them closer to those of their untreated control counterparts.

Influence of 3-hydrazinylquinoxaline-2-thiol hydrogel on collagen-1, TGFβ-1, and Nrf2 levels in experimental rats

The results depicted in Fig. 7 illustrate a notable increase in growth factor production following topical treatment with 0.2% QD hydrogel 21 days post-wounding. In comparison with untreated diabetic rats, the level of TGFβ-1was significantly elevated in the 0.2% QD hydrogel treated group ($P < 0.001$), reaching levels approximating those of the control non-diabetic group. Additionally, our findings indicate a significant decrease in Collagen-1

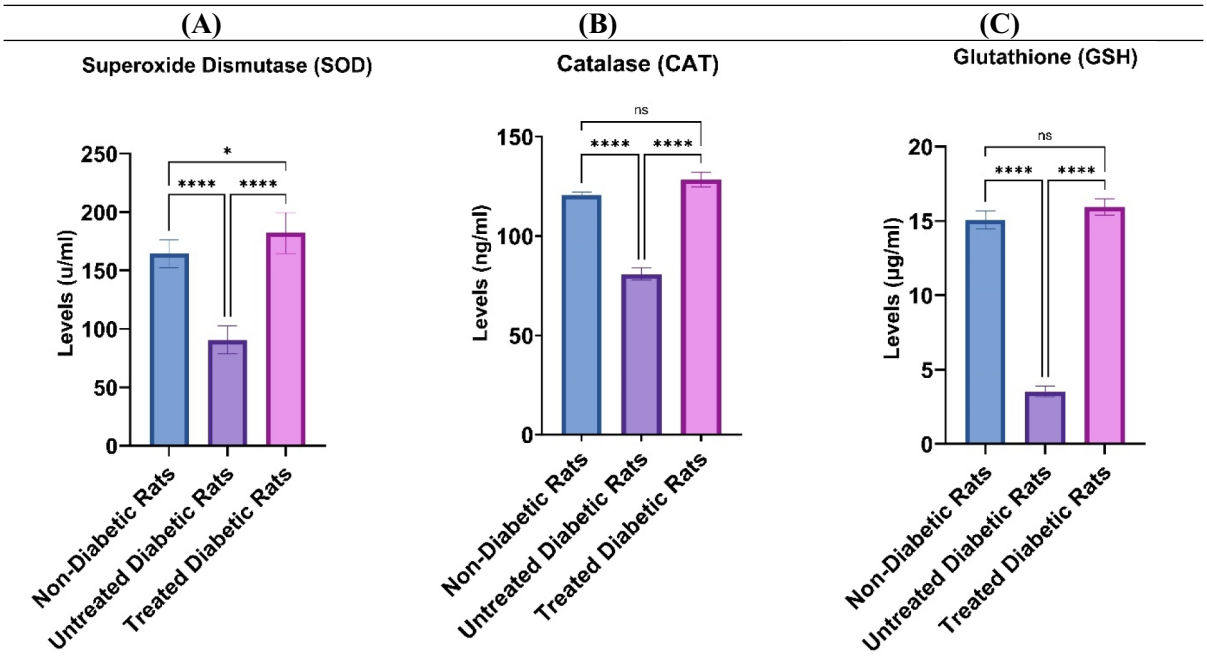


Fig. 5. The influence of 0.2% QD hydrogel application on the antioxidant markers (**A**) SOD, (**B**) CAT, and (**C**) GSH in both control and topically treated diabetic rats. The presented values (mean ± SEM) were obtained from each group of 10 animals. The statistical significance of * indicates a P-value of less than 0.05, while **** indicates a P-value of less than 0.0001.

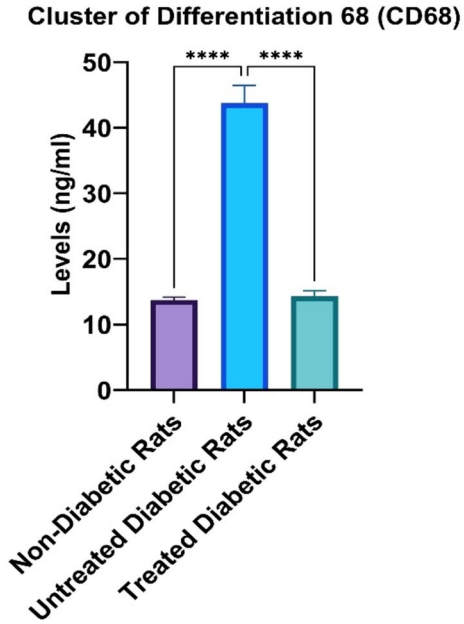


Fig. 6. Impact of 0.2% QD hydrogel on CD68 levels in control and topically treated diabetic rats. The reported values (mean ± SEM) were obtained from each group of 10 animals, demonstrating a significant effect with a P value of < 0.0001.

