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The Wound Healing Potential of 2,3 Dimethylquinoxaline Hydrogel in Rat Excisional Wound Model

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: 2,3-dimethylquinoxaline (DMQ) is a naturally occurring compound with documented antifungal activity. It showed also good in vitro physicochemical and pharmacokinetic characteristics.
Methods: The wound healing activity of 1% DMQ hydrogel in healthy adult male Wister rats were evaluated using the excision wound model. On day 7, the mean percentage closure of the wound area was determined. The animals were sacrificed on day 7 and skin was isolated for histology research and assessment of some inflammatory & oxidative markers, hydroxyproline and tissue growth factor.
Results and Discussion: Almost complete wound healing was observed after treatment with DMQ 1 % Jell for 7 days. The histological study confirmed marked attenuation of wound-induced histological changes. There was a marked reduction in TNF-α, IL-6 IL-β1 and NF-κB. levels. These data suggest the potential anti-inflammatory effect of DMQ.
Conclusion: DMQ has potential skin wound healing ability likely due to its anti-inflammatory mechanism. Further study is needed to confirm these preliminary findings and explore the molecular mechanism

Keywords: 2,3-dimethylquinoxaline; hydrogel; wound healing; inflammatory biomarkers.

1. INTRODUCTION

Wounds are defined as harm to the skin's structural integrity brought on by many causes such as burns, cuts, scalds, and lesions such as foot complications of diabetes [1]. Improper management of wounds is associated with many complications such as infections, wound dehiscence, inflammation, scarring, and improper angiogenesis and regeneration [2,3].

It is anticipated that chronic wounds will continue to be a significant challenge because of the ageing population, and the ongoing increase in diabetes, obesity, and infections around the globe [4]. These complications increase morbidity and mortality, negatively impact patients' quality of life, and place a tremendous financial load on healthcare systems. Different approaches are used to promote wound healing, including cell therapy, gene therapy, growth factor delivery, wound dressings, and skin grafts. However, none of these modalities is effective for all kinds of wounds [5]. Moreover, most sophisticated approaches are expensive and not readily available in developing countries. As a result, it is essential to create newer and creative therapy methods for multifaceted therapeutic regimens for chronic wounds.

The use of herbal natural products as possible helpful agents in the process of wound healing has been the subject of an increasing number of research papers [6]. Indeed, the main benefits of using these herbal remedies are their low cost, availability, and minimal side effects. In this context, the efficacy of phytochemicals in the management of wounds is attributed to their antimicrobial, anti-inflammatory, and oxidant effect. Some phytochemicals also influence the mitogen-activated protein kinase (MAPK) and transforming growth factor-beta (TGF-) signalling pathways [7-11]. Examples of extensively studied phytochemicals for potential wound healing include flavonoids [12]. The wound-healing activity of other active ingredients such as alkaloids, saponins, and terpenoids was also reported [13].

Some quinoxaline derivatives reported to possess wound-healing potential [14]. Quinoxaline derivatives were patented for anticancer and antimicrobial activity [15]. Charles and Minakiri reported the natural presence of 2,3-dimethylquinoxaline (DMQ) (Fig 1) in the *Chromolaena odorata* plant [16]. They suggested that the observed antimicrobial activity of *Chromolaena odorata* could be attributed to the presence of DMQ. Alfadil and his colleagues confirmed that DMQ had a broad-spectrum antifungal activity [17].

The lipophilicity of DMQ was determined by the distribution coefficient ($logD_{7.4}$) between n-Octanol and phosphate buffer at pH 7.4. It was significantly (P <0.05) higher than tolbutamide and lower than ketoconazole. DMQ had a significant (P <0.05) high permeability compared to antipyrine, atenolol, talinolol, and estrone 3-sulfate. It showed a good safety profile in both in vitro and animal toxicity studies [18].

Therefore, the present pilot study aims to explore the potential efficacy of DMQ for accelerating skin wound healing.

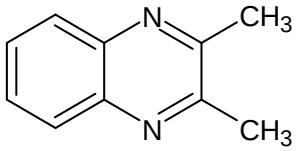


Fig. 1. 2,3-dimethylquinoxaline

2. MATERIALS AND METHODS

2.1 Materials

2,3-Dimethyquoxaline (DMQ), (Quinoxaline-2,3-dimethyl) (Cat. No. D184977; Chemical Abstracts Service, CAS #2379-55-7), Sigma. Hydroxypropyl methylcellulose (HPMC) and Methanol were pharmaceutical grades.

