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BIOMEDICAL SCIENCES

N-Methyl-(2*S*,4*R*)-*trans*-4-hydroxy-L-proline isolated from *Sideroxylon obtusifolium* attenuates TPA-induced irritant contact dermatitis in mice

PAULO IURY G. NUNES, ANA FLÁVIA S.C. VIANA, GREYCE L. SASAHARA, SABRINA M. DOS SANTOS, ANA PAULA N.N. ALVES, EDILBERTO R. SILVEIRA & FLÁVIA A. SANTOS

Abstract: Dermatitis is defined as a set of inflammatory diseases that affect the skin, with varied causes. Among the different types of dermatitis, contact dermatitis is the most prevalent. Although the current therapy is often effective, it is associated with adverse effects and the possibility of drug tolerance. *N*-Methyl-(2S, 4R)-*trans*-4-hydroxy-L-proline is a L-proline amino acid derivative found in the leaves of *Sideroxylon obtusifolium*, a species traditionally used to treat inflammatory diseases. The aim of this study was to investigate the topical anti-inflammatory effect of *N*-methyl-(2S, 4R)-trans-4-hydroxy-L-proline (NMP) in 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced irritant contact dermatitis in mice. Topically administered NMP, at doses of 0.03 – 0.50 mg/ear, reduced TPA-induced ear edema and neutrophil migration, as evidenced by low tissue myeloperoxidase activity and verified by histological examination. In addition, NMP (0.06 mg/ear) reduced tissue levels of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, INF-γ and MCP-1) and of the anti-inflammatory cytokine IL-10, and reduced gene expression of TNF-α, IL-6 and IL-1β increased by TPA. The data suggest that N-methyl-(2S, 4R)-trans-4-hydroxy-L-proline acts as a topical anti-inflammatory agent that decreases the expression of inflammatory cytokines, making it useful for the treatment of skin inflammation. Further investigations are necessary for its development as a therapeutic agent.

Key words: Anti-inflammatory effect, *N*-methyl-(2*S*, 4*R*)-*trans*-4-hydroxy-L-proline, *Sideroxylon obtusifolium*, TPA.

INTRODUCTION

The skin acts as the body's first line of defense against harmful chemical, physical and biological agents, and also plays a role in thermoregulation, water loss control, and tactile sensitivity (Egawa & Kabashima 2016). Pathological processes of the skin with exacerbated inflammatory character, such as psoriasis, allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD), occur with relevant frequency and have become more frequent in recent years (Johnson-Huang et al. 2009, Nguyen & Soulika 2019, Nguyen & Yiannias 2019).

It is estimated that the worldwide prevalence of contact dermatitis ranges from 12.5 to 40.6%, according to geographic location. In addition, because it is commonly related to occupational skin diseases, ICD has high prevalence especially among individuals of working age (Warshaw et al. 2017, Nguyen & Yiannias 2019).

Treatment of these dermatoses includes the use of emollients to improve barrier function, treatment with topical corticosteroids and UV-light, or application of systemic retinoids in severe cases (Lee et al. 2019, Mostosi & Simonart 2016, Mandlik & Mandlik 2021). The topical use of corticosteroids is the standard treatment of ICD, but its chronic use is associated with skin atrophy, telangiectasia, dryness and changes in the healing process (Chatzidionysiou et al. 2017, Wollenberg et al. 2018, Howell et al. 2020). Therefore, there is a need for safer and more effective therapies with fewer side effects compared to the drugs already in use (Wittmann et al. 2014). In this context, natural products are a promising source to develop innovative drugs (Calixto 2019, Khalifa et al. 2019, Atanasov et al. 2021).

Sideroxylon obtusifolium is a medicinal tree species common to Brazilian territory, popularly known as "quixaba", "saputiaba" or "rompe-gibão" (Lorenzi & Matos 2002, Delfino et al. 2005). In folk medicine, extracts of the bark and leaves of *S. obtusifolium* are used to treat gastrointestinal diseases, chronic inflammation, diabetes and pain (Agra et al. 2007, Albuquerque & Oliveira 2007, Beltrão et al. 2008, Santos et al. 2009, Araújo-Neto et al. 2010).

Extracts and fractions enriched in *N*-methyl-(2*S*,4*R*)-*trans*-4-hydroxy-L-proline (NMP), a constituent of *S. obtusifolium* leaves, have demonstrated biological activities as antioxidants (Aquino et al. 2020) and systemic anti-inflammatories (Aquino et al. 2017, 2019, 2020). NMP is a derivative of L-proline, an amino acid commonly used as a dietary supplement to stimulate collagen synthesis in the body (Vitagliano et al. 2001).