levels in untreated experimental rats, which was effectively reversed by the topical administration of 0.2% QD hydrogel, demonstrating a noteworthy enhancement in Collagen-1 content ($P < 0.0001$). Similarly, treatment with 0.2% QD hydrogel restored Nrf2 levels by 56.1% compared to untreated diabetic rats.

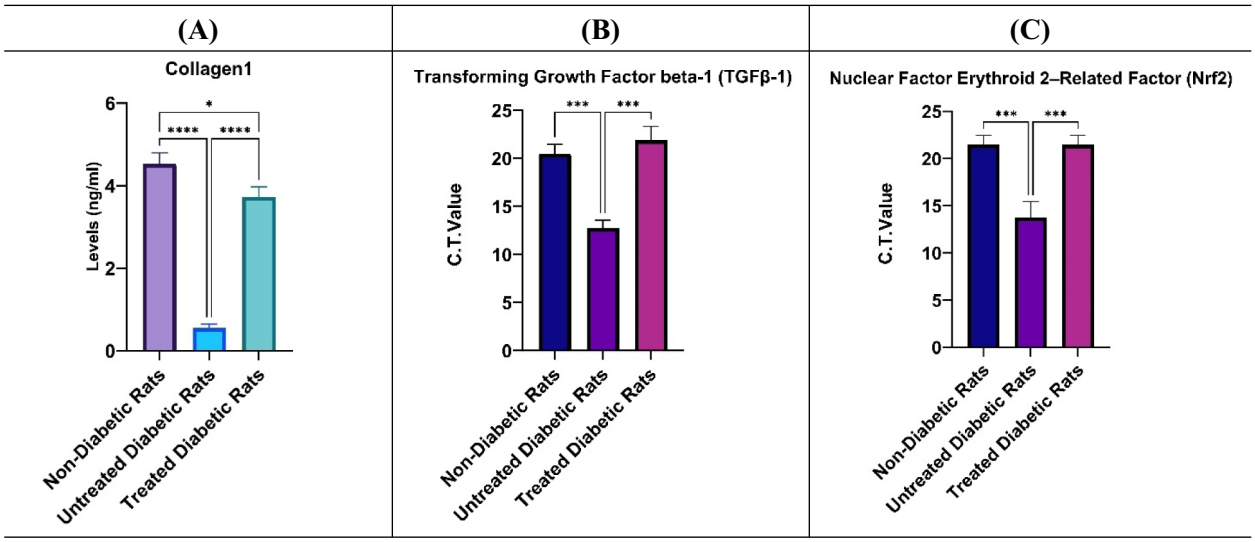


Fig. 7. Influence of 0.2% QD hydrogel on (**A**) Collagen-1, (**B**) TGFB1, and (**C**) Nrf2 levels in control and topically treated diabetic rats. The reported values (mean ± SEM) were obtained from each group of 10 animals, with statistical significance denoted by *P ≤ 0.05, **P ≤ 0.001, and ****P ≤ 0.0001.

Impact of 3-hydrazinylquinoxaline-2-thiol hydrogel on histopathological evaluation of wound healing

Histopathological evaluations were performed to analyze the progression of wound healing in each experimental group, as depicted in Fig. 8. On the 14th day, the control group (G1) exhibited mild granulation tissue, epithelial loss, and hyperkeratosis. These characteristics were more pronounced in the untreated diabetic group (G2). Conversely, the application of 0.2% QD hydrogel (G3) to the diabetic wounds resulted in significant improvements in wound healing, evidenced by restored epidermal continuity and the presence of inflammatory exudate in the epidermis. Histological examination after 21 days revealed complete healing of ulcers in non-diabetic rats (G1), while partial healing was observed in the other groups (G2 and G3). Additionally, epithelialization was complete in the control non-diabetic and treated experimental rats.

