

Therapeutic and technological potential of 7-chloro-4-phenylselanyl quinoline for the treatment of atopic dermatitis-like skin lesions in mice

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ABSTRACT

This study investigated the main effects of the oral treatment with 7-chloro-4-phenylselanyl quinoline (4-PSQ) on symptoms, inflammatory and oxidative parameters in an atopic dermatitis (AD) model in BALB/c mice. In addition, the possibility of antioxidant property of 4-PSQ improves the potential of a biofilm (based on chitosan/poly(vinyl alcohol) (PVA)/ bovine bone powder (BBP)) for the treatment of AD-like skin lesions was evaluated. 2,4-Dinitrochlorobenzene (DNCB) was applied to the dorsal skin on days 1–3 for sensitization. Mice were challenged with DNCB on the ear (on days 14–29) and dorsal skin (on days 14, 17, 20, 23, 26, and 29) and treated with 4-PSQ, dexamethasone, biofilm (biofilm sample without 4-PSQ) or 4-PSQ-loaded biofilms. On the day 30, skin severity scores and scratching behavior were determined. After that, animals were sacrificed, and ears and dorsal skin were removed for determination of inflammatory and oxidative parameters. DNCB induced the skin lesions, scratching behavior and ear swelling, increased myeloperoxidase (MPO) activity (ear and back) and reactive species (RS) levels (back). 4-PSQ, 4-PSQ-loaded biofilms and biofilm treatments ameliorated skin severity scores, scratching behavior and inflammatory response induced by DNCB. 4-PSQ and 4-PSQ-loaded biofilm treatments partially protected against the increase in the RS levels induced by DNCB. Our results revealed that the incorporation of 4-PSQ improved the therapeutic effect of the biofilm. The efficacy of 4-PSQ in treating AD-like lesions was similar or better than dexamethasone. In summary, 4-PSQ has a potential therapeutic advantage in the treatment and management of AD.

1. Introduction

Atopic dermatitis (AD) is a common chronic inflammatory skin disease that is characterized by intense itching and recurrent eczematous lesions. Patients with AD suffer from severe psychological stress, which markedly increases the prevalence rate of depression and anxiety disorders in later life [1]. The manifestation of AD is rising dramatically, especially in developed countries, and it now affects up to 30% of children and 10% of adults [2–4]. AD attacks are characterized by wound with intensely pruritic erythematous papules associated with excoriation and serous exudates. The skin affected by AD has redness and itching in the responsive skin, as well as excessive scratching can cause skin wounds and fluid leakage [5].

Treatment of AD consists mainly of steroids, such as

dexamethasone, applied to the skin [6]. However, steroids are immunosuppressants and increase the risk of bacterial infection [7]. Furthermore, long-term use of topical steroids also leads to thinning of the skin and to cracking and bleeding [8]. Therefore, these numerous side effects have disadvantaged long-term use of therapies used to AD [9]. In this way, there is a great need to develop new and effective treatment and strategies for AD.

Quinolines are synthetic or natural heterocyclic compounds with interesting biological activities [10–16]. Compounds with quinoline in their structure are receiving great attention in studies for the treatment of inflammatory diseases [17–19]. In turn, the essential element selenium (Se) is of fundamental importance to human health. As a constituent of the small group of selenocysteine containing selenoproteins, Se elicits important structural and enzymatic functions [20]. Dietary Se,

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plays an important role in inflammation and immunity. Adequate levels of Se are important for initiating immunity, but they are also involved in regulating excessive immune responses and chronic inflammation [21].

Based on major pharmacological properties of quinolines derivatives and Se, our research group targets the design of the 7-chloro-4-phenylselenanyl quinoline (4-PSQ), with the objective of structural improvement. A literature survey indicates that only a few publications have mentioned the incorporation of a selenium atom in the quinoline nucleus [22–24]. Consequently, synthesis and biological screening of selenoquinoline derivatives may be considered a relevant research area. Indeed, 4-PSQ revealed potential antioxidant, antinociceptive and anti-inflammatory actions [22,25]. Recently, our research group demonstrated that a single dose of 4-PSQ exerts anxiolytic activity in mice [23]. 4-PSQ exerts pharmacological actions without causing locomotor disorders and kidney, liver and brain toxicities [22,23].

In view of our continued interest in the pharmacology of this compound, the first objective of this study was to evaluate the effects of oral treatment with 4-PSQ on AD-like lesions induced by DNCB in mice. In line with our results of the first set of experiments, we were motivated to evaluate if the antioxidant property of 4-PSQ could improve the potential of a biofilm previously studied by our research group for the treatment of AD-like skin lesions.

2. Materials and methods

2.1. Animals and ethical approval

The experiments were conducted using female BALB/c mice (6–8 weeks old). Animals were kept in a separate animal room, on a 12 h light/dark cycles, with lights on at 7:00 a.m., at room temperature ($22 \pm 1^\circ\text{C}$), with free access to food and water. The biological assays were conducted according to the institutional and national guidelines for the care and use of animals. The Local Committee for Care and Use of Laboratory Animals of the Federal University of Pelotas (Pelotas, Brazil) approved the research (Code number: 4294-2015). The number of animals was the minimum necessary to demonstrate the consistent effects of the drugs treatments and all efforts were taken to minimize their suffering.

2.2. Chemicals and drugs

DNCB was obtained commercially and it was used as an inducer of AD. 4-PSQ (Fig. 1) was prepared and characterized in our laboratory by the method previously described by Duarte et al. [24]. Analysis of the ^1H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with 4-PSQ assigned structure. The chemical purity of 4-PSQ (99.9%) was determined by GC/MS. Dexamethasone was

obtained commercially and it was used as a reference drug. For oral treatment, 4-PSQ was dissolved in canola oil, while dexamethasone was dissolved in 0.9% saline solution. The oral administration (p.o.) of 4-PSQ or dexamethasone was by intragastric gavage, at a constant volume of 10 ml/kg body weight. For topical treatment, 4-PSQ was loaded into a biofilm (as described below) and applied as a bandage.

