Research Paper

Anti-Inflammatory Activity of 3-Hydrazinoquinoxaline-2-Thiol Topical Gel on Carrageenan-Induced Paw Edema in Wistar Rats

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Abdelbagi *et al*.: Anti-Inflammatory Effects of 3-Hydrazinoquinoxaline-2-Thiol in a Rat Paw Model

Inflammation, an adaptive response bring about by factors like infections, harmful chemicals, and immune reactions, induces the release of inflammatory mediators. These mediators play a role in significant protein denaturation and aggregation, which exacerbates the inflammatory condition. The existing anti-inflammatory medications, including non-steroidal anti-inflammatory drugs, come with undesirable side effects. Thus, there is a need to explore alternative therapeutics that offer anti-inflammatory benefits similar to non-steroidal anti-inflammatory drugs but with fewer adverse effects. Quinoxaline derivatives, categorized as small molecules, have exhibited promising anti-inflammatory activity and stand out as a potential avenue for further exploration. The objective of this study was to examine the inflammatory activity of 3-hydrazinoquinoxaline-2-thiol in a rat paw model and compare it to the effects of diclofenac. The *in vivo* activity of 3-hydrazinoquinoxaline-2-thiol was assessed using a rat paw model. The study employed histopathological examination and enzyme-linked immunosorbent assay as investigative methods. The anti-inflammatory efficacy of 3-hydrazinoquinoxaline-2-thiol was evident in the rat paw model, exhibiting a significant difference between the group treated with 3-hydrazinoquinoxaline-2-thiol and the positive control. Furthermore, no significant difference was observed between the group treated with 3-hydrazinoquinoxaline-2-thiol and the diclofenac group. Remarkably, the administration of 3-hydrazinoquinoxaline-2-thiol therapy resulted in a notable improvement in the inflammatory response, characterized by enhancements in inflammatory cell activity and the restoration of histological structures to a normal state. Suggesting that 3-hydrazinoquinoxaline-2-thiol has a good anti-inflammatory effect. The results of the study indicate that the topical administration of a hydrogel incorporating 0.2 % 3-hydrazinoquinoxaline-2-thiol (quinoxaline derivative) has proven to be successful in mitigating acute inflammation in the rat paw model. Subsequent research endeavors should delve into investigating different dosages of quinoxaline derivative with the aim of formulating a cost-effective and less toxic anti-inflammatory medication, potentially enhancing its utility in clinical settings.

Key words: Inflammation, inflammatory mediators, non-steroidal anti-inflammatory drugs, 3-hydrazinoquinoxaline-2-thiol, rat paw, diclofenac, quinoxaline derivative

Inflammation represents an adaptive physiological response triggered by various factors, including infections, exposure to harmful chemicals, and immune reactions[1]. This response is initiated in the presence of tissue damage and manifests through observable symptoms such as swelling, redness, warmth, and pain, potentially leading to a decline in tissue functionality[2]. The inflammatory process involves the dilation of blood vessels and heightened activity of white blood cells, accompanied by the release of inflammatory mediators[3]. If left unaddressed, acute inflammation has the potential

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to evolve into chronic inflammation, a persistent and often prolonged state[4]. Chronic inflammation is closely linked to various health conditions, including diabetes, cardiovascular dysfunctions, cancer, and arthritis, but not limited to these[5]. The prolonged activation of the immune system and the sustained release of inflammatory mediators characterize chronic inflammation, contributing to the pathogenesis of these diverse and often serious medical conditions[5]. Recognizing the transition from acute to chronic inflammation is crucial for understanding the underlying mechanisms of these health issues and developing targeted interventions for their prevention and management[4].