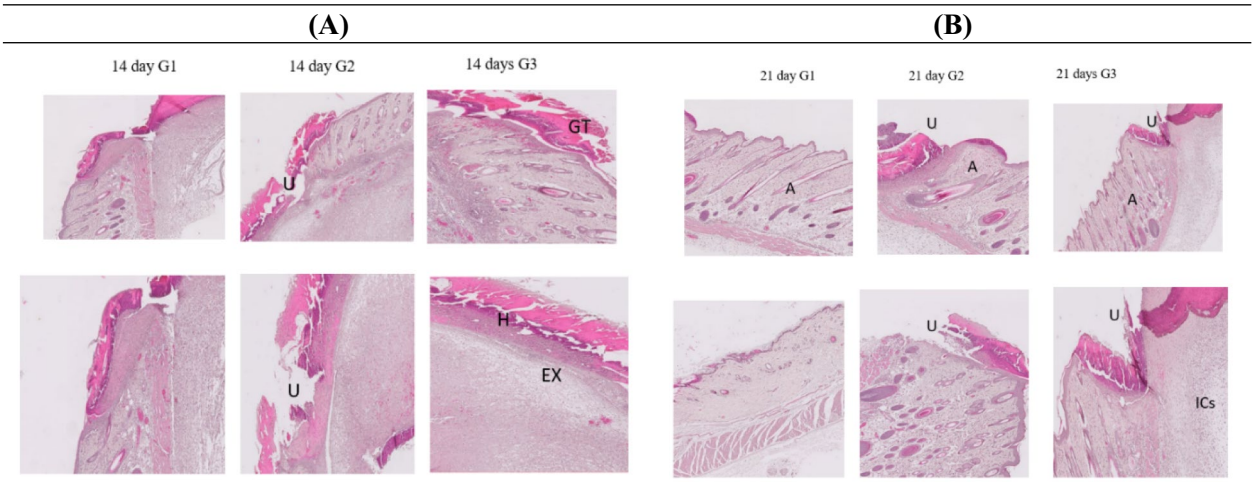


Fig. 8. Histopathological Examination of 0.2% QD Hydrogel. (**A**) Representative skin photomicrographs and histological sections stained with hematoxylin and eosin (H&E) at day 14. G1 (control non-diabetic), G2 (untreated diabetic rat), and G3 (0.2% QD Hydrogel Diabetic treated rat): noticeable differences include granulation tissue (labeled GT), hyperkeratosis (labeled H), and mild epithelial loss in G1, severe epithelial loss associated with ulceration (labeled U) in G2, and evidence of healing with restored epidermal continuity and increased inflammatory exudate (labeled EX) in the dermis in G3. (**B**) Skin photomicrographs and histological sections stained with hematoxylin and eosin (H&E) at day 21 featuring acanthosis (labeled A) in all groups, complete healing of the ulcer in G1 but partial healing in G2 and G3 (labeled U). On day 21, G2 exhibited less healing compared to G1 and G3. Epithelialization was complete in G1 and G3. Clinically, all groups showed healing, with complete intactness of the surface observed in the control group and 80% intactness in G3 (scale bar = 40 µm, 40× magnification).

Assessment of acute dermal irritation after 3-hydrazinylquinoxaline-2-thiol hydrogel treatment

Based on our current understanding, this study marks the first exploration into the potential acute dermal irritation of 0.2% QD hydrogel. Notably, throughout the experiment, animals exposed to the hydrogel did not display any adverse reactions, such as erythema, pruritus, edema, or inflammation. Moreover, no clinical signs of dermal toxicity were observed, and there were no noticeable behavioral changes in the animals throughout the entire 21-day observation period.

In silico study

Predicting ADME properties, molecular targets, and CYP P450 enzyme inhibition profile for 3-hydrazinoquinoxaline-2-thiol

As illustrated in Table 2, the results of the pharmacokinetic predictions indicate that the molecular weight of the 3-hydrazinoquinoxaline-2-thiol is 192.24 g/mol, which is considered within the range for orally active compounds. The Log Po/w value, which represents the logarithm of the octanol–water partition coefficient, is 1.39. This suggests that the compound has moderate lipophilicity (acceptable range is − 2.0 to 6.5). The Log S value, which represents the logarithm of the aqueous solubility, is − 3.13, indicating that the compound is soluble (recommended range is − 6.5 to 0.5). 3-Hydrazinoquinoxaline-2-thiol is not predicted to be a blood–brain barrier (BBB) permeant and is expected to have high gastrointestinal (GI) absorption. The Log Kp value, which represents the skin permeation coefficient, is − 7.21 cm/s, indicating a high and acceptable potential for skin permeation. According to the Rule of Five, 3-Hydrazinoquinoxaline-2-thiol does not violate any of the criteria, suggesting favorable drug-like properties. Moreover, the radar chart (Table 2A) indicates that all the predicted properties are within the recommended range except for instauration that is higher than recommended range.