Chitosan 85% deacetylated was purchased from Golden-Shell Biochemical (China). Poly(vinyl alcohol) (PVA) with M_w of 124,000 g/mol and 99% hydrolyzed was purchased from Sigma-Aldrich (USA). Sodium tripolyphosphate (TPP, $\text{Na}_5\text{P}_3\text{O}_{10}$) and acetic acid were purchased Synth (Brazil). Bovine bone powder (BBP, particle size < 80-mesh) was obtained according to the protocol described by Alves et al. [26]. All other chemicals of analytical grade were used as received without further purification.

2.3. Synthesis of 4-PSQ-loaded biofilms

The 4-PSQ-loaded biofilms were synthesized using conventional solvent casting method. PVA (200 mg) was solubilized in distilled water (30 ml) at 75°C for 4 h under stirring. Separately, chitosan (600 mg) was solubilized in acetic acid solution (30 ml, 2 v/v-%) at room temperature for 1 h. These two solutions were blended at room temperature and, then, BBP (40 mg) was added to the system. This resulting system was stirred for 30 min. Next, 4-PSQ (80 mg) was added under vigorous stirring for 15 min at room temperature. The biofilmogenic solution was poured into Petri dishes (polystyrene, round-plate shape 85×10 mm) and the solvent was evaporated in an oven (40°C for 48 h). The as-dried biofilm was peeled off from the Petri dishes and physically crosslinked by immersion in TPP solution (10 wt/v-%) for 1 h. During this step, the pH of the crosslinking medium was adjusted to 4 using HCl (1 M). Then, the crosslinked biofilms were soaked in NaOH solution (0.5 M) for a short while and subsequently, washed several times with distilled water up to neutral pH. Finally, the biofilms were oven-dried (40°C for 24 h) to a constant weight. A biofilm sample without 4-PSQ was synthesized using a similar procedure.

2.4. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of pure 4-PSQ, 4-PSQ-loaded biofilm, and pristine biofilm were recorded using a Shimadzu spectrometer (model Affinity, Japan) operating in the spectral region of $4000\text{--}600\text{ cm}^{-1}$ with a resolution of 4 cm^{-1} . The samples were ground with spectroscopic grade KBr and pressed into disks.

2.5. Experimental protocol

AD-like skin lesions in mice were induced by DNCB as previously described [27]. Mice were shaved of dorsal hair and $200\text{ }\mu\text{l}$ of 0.5 v/v-% DNCB in acetone/olive oil (3:1 ratio) was applied to the shaved area on experimental days 1–3 for sensitization. Mice were challenged with $20\text{ }\mu\text{l}$ of 1 v/v-% DNCB on the ear on days 14–29 and $200\text{ }\mu\text{l}$ on the dorsal skin on days 14, 17, 20, 23, 26, and 29 (Fig. 2). Mice were randomly divided into six groups ($n = 8\text{--}10$ animals/group): normal control mice (control group) were sensitized and challenged with acetone/olive oil (3:1); sensitized control mice (DNCB group) were sensitized and challenged with DNCB; and experimental mice were sensitized and challenged with DNCB and received the treatment with 4-PSQ at the dose of 5 mg/kg, biofilm (biofilm sample without 4-PSQ), 4-PSQ-loaded biofilms or dexamethasone at dose of 5 mg/kg (groups designated as DNCB + 4-PSQ; DNCB + biofilm; DNCB + biofilm-4-PSQ and DNCB + DEXA). Oral treatment with 4-PSQ or dexamethasone was by intragastric gavage, daily, from days 14 to 29. In order to compare with the 4-PSQ effect, the dose of dexamethasone used was 5 mg/kg. Biofilms treatments were applied in the dorsal region of animal and secured with a bandage starting on 14th day. On DNCB application days (14, 17, 20, 23, 26, and 29) in the dorsal region, the

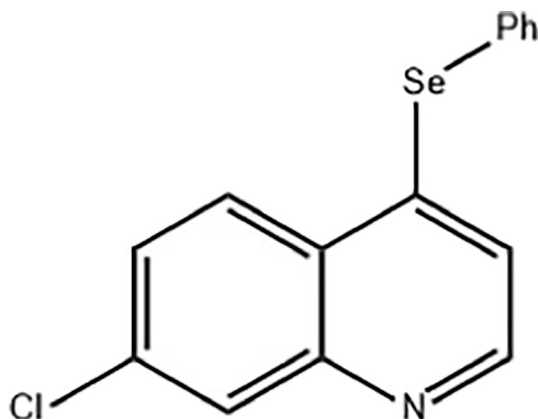


Fig. 1. Chemical structure of 4-PSQ.