Aquino et al. (2016) showed that topical administration of the methanolic extract of *S. obtusifolium* had an anti-edematogenic effect in *Croton*-oil and phenolic-induced ear edema in mice, demonstrating for the first time its topical anti-inflammatory potential, but not elucidating the mechanisms involved in its effect. Thus, this study aimed to evaluate the topical anti-inflammatory potential of NMP, the main constituent of the methanol extract of leaves of *S. obtusifolium*, against TPA-induced topical irritant contact dermatitis in mice and to investigate its underlying mechanisms.

MATERIALS AND METHODS

Plant material

The leaves of *S. obtusifolium* were collected in the Mauriti region (Ceará, Brazil). Voucher specimens were identified and deposited in the Caririense Dárdano de Andrade-Lima Herbarium of Regional University of Cariri (URCA) under Exsiccate no. 10.648. This study was registered with the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under number A5305D6.

Isolation and characterization of *N*-methyl-(2S,4R)-*trans*-4-hydroxy-L-proline

The *N*-methyl-(2*S*,4*R*)-*trans*-4-hydroxy-L-proline (NMP) (Figure 1) was isolated and characterized as the major constituent of the methanolic fraction of *S. obtusifolium* leaves, as previously reported by Aquino et al. (2017).

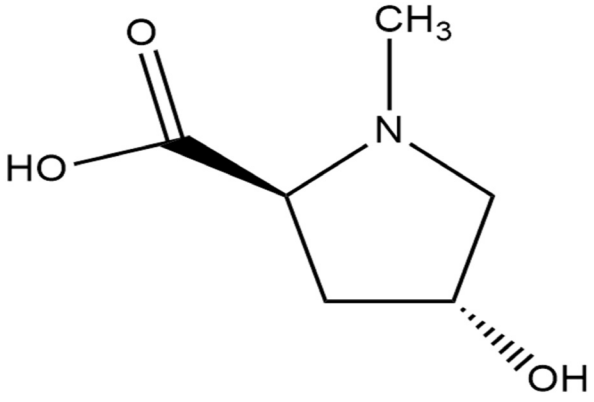

Figure 1. Chemical structure of the *N*-methyl-(2*S*, 4*R*)-*trans*-4-hydroxy-L-proline (NMP).

Briefly, the leaves of *S. obtusifolium* were previously dried, ground, and packed in cotton bags, which were then boiled in distilled water (100 g dry leaves/500 ml distilled water; 15 min; 2x). The obtained decoction was then freeze-dried and subjected to Soxhlet extraction with methanol (MeOH). The obtained MeOH solution was evaporated by rotation, and aliquots were dissolved in distilled water and chromatographed with a Phenomenex column in a Strata® reverse-phase solid-phase extraction chromatography. The aqueous fractions were pooled after thin-layer chromatography analysis and later lyophilized. A total of 1.7 g (1.7% yield) of the material identified as the compound *N*-methyl-(2*S*, 4*R*)-*trans*-4-hydroxy-L-proline ($C_6H_{11}NO_3$; 145.16 g/mol) was obtained. The final structure, including absolute stereochemistry, was determined based on the negative specific rotation of the compound, based on resonance (NMR), stereochemistry by NOESY spectrum analysis, and by comparison with data available in the literature.

Animals

Male Swiss mice (*Mus musculus*), 25-30 g, from Federal University of Ceará were used. The animals were kept in polypropylene cages, 8 animals per cage, at an average temperature of 24 ± 2 °C in a light-dark cycle of 12/12 hours, with standard feed (Purina Chow®) and drinking water *ad libitum*. After the experimental procedures, animals were euthanized with an overdose of sodium thiopental (100 mg/kg, i.p.) and lidocaine (10 mg/ml, i.p.). All animal protocols were approved by the Committee on Ethical Use of Animals of Federal University of Ceará under registration number 9255280218 (ID 000223).