Moreover, the pie chart presented in Table 2B represent the predicted molecular targets for 3-Hydrazinoquinoxaline-2-thiol. The largest section, comprising 33.3%, is dedicated to enzymes and family A G protein coupled receptor (GPCR), which are essential for various biological processes. The second-largest section, at 13%, is for other proteins, which may include structural or regulatory proteins. The remaining sections are smaller, with one at 6% representing proteins involved in cellular transport and signaling.

Additionally, the results of the CYP450 Enzymes Inhibition Profiling show that the 3-Hydrazinoquinoxaline-2-thiol inhibits only CYP1A2. While other CYP isoenzymes such as CYP2C19, CYP2C9, CYP2D6, and CYP3A4 are not affected. This means that the 3-Hydrazinoquinoxaline-2-thiol has a low potential to interact with multiple drugs that are metabolized by these enzymes. It is important to consider these findings when evaluating the safety and efficacy of the 3-Hydrazinoquinoxaline-2-thiol in combination with other medications.

Organ toxicity predictions for 3-hydrazinoquinoxaline-2-thiol

As summarized in Table 3, results of the study on the toxicity of a molecule named 3-Hydrazinoquinoxaline-2-thiol demonstrated that the molecule is classified as a non-blocker for hERG K^+ channels, thus no cardiotoxicity is predicted for his molecule. Moreover, the molecule was also found to have active toxicity in the liver (hepatotoxicity), with a probability of 70%. Additionally, the molecule showed active toxicity in terms of carcinogenicity, mutagenicity, and cytotoxicity, with probabilities of 61%, 68%, and 75%, respectively. However, the molecule was found to be inactive in terms of immunotoxicity, with a probability of 99%. Overall, our results suggests that the molecule is predicted to possess some potential toxicity in certain organs and endpoints, but further research is needed to fully understand its toxicity profile.

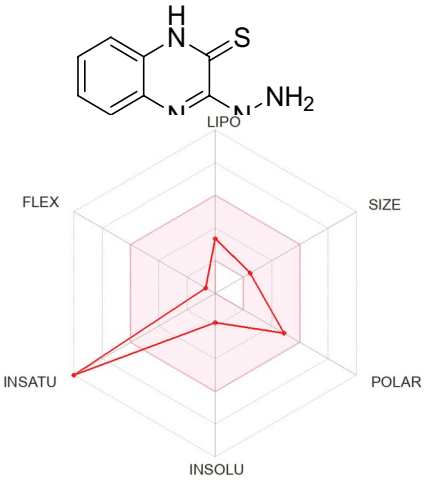
Discussion

Diabetes is experiencing a significant surge in its global prevalence, attracting considerable attention from both the general public and the scientific community. This condition has a profound impact on an individual's overall health, quality of life, and financial burden. Particularly alarming is the fact that 75% of the one million people worldwide who undergo leg amputations each year are diagnosed with diabetes. Uncontrolled hyperglycemia poses a grave risk, as it can lead to impaired wound healing, potentially resulting in gangrene and subsequent amputation if left unaddressed[30,31]. However, the currently available wound healing agents lack efficiency and safety. Therefore, there is an urgent need to develop new and effective therapeutic approaches[32]. Given the previous scientific studies that have demonstrated the pharmacological effects of quinoxaline derivatives, we hypothesized that 0.2% QD hydrogel could enhance wound healing properties, particularly in diabetic conditions. Thus, our objective in this study was to determine the influence of this compound on wound healing under hyperglycemic conditions.