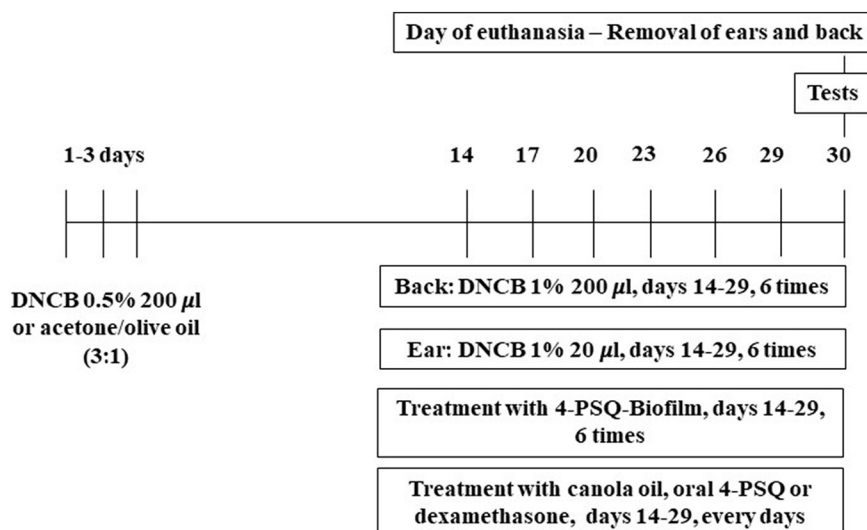


Fig. 2. A summary of the development of AD-like skin lesions in DNFB-treated mice. The dorsal skin of mice was shaved and treated with topical 0.5-% DNFB on days 1–3. Next, the mice were challenged with 1% DNFB on days 14, 17, 20, 23, 26, and 29. AD-like mice were treated with 4-PSQ, biofilm, 4-PSQ-loaded biofilm or dexamethasone on days 14–27. Finally, mice were sacrificed on day 30.

biofilms were changed.

2.6. Clinical skin severity scores

On the day 30, the skin severity scores were measured according to Park et al. [28]. The five signs of skin lesions were: (1) pruritus/itching, (2) erythema/hemorrhage, (3) edema, (4) excoriation/erosion, and (5) scaling/dryness. The above-mentioned symptoms were graded as follows: 0 (no symptoms), 1 (mild), 2 (moderate), and 3 (severe).

2.7. Scratching behavior

To investigate AD-like behavioral changes, it was measured and recorded at the time that mice spent rubbing their nose, ears, and dorsal skin with their hind paws for 20 min on the last day of the experiment (day 30) [29].

2.8. Inflammatory parameters

2.8.1. Ear swelling

On the day 30, after scratching behavior, mice were euthanized and both ears were cut at the base and the weight difference between the samples of the control ear (left) and the DNFB-treated ear (right) was established on an analytical balance. The results obtained are represented in weight (mg). Subsequently, ears were used to determine the myeloperoxidase (MPO) activity.

2.8.2. MPO assay

MPO activity was assayed according to the method previously described with some modifications [30]. Mice ears or the back fraction were minced, pooled and homogenized in PBS (20 mmol/l, pH 7.4) containing ethylenediaminetetraacetic acid (EDTA). The homogenates were centrifuged at 900 rpm for 10 min at 4 °C (fraction S1). Then, the S1 fraction was centrifuged again at 20,000 rpm at 4 °C for 15 min to yield a final pellet (P2) that was resuspended in medium containing potassium phosphate buffer (50 mmol/l, pH 6.0) and hexadecyltrimethyl ammonium bromide (0.5 wt/v-%). Samples were finally frozen and thawed three times prior the posterior enzymatic assay. For the MPO activity measurement, an aliquot of resuspended P2 (100 µl) was added to a medium containing the medium of resuspension and *N,N,N',N'*-tetramethylbenzidine (1.5 l). The kinetic analysis of MPO was started after H₂O₂ (0.01 v/v %) addition, and color reaction was measured at 655 nm at 37 °C. Results are expressed as optic density (OD)/mg protein/min. Protein concentration was measured by the Bradford method [31] using bovine serum albumin (1 mg/ml) as the

standard.

2.9. Oxidative parameters

Dorsal skin was removed and homogenized in 50 mM Tris-HCl, pH 7.4 (1:10) to determinate the oxidative parameters. The homogenate was centrifuged at 2000 rpm for 10 min and the S1 fraction was used for the reactive species (RS) and non-protein thiol (NPSH) measurement.

2.9.1. RS levels

The RS levels in the back of animals were determined by a spectrofluorimetric method, using 2',7' dichlorofluorescein diacetate (DCHF-DA) assay [32]. The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) was measured for the detection of intracellular RS. The DCF fluorescence intensity emission was recorded at 520 nm (with 480 nm excitation), 15 min after the addition of DCHF-DA to the medium (Shimadzu RF-5301 PC fluorometer). RS levels were expressed as arbitrary units of fluorescence.

2.9.2. NPSH levels

NPSH levels were determined by the method of Ellman [33]. A sample of S1 (portion of back in 50 mM Tris/HCl pH 7.4) was mixed (1:1, v/v) with 10% trichloroacetic acid. The samples were centrifuged at 3000 rpm for 10 min. After the centrifugation, the protein pellet was discarded and free-thiol groups were determined in the clear supernatant. An aliquot of supernatant (200 µl) was added in 1 M potassium phosphate buffer pH 7.4 and 10 mM 5,5'-dithiol-bis (2-nitrobenzoic acid). The color reaction was measured at 412 nm. NPSH levels were expressed as nmol NPSH/g tissue.

2.10. Statistical analysis

Data from the experiments were expressed as mean ± standard error medium (S.E.M.). Data were analyzed by Graphpad Prism® 5 using one-way ANOVA followed by the Newman-Keuls test when appropriated. Values of *p* < 0.05 were considered statistically significant. Pearson's correlation coefficient was used for correlation analysis.