2.2 Methods

2.2.1 Preparation of DMQ hydrogel

The composition of plain HMPC (2 % w/w) 100 g Jell is 2 gm HPMC and distilled water 100 gm. DMQ is soluble in methanol (100 mg/mL), The plain jell was prepared as follows: 2 g of the polymer was weighed and placed in a suitable beaker, 98 g of water was added to it, the container was covered and left for 24 hours to form the gel.

2.2.2 Formulation of the medicated Jell. 1%w/w

0.5 gram of DMQ was dissolved in 4.5 g of methanol (final concentration 100 mg/ g)

The drug solution in methanol was added gradually to 45 grams of the plain HPMC jell, with continued gentle mixing. Both plain and medicated jell were stored in suitable jars and stored at room temperature. The stability of DMQ was insured by spectrophotometric analysis.

2.2.3 Animal study

Male Wistar rats weighing about 200 g were used. Animals were kept at a relative humidity range of 40–70% and a temperature of 22 ± 2 °C, under a 12/12 h light–dark cycle. The animal handling procedures were approved by the Committee of Research Ethics, Faculty of Pharmacy, KAU.

2.2.4 Skin irritation test

This experiment was previously done in a previously published study.

2.2.5 Wound excision

The rodents were sedated with ketamine (90 mg/kg). To disinfect their shaved skin on the dorsal area, 75% ethanol was used. This was followed by the excision of a 100 mm^2 region of skin on the rat's dorsal surface. After disinfecting the wounds, the pain was reduced by injecting 4 mg/kg of lidocaine hydrochloride (2%) with 1:80,000 epinephrine (SC).

Each rat was put in its cage and given standard feed pellets and water ad libitum. The rats were split into three groups at random as follows: Group 1: no procedure, control (n=2); Group 2: treated daily with plain hydrogel (vehicle n=3); and Group 3: treated daily DMQ hydrogel (n= 3). Treatment was continued for 7 days. On days 0 & 7, wound diameters (WD) were measured, and lesions were photographed. On day 7, one randomly select rat from each group was decapitated, and the wounded skin was dissected. A portion of the skin was preserved in 10% neutral formalin for histology study. The other portion was thoroughly rinsed with ice-cold saline then immediately frozen in liquid nitrogen and stored at -80^o C for analysis of biochemical and immunological and other biomarkers in skin tissue homogenate.

2.2.6 Wound measurement

The percentage of wound contraction was determined according to the following formula:

Wound contraction % = (WD on day 0 − WD on day 7 or 14)/(WD on day 0) × 100

2.2.7 Preparation of tissue homogenate

The frozen skin specimens were delicately blotted between filter papers and weighed. Then 10% (w/v) homogenates were prepared in phosphate-buffered saline (PBS, 50 mM potassium phosphate, pH 7.4, ice-cooled) before spinning at 3000 rpm for 20 minutes at 4^{o}C. Using the supernatant, specific parameters were estimated.

2.2.8 Biochemical analyses

The following biochemical parameters (oxidative stress) were assessed in skin tissue homogenate

Assessments of the total protein, CAT, SOD, GSH, and MDA were performed using biochemical ELISA kits (Cayman® Chemical, Ann Arbor, MI, USA). based on the instructions provided by the manufacturer.

2.2.9 Analyses of pre-inflammatory markers

The following pre-inflammatory markers were assessed in skin tissue homogenate using ELISA Kits: TNF-α, IL-6 IL-β1 and NF-κB. levels. based on the instructions provided by the manufacturer

2.2.10 Histological examination

Skin tissues were preserved in a 10% neutral formalin solution for 24 hours before being dehydrated, embedded in paraffin, and then sectioned in a paraffin block (5µ m thickness). Following dewaxing, tissues were rehydrated, stained with hematoxylin and eosin (H&E),

3. RESULTS

Wound contraction % was about 97 % after 7 days after treatment with DMQ 1 % Jell compared to about 84 % in the case of the plain hydrogel.

Macroscopic wound closure at 0, 7 days after treatment with plain and DMQ 1 % Jell is presented in Fig 2. Almost complete wound healing was observed after treatment with DMQ 1 % Jell. Treatment with plain hydrogel was associated with a reduction in wound size but incomplete healing.

Micrographs of H&E-stained wounded tissues of Wister rats are presented in Fig. 3. The histological study confirmed that treatment with DMQ 1 % Jell for 7 days lead to marked attenuation of wound-induced histological changes of the skin and documented as the resolution of neutrophile infiltration that was still observed in the skin of rats treated with plain jell.

A panel of biochemical markers of oxidative stress markers in skin tissue homogenate is presented in Table 1. These data show a slight decrease in GSH and an increase in SOD levels after treatment with DMQ 1 % Jell but the level of CAT & MDA are similar to those after treatment with plain hydrogel.