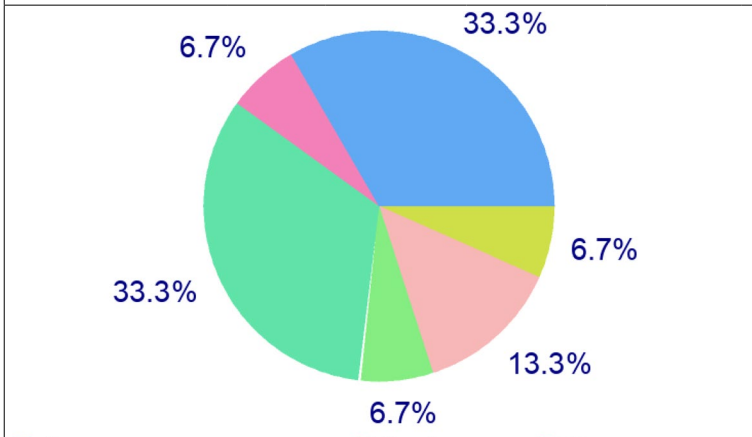
Following the confirmation of the safety and the characteristics of topical formulation, our focus shifted towards investigating the impact of quinoxaline hydrogel preparation on the wound healing process in diabetic rats. By day 14, diabetic rats treated with a 0.2% hydrogel containing 3-hydrazinylquinoxaline-2-thiol exhibited noteworthy rates of wound closure in excision wound models, surpassing both the normal control and diabetic control rats. This observation strongly suggests the efficacy of our treatment in promoting wound healing in diabetic rats. Previous research on quinoxaline derivatives has reported similar findings, where the topical application of a 1% hydrogel containing 2, 3-dimethylquinoxaline enhanced the healing of excision wounds in diabetic rats through its anti-inflammatory mechanism[20].

Pro-inflammatory cytokines, including IL-1β, TNF-α, and IL-6, play a crucial role in the initial inflammatory phase of skin wound healing. Their proper expression is essential for attracting neutrophils and removing bacteria and contaminants from the wound site[33,34]. The transcription factor NF-κB induces the expression of multiple pro-inflammatory genes. However, diabetic wounds are characterized by a prolonged inflammatory phase and suppressed growth factors such as VEGF and TGF-β during chronic wound healing. In the current

Molecule Name	Molecular weight (g/mol)	Log Po/w	Log S	BBB permeant	GI absorption	Log Kp (skin permeation, cm/s)	Rule of five (ROF)
3-Hydrazinoquinoxaline-2-thiol	192.24	1.39	− 3.13 (soluble)	No	High	− 7.21	Yes; 0 violation



(A) Radar chart for pharmacokinetics



(B) Pie Chart for molecular targets

Cytochromes (CYP) P450 Enzymes Inhibition Profiling				
CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
Yes	No	No	No	No

Table 2. The predicted ADME properties for 3-hydrazinoquinoxaline-2-thiol chemical structure using SWISSADME webserver.

	Classification					
	Organ toxicity (%confiabilty)	Organ toxicity (% probability)	Toxicity endpoint (% probability)			
Molecule name	Cardiac toxicity	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
3-Hydrazinoquinoxaline-2-thiol	Non-blocker (84.27)	Active (0.70)	Active (0.61)	Inactive (0.99)	Active (0.68)	Inactive (0.75)

Table 3. Organ and endpoint toxicity predicted for 3-hydrazinoquinoxaline-2-thiol using the ProTox-II and predherg web servers.

study, treatment with the 0.2% QD hydrogel formulation demonstrated significant improvements in diabetic wounds. We observed a notable reduction in the levels of pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6) and NF-κB in addition to an increase in TGFβ-1levels. TGFβ-1 plays a crucial role in the healing process by directing inflammatory cells to the wound site during the inflammation phase, promoting ECM deposition and granulation tissue formation during the proliferation phase[35], and facilitating the transition from collagen type 3 to collagen type 1 during the remodeling phase. These findings are supported by previous studies[36] in which wound healing was improved in hyperglycemic conditions through the down-regulation of pro-inflammatory cytokine expression and activating granulation tissue formation through TGF-β signaling pathways, which is consistent with our findings.