3. Results

3.1. FTIR analysis

The encapsulation of 4-PSQ into the biofilm matrix was investigated

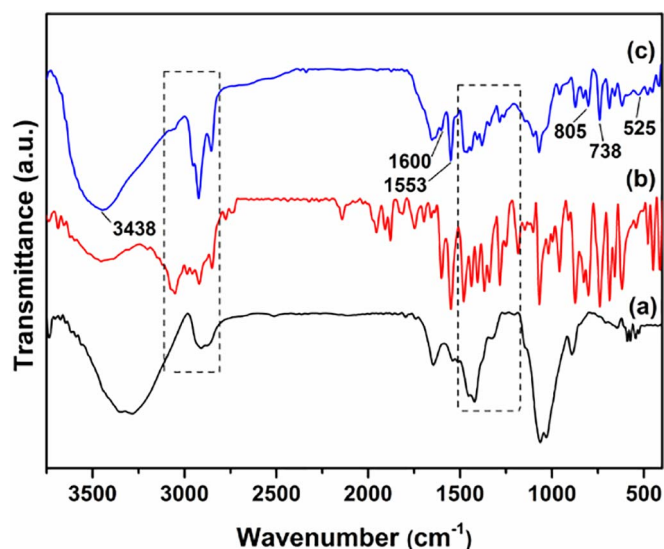


Fig. 3. FTIR spectra of (a) Cs/PVA/BBP, (b) 7-chloro-4-phenylselanyl quinoline (4-PSQ) and (c) 4-PSQ-loaded Cs/PVA/BBP.

by FTIR analysis. As demonstrated in Fig. 3a, the spectrum recorded to the pristine biofilms shows the characteristics bands of its precursor materials; Cs, PVA, and BBP. The broad band in the 3650–3100 cm^{-1} region is assigned to the stretching of the hydroxyl groups from Cs and PVA [26,34]. Also, in this wavenumber region, it is observed the stretching of N–H bond of the amino groups from Cs. Bands in the 2990–2800 cm^{-1} region are assigned to C–H stretching from alkyl groups of Cs and PVA [26,34]. The band at 1645 cm^{-1} is related to the protonated amino groups ($-\text{NH}_3^+$) that interact with phosphoric ions from TPP [35]. Bands in the 1160–890 cm^{-1} region are assigned to the C–O and C–O–C bonds that form the saccharide structure of Cs, the backbone of PVA and BBP. Bands at 1474 cm^{-1} (vibration of CO_3^{2-} groups), 1244 cm^{-1} (antisymmetric stretching of PO_2^- groups), 1380 cm^{-1} (symmetric deformation of C–H bond) and 1140 cm^{-1} (P=O stretching) are related to the TPP and BBP structures [26,36]. All these finds confirm the ionic crosslinking of Cs with TPP ions and the composite formation. The 4-PSQ spectrum (Fig. 3b) shows bands in the 3100–3000 cm^{-1} region related to the aromatic C–H stretching, bands at 1652 and 1553 cm^{-1} assigned to C=N stretching and bands at 1600, 1482 and 1435 cm^{-1} related to aromatic C=C stretching [37,38]. In addition, bands at 1068 cm^{-1} (C–N stretching), 735 cm^{-1} (C–Cl stretching) and 525 cm^{-1} (C–Se stretching) were observed [39]. Bands in the 810–730 cm^{-1} and 690–670 cm^{-1} regions are related to C–H out of plane bending.

The FTIR spectrum recorded for the 4-PSQ-loaded biofilm (Fig. 3c) shows the significant changes as compared to the spectrum recorded for the bare biofilm. The broad band centered at 3438 cm^{-1} assigned to O–H stretching suggests that the presence of 4-PSQ into the biofilm matrix reduces the number of hydrogen bonds among the Cs, PVA and BBP [40]. For this reason, such band was shifted to higher wavenumber region as compared to the same band observed in the biofilm spectrum. Further, the characteristic bands related to the unsaturated structure of 4-PSQ are observed in this spectrum, which suggests the effective incorporation of this compound into the biofilm matrix. The absences of band shifting to different wavenumber regions or new bands allow suggesting that any chemical or physical interaction is verified between 4-PSQ and the biofilm matrix.

3.2. Clinical skin severity scores

Effects of 4-PSQ treatment (oral or topical) on severity of skin lesions in mice are demonstrated in Fig. 4a and b [$F_{5,40} = 16.96$,

$p < 0.0001$]. One-way ANOVA followed by Newman-Keuls' post-hoc test showed that DNCB significantly increased (around 3 times) skin severity scores when compared with the control group ($p < 0.0001$) (Fig. 4b). Treatment with 4-PSQ ($p < 0.01$), 4-PSQ-loaded biofilm ($p < 0.05$) or biofilm ($p < 0.05$) was partially effective in reducing the severity of skin lesions induced by DNCB (Fig. 4b). In addition, no significant difference in severity of skin lesions was observed after treatment with dexamethasone, when compared to DNCB group ($p > 0.05$) (Fig. 4a and b).

3.3. Scratching behavior

Effect of 4-PSQ treatment (oral or topical) on scratching behavior is showed in Fig. 5a [$F_{5,40} = 20.63$, $p < 0.0001$]. One-way ANOVA followed by Newman-Keuls' post-hoc test revealed that DNCB-exposed animals increased (around 15 times) the scratching time, when compared with the control group ($p < 0.0001$) (Fig. 5a). Biofilm treatment was partially effective in reducing scratching time, when compared with DNCB group ($p < 0.05$). As observed in Fig. 5a, treatment with 4-PSQ ($p < 0.0001$) or 4-PSQ-loaded biofilm ($p < 0.01$) protected against the increase of scratching time induced by DNCB. Dexamethasone treatment increased the scratching time when compared with DNCB exposed mice ($p < 0.05$). Treatment with 4-PSQ ($p < 0.05$) or 4-PSQ-loaded biofilm ($p < 0.05$) was more effective in reducing the scratching time than biofilm treatment.

3.4. Inflammatory parameters

3.4.1. Ear swelling

Fig. 5b illustrates the effects of 4-PSQ treatment (oral or topical) on ear swelling in mice [$F_{5,40} = 67.63$, $p < 0.0001$]. One-way ANOVA followed by Newman-Keuls' post-hoc test demonstrated that DNCB substantially increased right ear swelling (175 times) as compared with the control group ($p < 0.0001$) (Fig. 5b). Treatments with 4-PSQ ($p < 0.0001$), biofilm ($p < 0.05$), 4-PSQ-loaded biofilm ($p < 0.0001$) or dexamethasone ($p < 0.0001$) partially reduced the ear swelling induced by DNCB (Fig. 5b). 4-PSQ-loaded biofilm was more effective in reducing the ear swelling than biofilm ($p < 0.0001$) and dexamethasone ($p < 0.05$) treatments.