The process of inflammation triggers the release of inflammatory mediators, encompassing Interleukin-1Beta (IL-1β), IL-6, C-Reactive Protein (CRP), and Tumor Necrosis Factor-Alpha (TNF-α). Notably, these mediators are found in both acute and chronic inflammatory conditions. Their presence plays a pivotal role in inducing substantial protein denaturation and aggregation, thereby amplifying the inflammatory response[6]. Additionally, inflammation sets in motion oxidative stress, which, in turn, serves as a catalyst for the escalation of protein denaturation. This interconnected relationship underscores the complexity of the inflammatory cascade, highlighting the multifaceted impact that inflammatory mediators and oxidative stress collectively exert on the progression and perpetuation of inflammation[7]. In the broader context, inflammation and its repercussions can set in motion a self-perpetuating cycle of deteriorating health conditions. The key strategy for effectively managing both acute and chronic inflammation is to identify and isolate phytochemicals with antioxidant properties and the ability to inhibit protein denaturation. These phytochemicals hold significant therapeutic promise in addressing the complexities of inflammatory processes[8]. An integral aspect of the inflammatory pathway involves Cyclooxygenase (COX), a key enzyme in prostaglandin biosynthesis, with two distinct isoforms, COX-1 and COX-2[9]. Conventional Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) function as non-selective inhibitors of these enzymes. Furthermore, selective COX-2 inhibitors are employed in the treatment of inflammatory diseases, aiming for a more targeted approach[10,11]. Another class of drugs, glucocorticoids, is frequently utilized for managing inflammation. Despite the associated side effects, glucocorticoids play an indispensable role in mitigating inflammation. Breaking the cycle of inflammation-related deterioration involves a multifaceted approach, including the exploration of phytochemicals with specific properties, the targeting of COX isoforms, and the cautious use of glucocorticoids. This comprehensive strategy is vital for addressing the intricate dynamics of inflammation and fostering effective therapeutic interventions[12]. In drug development, molecular design aims to create innovative compounds characterized by optimal efficacy and minimal side effects. The foundational quinoxaline scaffold plays a pivotal role in the synthesis of substances boasting diverse biological activities. Quinoxalines have been recognized for a spectrum of functions, encompassing antimicrobial[13], anti-inflammatory[14], bactericidal, bacteriostatic[15], anticonvulsant[16], Central Nervous System (CNS) depressant, antitumor[17], antioxidant[18], and neuroprotective properties[19].

The synthesis of quinoxalines can be achieved through various routes, and they are alternately referred to by different names such as 1,4-benzodiazine, benzopyrazine, and 1,4-diazonaphthalene[20,21]. Over the years, dedicated efforts within the domains of organic and medicinal chemistry have been consistently directed toward the design, synthesis, and evaluation of molecules specifically tailored to exhibit anti-inflammatory properties. This persistent exploration reflects a commitment to advancing the understanding of molecular structures that can contribute to the development of therapeutics with targeted anti-inflammatory effects[22].

The importance of quinoxaline molecules in medical research cannot be overstated. In light of the pressing need for alternative treatment modalities, our research is centered on a particular Quinoxaline Derivative (QD) (3-hydrazinoquinoxaline-2-thiol). This specific derivative, denoted as QD and characterized by the molecular formula $C_8H_8N_4S$, is the focal point of our investigation. The primary aim of our study is to explore the potential anti-inflammatory effects of QD in a rat model and compare them to those of diclofenac. Additionally, we conducted a comprehensive assessment of the compound's *in silico* pharmacokinetic properties, scrutinizing aspects such as lipophilicity, skin permeation, and its overall toxicity profile. Through this evaluation, our intent is to gauge and appraise the therapeutic viability of QD.

MATERIALS AND METHODS

Chemicals:

The compound with the molecular formula $C_8H_8N_4S$, identified by the Chemical Identifier (CID) 781248 and coded as QD, as well as carrageenan, was procured commercially from Sigma Chemical in Munich, Germany. Diclofenac gel, specifically Voltaren Emulgel 1 % 50 g, was obtained from a local pharmacy. The ketamine injection, supplied by Hikma Pharmaceuticals PLC at a concentration of 10 mg/ml (10 ml), was sourced from the pharmacy department at King Abdul-Aziz University Hospital (KAUH). Additionally, xylazine hydrochloride was utilized in the study. It is imperative to note that all solvents and reagents used in the experimentation were of pharmaceutical grade, ensuring the highest quality and standards for the research.