A panel of inflammatory markers in skin tissue homogenate is presented in Table 2: There was a marked reduction of the level of all tested parameters (TNF-α, IL-6 IL-β1 and NF-κB.) after treatment with DMQ 1 % Jell, These data suggest a potential anti-inflammatory effect of DMQ.

Hydroxyproline and TGFB1 in skin tissue homogenate are presented in Table 3. The level of both Hydroxyproline and TGFB1 was increased after treatment with DMQ 1 % Jell suggesting an acceleration of wound proliferation.

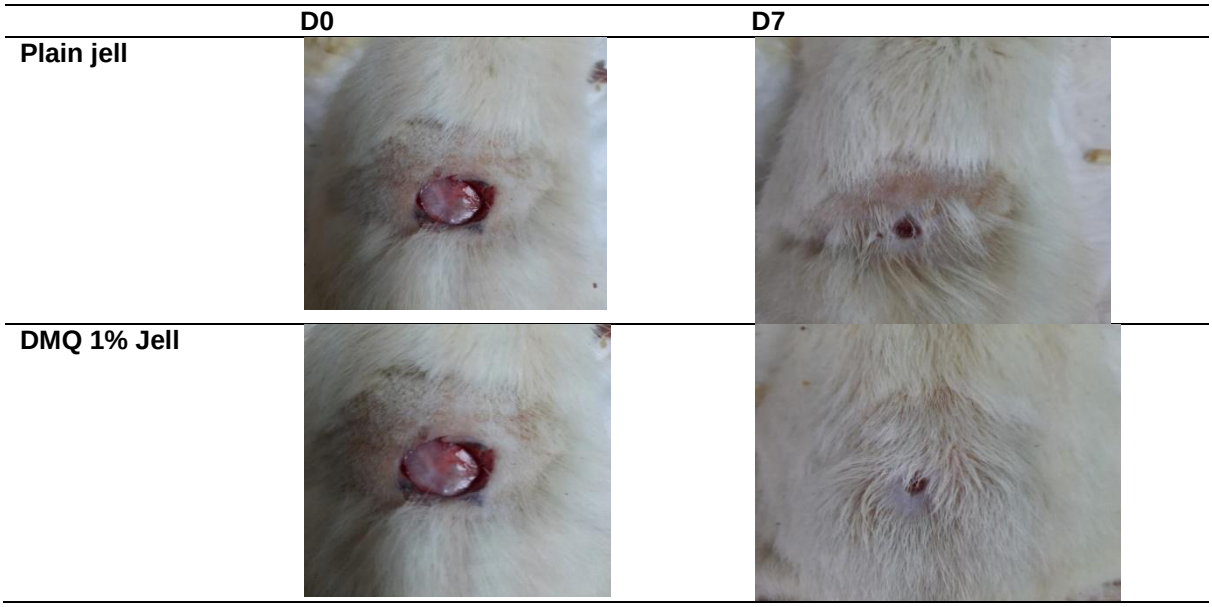
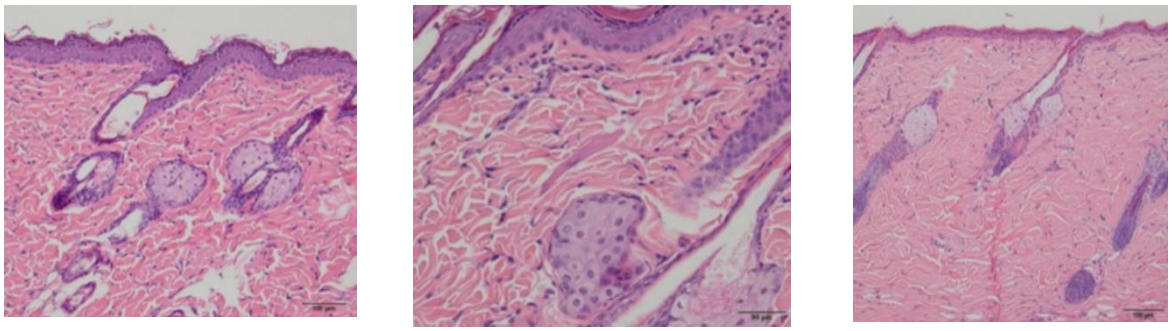


Fig. 2. Wound healing evaluation in Wistar albino rodents with complete thickness excision wounds. Macroscopic wound closure at 0, 7 days after treatment with plain and DMQ 1 % Jell



A B C

Fig. 3. Micrographs of H&E-stained wounded tissues of wister rats
A: control, B plain jell treated, C: 1 % DMQ Jell treated after 7 days of treatment