3.4.2. MPO activity

Results depicted in Fig. 6a and b show effects of oral or topical administration of 4-PSQ on the ear and back MPO activities [$F_{5,40} = 8.82$, $p < 0.0001$ (ear); $F_{5,40} = 15.07$, $p < 0.0001$ (back)]. One-way ANOVA followed by Newman-Keuls' post-hoc test revealed that DNCB significantly increased the MPO activity in the mouse ear (379 times; $p < 0.0001$) and back (230 times; $p < 0.0001$) when compared with the control group (Fig. 6a and b). The statistical analysis of data revealed that 4-PSQ ($p < 0.0001$), biofilm ($p < 0.001$), 4-PSQ-loaded biofilm ($p < 0.0001$) or dexamethasone ($p < 0.0001$) restored ear MPO activity to the control levels (Fig. 6a).

The results presented in Fig. 6b demonstrated that treatment with 4-PSQ ($p < 0.0001$) or 4-PSQ-loaded biofilm ($p < 0.0001$) protected against the increase of back MPO activity induced by DNCB. 4-PSQ and 4-PSQ-loaded biofilm treatments were more effective in reducing the back MPO activity than biofilm ($p < 0.0001$) and dexamethasone ($p < 0.0001$) (Fig. 6b). The correlation analysis (Pearson's correlation coefficient) revealed a positive correlation between the back MPO activity and scratching behavior ($r = 0.7548$, $p < 0.01$).

3.5. Oxidative parameters

3.5.1. RS measurement

Fig. 7a illustrates the effects of 4-PSQ treatment (oral or topical) on the of dorsal skin RS level in mice [$F_{5,40} = 9.749$, $p < 0.0001$]. One-way ANOVA followed by Newman-Keuls' post-hoc test revealed that

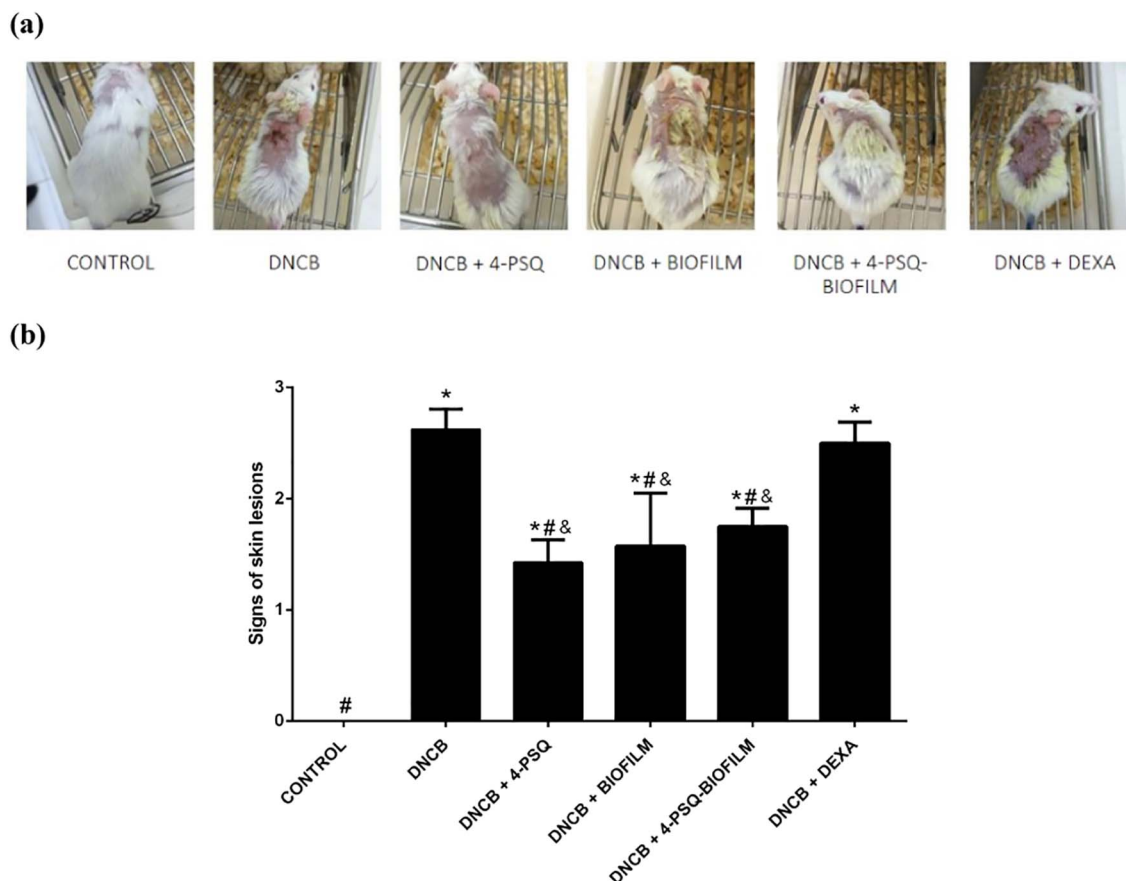


Fig. 4. Effect of 4-PSQ treatment (oral or topical) on DNCB-induced AD-like symptoms in mice. (a) Images of skin and ear lesions from the groups of mice taken on the last day of the experiment (day 30). (b) Dermatitis scores evaluated on day 30. Data represent the mean $\pm$ S.E.M. (one-way ANOVA followed by the Newman-Keuls' test). * $p < 0.05$ compared with the control group, # $p < 0.05$ compared with the DNCB group, & $p < 0.05$ compared with the dexamethasone group.