Preparation of plain and medicated hydrogel:

The formulation of the unmedicated Hydroxypropyl Methylcellulose (HPMC) gel at a concentration of 2 % w/w, totaling 100 g, commenced by dissolving 2 g of HPMC in an adequate amount of distilled water to achieve the desired weight. The meticulous procedure involved precisely measuring 2 g of HPMC, transferring it to a suitable beaker, and adding 98 g of distilled water. This mixture was covered and allowed to sit undisturbed for a duration of 24 h, facilitating complete hydration of HPMC and the formation of a uniform gel. In the case of the medicated gel, which incorporated the QD at 0.2 % w/w, the formulation process was executed with precision. Initially, 20 mg of QD was dissolved in 1 g of Dimethyl Sulfoxide (DMSO). Subsequently, this solution was judiciously integrated into 9 g of the previously prepared plain HPMC gel with a continuous and gentle stirring mechanism to ensure a homogeneous distribution of the medication within the gel matrix. Both the unmedicated and QD-infused gels were then carefully transferred into suitable containers and stored under refrigeration at a temperature of 8° to preserve their stability and efficacy. It is noteworthy that these procedures adhere to established compounding standards prevalent in the United States Pharmacopeia (USP) references for pharmaceutical formulations[23,24].

Animals:

For this investigation, a total of 24 adult male Wister albino rats, weighing between 150 g and 200 g, were procured from the Faculty of Pharmacy at King Abdul-Aziz University. The rats were acclimated to standard laboratory conditions, provided with a regular laboratory diet, and had unrestricted access to water. Their housing environment adhered to a 12 h light-dark cycle, and the room temperature was consistently maintained between 22° and 25° within the animal house. Ethical considerations were paramount in the execution of all animal experiments. The biomedical ethics committee, having reviewed and approved the experimental protocol at the Faculty of Pharmacy, King Abdulaziz University, granted approval under the reference number (PH-1443-76). This ensured that the research involving the use of these animals was conducted in accordance with established ethical standards and guidelines.

Experimental design:

The experimental design involved the categorization of rats into four distinct groups, each comprising six animals (n=6), as follows: Group A served as the healthy control and received a subcutaneous injection of normal saline solution. Group B received carrageenan to induce inflammation and were treated with plain HPMC gel. Group C also received carrageenan and was subsequently treated with a gel containing 0.2 % w/w of the QD; and group D received carrageenan followed by treatment with a gel containing 1 % w/w of the anti-inflammatory drug diclofenac. This design aimed to evaluate the comparative efficacy of the treatments in mitigating inflammation induced by carrageenan.

To ensure the well-being and comfort of the animals during the procedures, all rats were anesthetized using a combination of xylazine hydrochloride (3 mg/kg) and ketamine hydrochloride (50 mg/kg) administered intraperitoneally. The control group received a subcutaneous injection of 0.2 ml saline on the plantar surface of the right hind paw. Groups B, C, and D received a similar injection of 0.2 ml carrageenan (1 % solution in isotonic saline) in the same location to induce inflammation, following the methodology outlined. 1 h prior to the induction of inflammation, the respective gels (plain, QD, or diclofenac gels) were applied to the hind paws of the rats in Groups B, C, and D. The experiment concluded 4 h after the carrageenan injection. The rats were then anesthetized, euthanized, and their right hind paws were collected. Paw collection involved cutting the paws at the proximal end of the lateral malleolus. For subsequent histological examinations, portions of

the tissue were preserved in a 10 % formalin solution for 24 h. Additionally, for biochemical analyses, other tissue samples were rinsed with ice-cold buffer, rapidly frozen in liquid nitrogen, and stored at -80° until further analysis[25,26].