Elevated blood glucose levels are known to induce oxidative stress, which disrupts the wound healing process during uncontrolled inflammation[37]. Reactive oxygen species (ROS) typically play beneficial roles in combating microbial invasions and regulating intracellular signaling pathways. Nuclear-related factor 2 (Nrf2) acts as a cellular defense mechanism in response to oxidative stress. Upon oxidative damage, Nrf2 dissociates from its cytoplasmic repressor Keap1 and activates genes encoding antioxidant enzymes (SOD, GSH, CAT, and GST) in the nucleus[38]. In our study, we aimed to investigate whether the hydrogel formulation could control oxidative stress after three weeks of topical application. The diabetic control rats exhibited delayed wound healing due to a deficiency in the antioxidant defense response, resulting from Nrf2 deprivation. Conversely, the topical treatment with 0.2% QD hydrogel significantly activated nuclear Nrf2, leading to an increase in antioxidant enzymes. This, in turn, accelerated wound healing by activating ECM-related genes such as collagen-1 and TGFβ-1. These findings are consistent with the work of Sakshi et al.[39], who confirmed the positive impact of Nrf2 on diabetic wound healing.

Macrophages play a crucial role in tissue regeneration and wound healing by releasing pro-inflammatory cytokines that eliminate foreign agents and generate growth factors essential for managing inflammation and tissue restoration. The glycoprotein CD68 serves as a marker for macrophages involved in the wound healing process[40]. In our study, we observed a significant elevation in CD68 levels in diabetic rats, indicating its involvement in inflammation and phagocytosis. However, this increase was significantly reduced in the group treated with 0.2% QD hydrogel. Our findings align with the observations made by Phan et al.[41], which indicated an increased expression of CD68 during wound healing.

The histopathological studies conducted in this research corroborated the findings from the biochemical and molecular analyses. After 14 days, the control group displayed mild granulation tissue, epithelial loss, and hyperkeratosis, with more pronounced severity observed in the untreated diabetic group. In contrast, the administration of quinoxaline hydrogel effectively accelerated wound healing in diabetic rats, as evidenced by the restored continuity of the epidermis and the presence of inflammatory exudate in the dermis. Histological examination after 21 days revealed complete epithelialization in the quinoxaline hydrogel-treated rats. These histopathological observations provide compelling evidence supporting the wound-healing properties of the hydrogel containing quinoxaline.

Our in-silico findings on the pharmacokinetic properties, molecular targets, and toxicity profile of the compound 3-Hydrazinoquinoxaline-2-thiol indicates that the compound appears to have favorable drug-like properties, with a molecular weight within the range for orally active compounds, moderate lipophilicity, and acceptable aqueous solubility. The compound is not predicted to be a blood–brain barrier permeant and is expected to have high gastrointestinal absorption. The Log Kp value suggests a high potential for skin permeation. The molecular target prediction suggests that the molecule could act through GPCR or enzyme biological targets.

The inhibition profile of the 3-hydrazinoquinoxaline-2-thiol indicates that it only affects CYP1A2, which is a single enzyme among the many CYP isoenzymes responsible for metabolizing drugs. This means that the compound has a low potential to interact with multiple drugs that are metabolized by other CYP isoenzymes. This is crucial to take into account when assessing the safety and efficacy of the compound when used in conjunction with other medications.

The study on the toxicity of the 3-hydrazinoquinoxaline-2-thiol revealed that it does not block hERG K^+ channels, thus reducing the risk of cardiotoxicity. However, the compound was found to have a significant level of hepatotoxicity. Furthermore, the compound exhibited active toxicity in terms of carcinogenicity, mutagenicity, and cytotoxicity which needs to be confirmed experimentally. On the other hand, the compound was found to be inactive in terms of immunotoxicity.

Conclusion

This research offered a beneficial insight to enhance our understanding of the possible mechanism underlying the wound healing action of 3-hydrazinylquinoxaline-2-thiol 0.2% hydrogel in diabetic animal models. The study revealed that the compound has the ability to enhance wound contraction, promote epithelialization, and modulate inflammatory mediators. The current findings provide valuable information about the pharmacokinetic properties, molecular targets, and toxicity profile of the compound 3-Hydrazinoquinoxaline-2-thiol further investigation is required to investigate deeply the molecular mechanisms that contribute to its biological effects and to understand their mechanisms of action.

However, there are several limitations in our experiment. First, inducing a type 2 diabetes model in rats using STZ and a high-fat diet creates a broader understanding of the pathological features of the wound repair mechanism. Second, multiple risk factors might complicate wound healing in hyperglycemic conditions. Such as infection, ischemia, or neuropathy, which is different from our current experiment design. To gain a better comprehensive understanding of the outcome, further studies on type II diabetes and other animal genders in addition to the compound's stability study are recommended to facilitate the development of effective wound-healing formulations.

Data availability

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

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Competing interests
The authors declare no competing interests.

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