DNCB-exposed animals increased the RS levels (2 times) when compared with the control group ($p < 0.0001$). 4-PSQ ($p < 0.05$) and 4-PSQ-loaded biofilm ($p < 0.05$) treatments partially protected against the increase of RS levels induced by DNCB exposure. Dexamethasone and biofilm treatments had no effect in decreasing levels of RS compared to the DNCB group ($p > 0.05$). The correlation analysis (Pearson's correlation coefficient) revealed a positive correlation between the RS levels and scratching behavior ($r = 0.6722$, $p < 0.01$).

3.5.2. NPSH levels

As verified in Fig. 7b, statistical analysis demonstrated that NPSH levels of dorsal skin were not altered by DNCB or the other treatments, when compared to the control group [$F_{5,40} = 1.1962$, $p > 0.05$].

4. Discussion

Given that 4-PSQ has antioxidant and anti-inflammatory activities, in the present study we hypothesized, that 4-PSQ might inhibit the mechanisms underlying AD. Indeed, oral treatment with 4-PSQ attenuates signs of skin lesions, scratching behavior, ear swelling and the increase of MPO activity and RS levels induced by DNCB exposure. In addition, the incorporation of 4-PSQ improved the therapeutic effect of the biofilm. The efficacy of 4-PSQ (oral or topical) in treating AD-like lesions was similar or better than dexamethasone, a glucocorticoid frequently used in the treatment of AD.

Topical application of hapten in mice, such as DNCB, is commonly used to induce skin contact hypersensitivity and it is widely used as animal model for allergic contact dermatitis [41]. AD model induced by DNCB is used to study this disease [28,29,42–44] since it mimics the inflammatory process of the skin involved in development and

maintenance of AD [5,45]. Considering the numerous side effects resulted from the long-term use of therapies to AD, there is a great need to develop new and effective therapeutic strategies for AD. In this context, researchers have sought to identify new treatments to AD [46–50].

It is important to highlight the advantages that justify the objective in extending our knowledge of the pharmacological potential of this quinolinic compound. Firstly, the antioxidant activity of 4-PSQ [22,25]. The use of compounds with a potential antioxidant effect has received attention from researchers and they are promising therapies by acting as a “multi-target” drug, which is important in the treatment of several pathologies [51–54]. Second, it should be noted that 4-PSQ did not cause renal and hepatic impairment [22,23]. Third, 4-PSQ is a compound synthesized in high yields and under simple reaction conditions [24]. Additionally, 4-PSQ presented antinociceptive and anti-inflammatory actions at low doses (0.1 and 1 mg/kg), reinforcing its pharmacological potential [22].

The experimental design of the present study started with the evaluation of the protective effect of oral treatment with 4-PSQ on AD induced by DNCB in mice. Studies have demonstrated that the method used to deliver the compound to target tissues/cells is important [55–57]. Oral and topical administration routes are widely used in AD models. Oral administration of effective compounds is of particular interest in the AD because it allows close control of the dose over long-term periods. In addition, oral administration is particularly important for adequately treating children or non-compliant patients in the clinic. Importantly, the present study showed that when mice were treated orally with 4-PSQ, both local and systemic AD manifestations were ameliorated.

In line with our results of the first set of experiments, we were motivated to evaluate the effects of topical treatment with 4-PSQ.

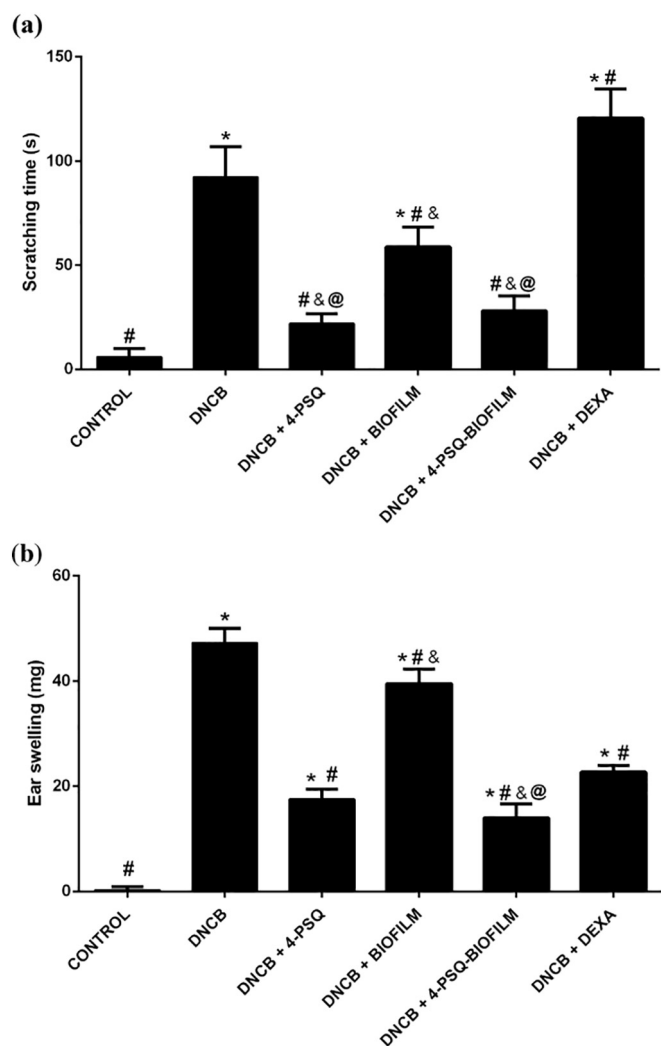


Fig. 5. Effect of 4-PSQ treatment (oral or topical) on (a) DNCB-induced scratching incidence and (b) ear swelling. Scratching time and ear swelling were evaluated on day 30. Data represent the mean $\pm$ S.E.M. (one-way ANOVA followed by the Newman-Keuls' test). * p < 0.05 compared with the control group, # p < 0.05 compared with the DNCB group, & p < 0.05 compared with the dexamethasone group and @ p < 0.05 compared to the biofilm group.