Quantification of chosen inflammatory markers using an Enzyme-Linked Immunosorbent Assay (ELISA) methodology:

In the evaluation of inflammation, we employed ELISA immunoassay kits, specifically utilizing the ELISA inos kit. This kit comprised ELISA plates that were pre-coated with monoclonal antibodies designed to target the specific cytokine under investigation. The methodology employed a quantitative sandwich enzyme immunoassay technique. To precisely determine the levels of the selected inflammatory marker, standards, and samples were added to the wells of the ELISA plate. Following this, any unbound substances in the wells were removed using a wash buffer. Subsequently, an enzyme-linked polyclonal antibody specifically designed to target the cytokine of interest was introduced into the wells. After the addition of the enzyme-linked antibody reagent, washing steps were performed to eliminate any unbound antibodies. Finally, a substrate solution was introduced into the wells, and the development of color was terminated. The intensity of the resulting color was directly proportional to the quantity of the targeted cytokine that had bound, and this was quantified using a microplate reader[27].

Histopathological study:

Upon completion of the 7 d regimen and the administration of the final dose of the anti-inflammatory agent or saline solution, the animals were humanely euthanized. Euthanasia was carried out using an overdose of a ketamine-xylazine cocktail administered *via* intraperitoneal injection, followed by cervical dislocation. The tongues were extracted and fully immersed in Bouin solution for a minimum of 48 h to achieve fixation. Subsequently, the tongues were collected from the euthanized animals, fixed in a 20 % formalin solution, and embedded in paraffin. Thin 5 µm sections were meticulously prepared using a Leica microtome (Leica Microsystems Inc., Buffalo, NY, United States of America (USA)). These sections underwent staining with Hematoxylin and Eosin (H&E) as well as Periodic Acid-Schiff (PAS) stains to facilitate examination under a light microscope (Olympus Optical Co., Ltd., Japan) for comprehensive histopathological analysis[26].

Statistical analysis:

Statistical analysis for this study was conducted using GraphPad Prism. Each experiment was replicated at least twice, and average values were calculated. Unpaired t-tests were employed to evaluate significant differences and perform statistical comparisons across the experimental groups. A significance level of 0.05 was employed to determine statistical significance, with values equal to or less than 0.05 considered significant. The p-values were represented as follows: *$p<0.05$, **$p<0.01$, ***$p<0.001$ and ****$p<0.0001$.

RESULTS AND DISCUSSION

In our pursuit to comprehend the clinical manifestations associated with concurrent inflammation in the rat paw, we initiated a comprehensive examination utilizing histopathological analysis of tissue specimens. The histological landscape of the rat paw is distinctly characterized by identifiable tissue lesions, discernible in sagittal sections. These lesions exhibit a pathological spectrum, including prominent edema and a moderately diffuse infiltration of inflammatory cells, particularly neutrophils. Additionally, moderate lesions were observed, illustrating mild edema and severe diffuse infiltration of inflammatory cells, accompanied by a notable presence of neutrophils.

Significantly, upon the administration of 3-hydrazinoquinoxaline-2-thiol to the rat, histopathological slides unveiled notable enhancements in tissue conditions. These improvements were conspicuous, manifesting as a marked amelioration evidenced by a diminished inflammatory reaction and a reduction in infiltrated inflammatory cells, ultimately showcasing a restoration to a histological structure indicative of normalcy. This evidence underscores the positive impact of intervention or treatment in the restoration of tissue integrity, emphasizing the potential therapeutic efficacy of 3-hydrazinoquinoxaline-2-thiol in mitigating inflammatory responses in the rat paw model (fig. 1).