Table 1. Panel of Biochemical markers of oxidative stress markers in skin tissue homogenate

Groups	SOD	CAT	GSH	MDA
	u\ml	u\ml	ng\ml	nmol\ml
DMQ 1%	68	122	2.9	2.00
Plain jell	80	121	4.0	1.98

Table 2. Panel of inflammatory markers in skin tissue homogenate

Groups	TNF-a	IL-6	IL-B1	NF-Kb
	pg\ml	pg\ml	pg\ml	ng\ml
DMQ 1%	56	16.3	38	44
Plain jell	82	18	56.2	67

Table 3. Hydroxyproline and TGFB1 in skin tissue homogenate in skin tissue homogenate

Groups	hydroxyproline	TGFB1
	pg/ml	pg/ml
DMQ 1%	1.5	10.4
Plain jell	0.94	7.8

4. DISCUSSION

Wound healing is an optimized biological mechanism that occurs in a series of contiguous stages that include hemostasis, inflammatory scenarios, the proliferation of cells, and tissue remodelling [19,20].

The present pilot study suggested the potential healing properties of topically applied DMQ. The exact mechanism needs more detailed studies. However, currently, limited data suggest that its anti-inflammatory effect is likely involved in its obvious ability to accelerate skin wound healing in healthy rats.

It has been established that reactive oxygen species (ROS) play an important part in several stages of healing. ROS are fundamental to all wound healing processes because low levels of ROS production are required for the fight against invading microorganisms and cell survival signalling. However, excessive ROS generation or impaired ROS detoxification causes oxidative damage, which is the primary cause of chronic wounds that do not resolve. In this context, experimental and clinical research has shown that antioxidant and anti-inflammatory strategies can help to speed up wound healing [21]. As a result, various antioxidant approaches help improve wound healing [22-24].

Inflammation is a physiological process in the healing process [25]. However, prolonged amounts of ROS production in the wound can lead to chronic inflammation which unfavourably affects wound healing. The inflammation begins with the neutrophil influx, which is aided by mast cell histamine production. The neutrophils promote the release of pro-inflammatory cytokines (e.g., TNF- α, IL-1, and IL-6), phagocytosis, and protease secretion, which contribute to the attraction of other inflammatory cells, the amplification of the inflammatory response, the death of microbial pathogens, and the stimulation of regenerative and remodelling factors [26].

Interleukin IL-6 (a proinflammatory cytokine)is essential for wound healing and plays an important part in acute inflammation. It is released early in reaction to injury and induces the release of proinflammatory cytokines from tissue-resident macrophages, keratinocytes, endothelial cells, and stromal cells [27]. The present study showed a moderate reduction of IL-6 in the skin tissues of rats treated with DMQ hydrogel

Hydroxyproline, a non-essential amino acid, is necessary for the production of collagen and the thermodynamic equilibrium of collagen's triple-helical conformation and associated tissues. Its low amount indicates poor wound healing [28]. Transforming growth factor-beta 1 (TGF-β 1) is a multifunctional cytokine implicated in a variety of human illnesses. It is thought to play an essential role in wound healing and repair because it is a key regulator of extracellular matrix production and remodelling via its impact on mesenchymal cells [29-31]. In the present pilot study, an increase in tissue content of Hydroxyproline, and TGF B1 was noticed after treatment with DMQ hydrogel.

Several pharmacological approaches for the management of wounds target the inflammatory response [32]. Certain phytochemicals and natural products can modulate inflammation through a variety of pathways, including the regulation of growth factors and cytokines. Examples of topical application of natural compounds for enhancing wound healing include aloe vera [33,34], It has a broad spectrum of effects that synergistically enhance wound healing. One of these effects is its capacity to suppress inflammatory responses by inhibiting IL-6 and IL-8, reducing leukocyte adhesion, increasing IL-10 levels, and decreasing TNF- α levels [35]. Another example is curcumin which works by decreasing TNF- α, IL-1, and MMP-9 expression while increasing anti-inflammatory cytokine IL-10 and antioxidant enzymes at the lesion site [36].

5. CONCLUSION

DMQ has potential skin wound healing ability likely due to its anti-inflammatory mechanism. Further study is needed to confirm these preliminary findings and to explore the molecular mechanism.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable (The Biomedical Ethics Committee approved the experimental protocol at King Abdulaziz University, KAU and the National Committee of Bioethics, NCBE, Registration No (PH-1443-76).

All experiments have been examined and approved by the appropriate ethics committee. Animal handling was performed in strict compliance with the ethical guidelines for treating animals as defined by KAU and NCBE

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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