Topical administration results in the relatively rapid action of the compound, albeit at a limited site. Recently, our research group verified that a biofilm based on chitosan, PVA, and BBP had potential for the treatment of AD-like skin lesions [26]. This biocomposite showed reliable physicochemical properties that encourage its use as a potential biomaterial. It is worth mentioning that the use of this biofilm allows the application as a bandage, which makes its use practical and easy. In this regard, we believed that the antioxidant property of 4-PSQ could improve the biofilm potential for the treatment of AD. Our results revealed that the incorporation of 4-PSQ into chitosan/PVA/BBP biofilm improved the therapeutic effect on scratching behavior, ear swelling, back MPO activity and RS levels when compared to biofilm. It is important to highlight that no difference was observed between oral and topical treatments with 4-PSQ. However, an important advantage of topical treatment was the necessity of only 6 applications, while the oral administration was by 14 days.

It is well established that AD is a chronic inflammatory skin disease resulting from skin barrier dysfunction and leading to eczematous and itchy lesions [58]. Itch causes skin barrier damage by inducing scratching, exposing antigen-presenting cells resident in the skin to external factors, such as allergens and bacterial [58]. In the present

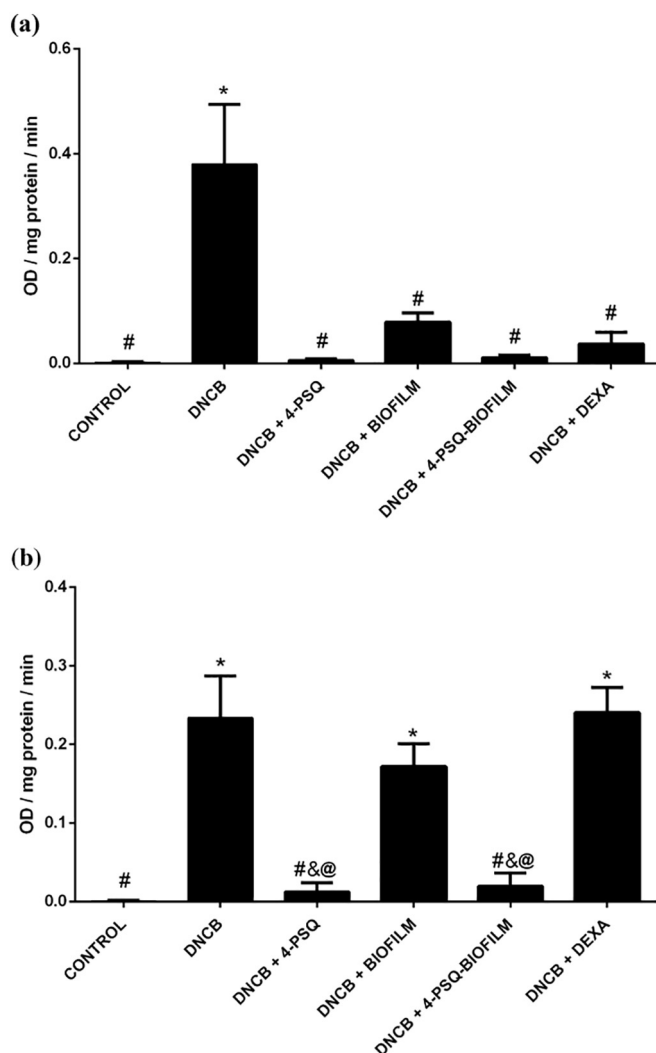


Fig. 6. Effect of 4-PSQ treatment (oral or topical) on (a) ear and (b) back MPO activities in mice. Data represent the mean $\pm$ S.E.M. (one-way ANOVA followed by the Newman-Keuls' test). * p < 0.05 compared with the control group, # p < 0.05 compared with the DNCB group, & p < 0.05 compared to the dexamethasone group and @ p < 0.05 compared with the biofilm group.

study, the DNCB exposure induced the AD-like symptoms in mice. It was evidenced by increase in the severity of skin lesions (erythema, erosion, and dryness) and scratching behavior. Treatments with 4-PSQ, biofilm and 4-PSQ-loaded biofilm attenuated the skin lesions severity, however, no significant difference was observed after treatment with dexamethasone when compared to DNCB group. As evidenced by Alves and collaborators [26], biofilm treatment attenuated DNCB-induced scratching behavior. Additionally, treatment with 4-PSQ (oral or loaded in biofilm) completely protected against the increase of scratching time induced by DNCB and as expected, the incorporation of 4-PSQ into biofilm improved its therapeutic effect on scratching behavior. These results are important in order to reduce the itching and scratching to prevent aggravation of the skin lesion on pruritic diseases and to upgrade the quality of life of patients. On the other hand, our results revealed that dexamethasone treatment increased scratching time when compared with DNCB group. This data agrees with the fact that long-term topically applied steroids also lead to thinning of the skin and to cracking and bleeding [8]. Together, these results reinforce the potential of 4-PSQ for AD treatment.