In a rat model, we evaluated the *in vivo* efficacy of QD in treating carrageenan-induced inflammation. Administering QD at concentrations (0.2 mg/ml) led to a notable decrease in TNF-α levels compared to the untreated infected rat group, with a p-value below 0.05. Additionally, a significant difference was observed between rats treated with diclofenac

and those treated with QD. This indicates that QD exhibits anti-inflammatory effects akin to diclofenac against TNF-α, underscoring its potential to regulate immune responses during inflammation *in vivo*. Furthermore, a noteworthy decrease in IL-6 levels was evident in the QD-treated group compared to the untreated infected rat group, with a p-value below 0.05. Interestingly, the reduction in IL-6 levels was more pronounced in rats treated with diclofenac compared to those treated with QD. However, no significant difference was observed between these treated groups. Similarly, we observed a parallel outcome for IL-6 in the QD treated group. The decrease in IL-1β levels was apparent in the QD treated group compared to the untreated infected rat group, with a p-value below 0.05. Notably, the reduction in IL-1β levels was more pronounced in rats treated with diclofenac compared to those treated with QD. Nonetheless, no significant difference was observed between these treated groups.

Also, in a rat model, we conducted an assessment of the *in vivo* efficacy of QD in treating carrageenan-induced inflammation. The administration of QD at concentrations (0.2 mg/ml) resulted in a significant reduction in COX levels compared to the untreated infected rat group, with a p-value below 0.05. Notably, there was no significant difference observed between rats treated with diclofenac and those treated with QD. This suggests that QD possesses anti-inflammatory effects similar to diclofenac against COX, highlighting its potential to modulate immune responses during inflammation *in vivo*.

A noteworthy decrease in inducible Nitric Oxide synthase (iNOs) levels was observed in the QD-treated group compared to the untreated infected rat group, with a p-value below 0.05. Remarkably, the reduction in iNOs levels was more pronounced in rats treated with diclofenac compared to those treated with QD. Importantly, a significant difference was observed between these treated groups. A significant reduction in Interferon-Gamma (IFN-γ) levels was observed among the group treated with QD in comparison to the untreated infected rat group, with a p value below 0.05. Notably, the reduction in IFN-γ levels was more pronounced in rats treated with diclofenac compared to those treated with QD. However, there was no significant difference observed between these treated groups. This suggests that QD demonstrates anti-inflammatory effects comparable to diclofenac across various inflammatory markers, emphasizing its potential to modulate immune responses during inflammation *in vivo* (fig. 2).