AD is one of many autoimmune diseases, and over-activation of immunity induces AD by promoting inflammation of the skin barriers. Thus, assuming the close linkage of inflammation in the

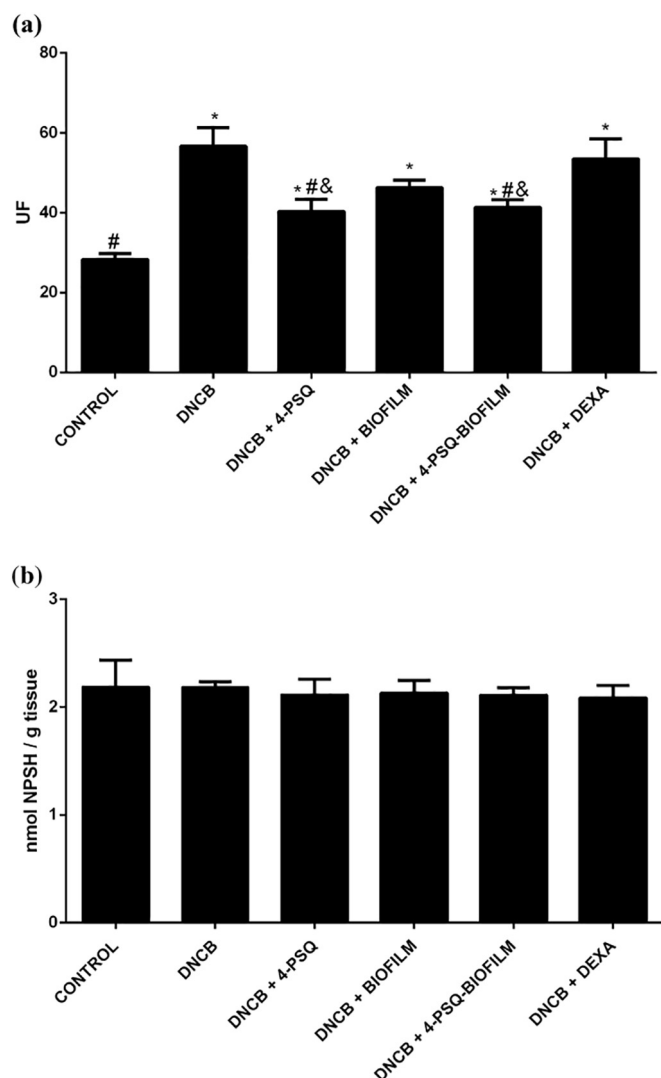


Fig. 7. Effect of 4-PSQ treatment (oral or topical) on (a) RS and (b) NPSH levels of back of mice. Data represent the mean $\pm$ S.E.M. (one-way ANOVA followed by the Newman-Keuls' test). * $p < 0.05$ compared with the control group, # $p < 0.05$ compared with the DNCB group, & $p < 0.05$ compared with the dexamethasone group and @ $p < 0.05$ compared with the biofilm group.

pathophysiology of AD, we evaluated ear swelling and MPO activity. Here, treatment with 4-PSQ, biofilm, 4-PSQ-loaded biofilm or dexamethasone attenuated the ear swelling induced by DNCB, indicating a reduction in the severity of ear inflammation. In line with our results, it is important to highlight that protective effect of the 4-PSQ was similar to dexamethasone and biofilm based on chitosan, PVA and BBP. Importantly, the incorporation of 4-PSQ into biofilm improved its therapeutic effect on ear swelling. In ear, all treatments decreased the MPO activity induced by repeated DNCB challenges, corroborating with the ear swelling results. Of particular importance, treatments were applied orally or in the back of animals, suggesting a systemic action. When the back MPO activity was evaluated, it was verified that only treatments with 4-PSQ or 4-PSQ-loaded biofilm had a protective effect in reducing neutrophil influx into tissue. As evidenced here, DNCB exposure causes a skin irritation and local inflammation due to increasing vascular permeability, thereby, polymorphonuclear leukocytes migrate to the inflammation local, increasing the MPO activity in the site [59]. Thus, the current findings suggest that the modulation of MPO activity is implicated in the protective effect of 4-PSQ on AD-like skin lesions.

Considering the background regarding the antioxidant potential of

4-PSQ [22], the knowledge of the involvement of this action on the protective effect against AD-like skin lesion induced by DNCB was evaluated. All types of skin cell, including melanocytes and keratinocytes, produce RS [60,61]. In addition, RS from skin cells can also produce reactive lipid species, such as lipid radicals, peroxides, hydroperoxide or aldehydes by reacting with lipid molecules or redox-sensitive lipid-metabolizing enzymes, such as lipoxygenases and cyclooxygenases. AD, which is a chronic inflammatory disease, can disrupt the redox balance, resulting in the overproduction of RS, and high levels of lipid peroxidation products. This exacerbates the disease state and shifts the response toward a Th2 skewed immune response [62,63]. Indeed, repeated DNCB challenges substantially increased back RS levels. 4-PSQ treatment (oral or loaded in biofilm) alleviates the increase on RS levels, indicating that its antioxidant effect probably also contributes to protective effect on AD. No alteration on NPSH levels was observed after the treatments, suggesting that NPSH is not involved in the alterations induced by DNCB exposure or 4-PSQ/biofilm treatments. Accordingly, antioxidants as thiol *N*-acetyl-L-cysteine or glutathione downregulated Th2 cytokines, IL-4 and IL-5 in human T cells [64,65]. In this sense, supplementation with antioxidants may, therefore, be a good approach to the treatment of AD and other skin disorders.

Taking into account these results, the present study revealed for the first time that 4-PSQ attenuates AD-like symptoms in mice by suppressing the increase of MPO activity and RS levels induced by DNCB. Importantly, the efficacy of 4-PSQ in treating AD-like lesions was similar or better than dexamethasone, reinforcing the potential of 4-PSQ for AD treatment. In addition, the incorporation of 4-PSQ into biofilm improved its therapeutic effect on scratching behavior, ear swelling, back MPO activity and RS levels when compared to biofilm.

5. Conclusion

In conclusion, our results suggest that 4-PSQ (oral or loaded in biofilm) has a potential therapeutic advantage in the treatment and management of AD. Although our previous studies no demonstrated acute toxicity [22,23], the evaluation of toxicological effects of repeated administrations of 4-PSQ should be elucidated in further studies.

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Conflict of interest

The authors declare that they have no conflict of interest.

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