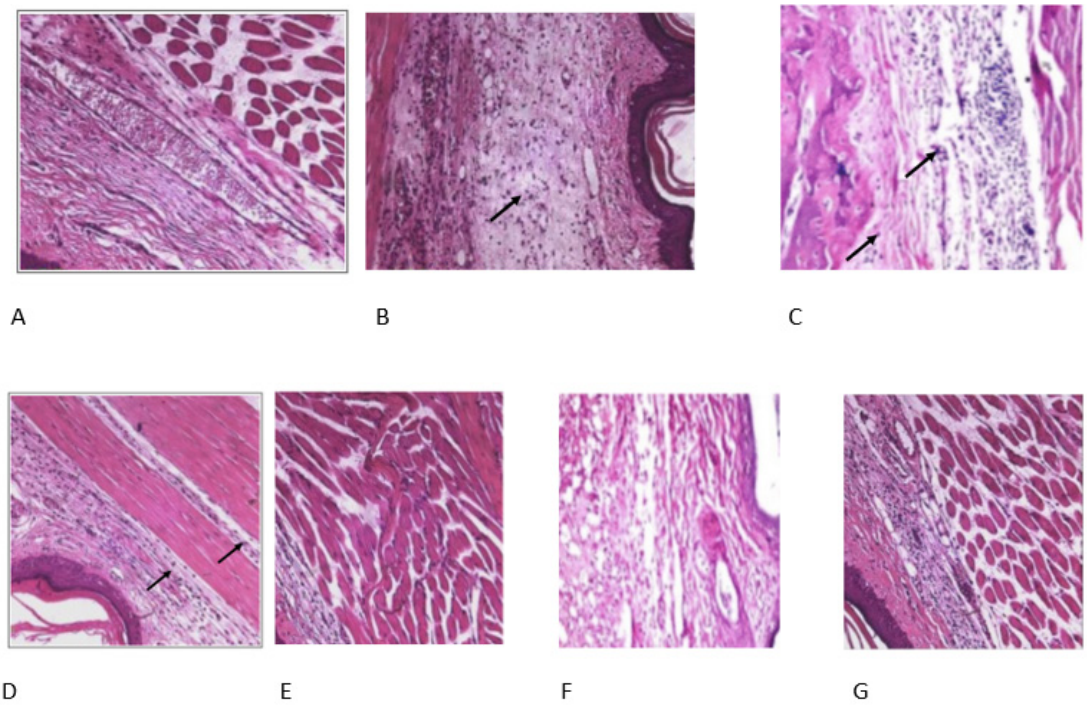

Fig. 1: Utilizing photomicrographs of H&E stained sections from rat paws, the histological examination reveals distinct observations. A: Depicts a control rat displaying a histologically normal structure in the paw tissue; B and C: The positive control exhibits pronounced edema and moderate diffuse infiltration of inflammatory cells, notably including neutrophils (indicated by black arrows); D: Illustrates mild edema and severe diffuse infiltration of inflammatory cells, along with some neutrophils (black arrows); E and F: Collectively showcase a marked improvement, evidenced by a decreased inflammatory reaction and reduced infiltrated inflammatory cells and G: Displays a return to a normal histological structure, indicating the positive impact of intervention or treatment in restoring tissue integrity

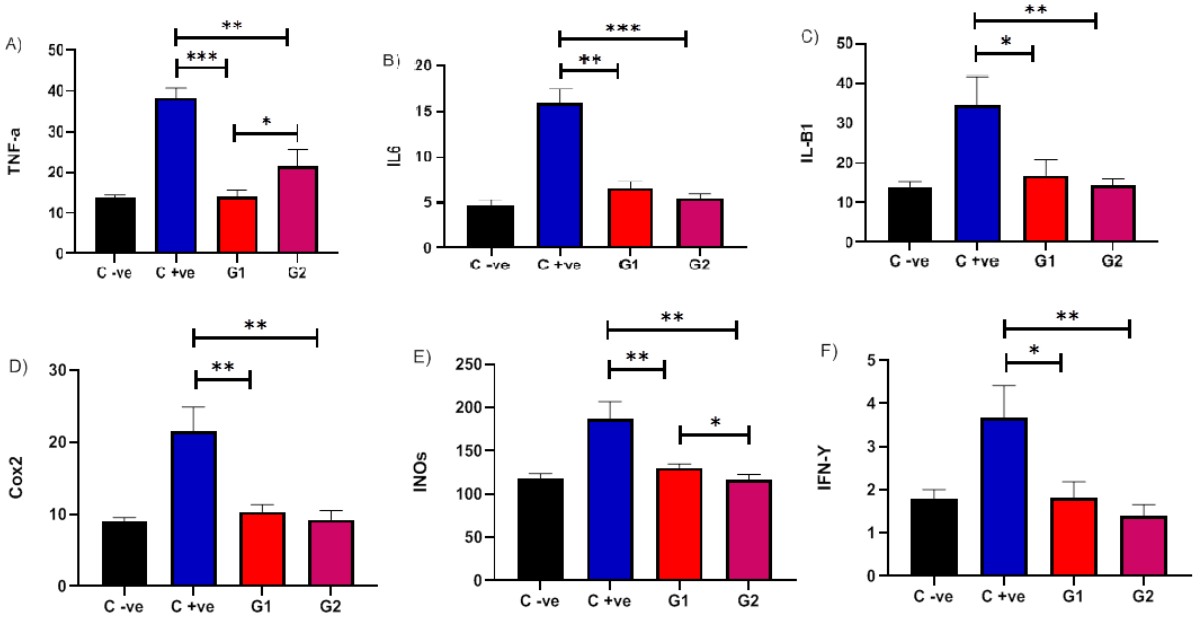

Fig. 2: The *in vivo* effectiveness of 3-hydrazinoquinoxaline-2-thiol using rat model as anti-inflammatory agent. The reduction was statistically significant, with a p-value below 0.05. G1 represents group received 0.2 mg/ml, G2 represents group received diclofenac. A: TNF-α; B: IL-6; C: IL-1β; D: Cox2; E: iNOs and F: IFN- γ
Note: (■) C^{-ve}; (■) C^{+ve}; (■) G1 and (■) G2

Inflammation and pain persist as significant global issues, adversely affecting the overall well-being of numerous individuals worldwide. Moreover, the financial strain imposed by the high costs of pain inhibitors, including pain rehabilitation programs for chronic pain, exacerbates the challenge[28]. Presently, the existing medications for addressing inflammation and pain encompass NSAIDs and antinociceptive drugs. Although, these treatments frequently come with numerous adverse and toxic side effects. In cases where acute inflammation remains unresolved, a range of health complications may arise, potentially disrupting the normal functioning of the body's immune system. This can lead to the development of chronic inflammatory conditions such as rheumatoid arthritis and osteoarthritis[29]. Therefore, it is crucial to explore alternative therapeutics characterized by minimal side effects from a scientific standpoint. In a groundbreaking development, we have successfully showcased the anti-inflammatory effects of QD for the first time in a rat model. Histopathological analysis revealed a notable enhancement in the inflammatory response following QD therapy, characterized by increased inflammatory cell activity and the restoration of histological structures to normalcy. Additionally, we have presented evidence indicating that the inflammatory markers nearly reverted to baseline levels comparable to the negative control, with no statistically significant differences observed between the rats treated with QD and the negative control group. Furthermore, our findings revealed that the effectiveness of QD in reducing inflammatory markers is nearly equivalent to that of diclofenac, as no statistically significant distinctions were recorded between the two treatment groups. Notably, we observed that QD exhibited superior efficacy in reducing TNF-α compared to rats treated with diclofenac. Conversely, the reduction in iNOS was more pronounced in the group treated with diclofenac compared to the cohort treated with QD.

The exploration of novel anti-inflammatory therapies with minimal side effects presents an intriguing avenue for the development of new medicines. This approach is not only attractive but also imperative for addressing the limitations associated with existing treatments[30]. It has been reported that the methanol leaf extract derived from *Ximenia caffra* (*X. caffra*) has been demonstrated to exert inhibitory effects on the mRNA expression of pro-inflammatory genes, specifically IL-6, iNOS, and TNF-α, as assessed through Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR) *in vitro*. Notably, this extract exhibited a remarkable dose-dependent reduction of approximately 60 % in Nuclear Factor Kappa B (NF-κB) transcriptional activity compared to the control. Moreover, the extract elicited a substantial decrease in the expression of IL-6, reaching an almost 100 fold reduction when compared to untreated cells induced with lipopolysaccharide[31]. In a separate study,

Aspalathus linearis, commonly known as rooibos tea, demonstrated significant efficacy in decreasing TNF-α and IL-6 levels in the liver of mice, as highlighted by Oguntibeju *et al.*[32]. These findings underscore the potential anti-inflammatory properties of *X. caffra* and *Aspalathus linearis*, suggesting their promising roles as therapeutic agents in modulating inflammatory responses[31,32]. Our results are consistent with prior studies, the imperative to scrutinize and establish the safety profile of QD remains a pivotal aspect of its evaluation. This careful examination contributes to the broader landscape of medical knowledge, enhancing our understanding of the potential benefits and risks associated with this medicine and ultimately fostering a safer and more effective therapeutic approach.

Emerging scientific investigations have brought to light a recently identified group of compounds, namely 4-alkoxy-6,9-dichloro triazolo[4,3-a]quinoxalines, showcasing notable inhibitory capabilities against pro-inflammatory cytokines, particularly TNF-α and IL-6. The discernment of this inhibitory effect hints at a conceivable correlation between the efficacy of quinoxalines as anti-inflammatory agents and their specific targeting prowess directed at these cytokines. This revelation underscores the potential significance of QD in modulating inflammatory responses through the precise inhibition of key mediators like TNF-α and IL-6, thus presenting a promising avenue for further exploration in the realm of anti-inflammatory therapeutics[14]. The preceding investigation aligns with our own research findings, wherein we demonstrated the capacity of the QD to diminish inflammatory markers, encompassing but not limited to COX, IFN-γ, IL-6, IL-1β, iNOS and TNF-α. This evidence strongly implies an anti-inflammatory effect exerted by QD. However, a comprehensive exploration of the underlying mechanistic pathways and safety profiles is imperative to validate and elucidate the potential therapeutic implications of QD in the context of inflammation.

Topical drug administration targets localized areas through routes such as ophthalmic, rectal, vaginal, and skin applications. The skin, being easily accessible, is the primary route for delivering medications, especially for treating fungal infections like athlete's foot and ringworm. It has been shown that miconazole, an imidazole antifungal, effectively treats both local and systemic fungal infections, but its oral use is limited due to significant side effects. To enhance patient compliance, reduce dosage, and minimize side effects, a topical gel formulation of miconazole was developed. This formulation was characterized for drug content, pH, viscosity, diffusion, antifungal activity, and skin irritation, with the F1 formulation identified as the best[33]. Topical drug administration provides a localized method for delivering medication through various routes, including ophthalmic, rectal, vaginal, and skin applications. Among these, the skin is the most accessible and commonly used route for topical therapies, especially for treating dermatological conditions such as fungal infections, including athlete's foot, ringworm, and candidiasis. Miconazole, an imidazole antifungal agent, is effective in treating both local and systemic fungal infections. However, the oral administration of miconazole is often associated with undesirable side effects, such as liver and kidney damage. To address these issues and improve patient outcomes, a topical gel formulation of miconazole has been developed. This approach aims to provide a more effective and targeted delivery of the antifungal medication directly to the affected area. The gel formulation offers several advantages; it enhances patient compliance by making the application process more convenient and less intrusive compared to oral medications. Additionally, it allows for a reduced dosage of the drug while minimizing systemic side effects, which are often a concern with oral administration[33]. This could enhance using gel formulation it may represent a significant advancement in topical antifungal therapy. It enhances the drug's delivery to the site of infection, reduces the required dose, and minimizes the risk of systemic adverse effects, making it a promising approach for more effective and safer treatment of fungal infections. Another study has shown possible novel antifungal agents. Study focused on developing a topical hydrogel formulation of oxiconazole nitrate, an antifungal agent. It demonstrated good antifungal activity with a 20 mm zone of inhibition for *Aspergillus Niger*[34].

For the first time, the study's outcomes indicate that the topical application of a hydrogel containing 0.2 % QD has demonstrated efficacy in mitigating acute inflammation in a rat paw model. This anti-inflammatory effect is likely attributable to the inhibition of the inflammatory marke such as iNOS and COX. As a result, QD emerges as a promising candidate for a potent anti-inflammatory agent. Nevertheless, further in depth investigations into the underlying mechanisms are essential to substantiate these initial observations. Future research endeavors

should delve into exploring different dosages of QD with the aim of developing an economically viable and less toxic anti-inflammatory medication, potentially yielding greater clinical benefits.

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Conflict of interests:

The authors declared no conflict of interests.

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