TPA-induced acute irritant contact dermatitis

The acute irritant contact dermatitis was induced as described by Recio et al. (2000), in which mice were divided into groups (n = 8/group) and received a single topical application of 12-O-tetradecanoylphorbol-13-acetate (TPA; Sigma Aldrich®, USA) diluted in acetone (2.5 µg/ear; 20 µL) in the right ear. Immediately after TPA application, vehicle (2% Tween 80 in distilled water; 20 µL), NMP (0.03 – 0.50 mg/ear; 20 µL), or dexamethasone (0.10 mg/ear; 20 µL) was administered topically at the same site of TPA application. A group of untreated (naive) animals was included. Edema was determined by measuring ear thickness 0 h (before TPA administration), 4 h, 6 h, and 24 h after TPA administration using a digital caliper (100.174B/Digimess®). Immediately after the last edema measurement, the animals were euthanized and ear tissue samples were collected (6 mm diameter) for subsequent analyses.

Determination of the enzymatic activity of myeloperoxidase (MPO)

Approximately 100 mg of ear tissue was homogenized in 1 ml of PBS buffer (50 mM; pH 6) containing 0.5% hexadecyltrimethylammonium bromide. The homogenate was then subjected to three cycles of freezing and thawing. After this procedure, samples were centrifuged at 2,000 *g* and 4 °C, for 15 minutes, and MPO activity in the supernatant was measured after adding PBS containing 0.167 mg/ml of o-dianisidine hydrochloride and 0.0005% hydrogen peroxide. The kinetics of the change in absorbance was measured at time points 0 and 5 min at 470 nm (Bradley et al. 1982).

Histological analysis

Ear tissue samples were fixed in a 10% buffered formalin solution for 24 h, then dehydrated, included in paraffin blocks, and cut into 3-5 µm sections with a microtome. The sections were mounted on slides for histology and stained with hematoxylin and eosin (H&E). Tissues from the

ears (n = 8) were analyzed using specific scores as described by Biondo-Simões et al. (2006), and the relative thickness of edema was measured as described by Pinto et al. (2015).

Levels of TNF-α, IL-6, IL-1β, IL-10, INF-γ and MCP-1 in ear tissues

The collected ear tissue samples were immediately homogenized in Tris-HCl buffer (50 mM; pH 7.5) with EDTA (1 mM) and 10x Halt ™ protease inhibitor cocktail (1:10, Thermo Scientific®, USA). Then the homogenates were centrifuged at 10,000 *g* for 10 min at 4 °C. After centrifugation, the supernatants were collected and the concentrations of TNF-α, IL-6, IL-1β, IL-10, INF-γ, and MCP-1 were determined using specific ELISA kits (Merck®, USA) according to the manufacturer's instructions, and the results were expressed in pg/ml.

Gene expression of TNF-α, IL-6, and IL-1β by RT-qPCR

To analyze the relative changes in mRNA expression of the cytokines TNF-α, IL-6, and IL-1β, the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was used. Total mRNA was isolated from ear tissue using the QIAzol Lysis RNeasy Lipid Tissue Mini Kit (Qiagen, Germany), and its purity was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Subsequently, cDNA was synthesized using the High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific) and the RT-qPCR technique was performed using the GoTaq® Master Mix kit containing an SYBR green® probe (Promega, USA) in an Mx3005p PCR thermocycler system, with all steps performed according to the protocols described by the manufacturer. PCR was performed with a pre-degeneration step at 95 °C for 2 minutes, followed by 40 cycles of amplification/degeneration at 95 °C for 15 seconds and an annealing/extension step at 59/60 °C for 60 seconds. The $2^{-\Delta\Delta Ct}$ method of Livak & Schmittgen (2001) was used for the relative quantification of samples. The primers used in these procedures are described in Table I.

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM) or median (minimum-maximum). For multiple comparisons of data, one-way analysis of variance (ANOVA) was used, and the level of significance between groups was determined by the Tukey post-test or Kruskal-Wallis test followed by the Dunn post-test. RT-qPCR results were analyzed by the Kolmogorov-Smirnov test. The difference between the means of the normal distribution (parametric data) was analyzed with Student's t-test. $p < 0.05$ was considered statistically significant. All tests were performed using the GraphPad Prism® 5.03

Table I. Gene sequence and annealing temperature (°C) of primers used in RT-qPCR.

Gene	Sequence	Annealing temperature (ºC)	Described by
TNF-α	Forward: 5´ CCCACTCTGACCCCTTTACT 3´ Reverse: 5´ TTTGAGTCCTTGATGGTGGT 3´	59	Syed et al. (2015)
IL-1β	Forward: 5´ GCCACCTTTTGACAGTGATG 3´ Reverse: 5´ AAGGTCCACGGGAAAGACAC 3´	60	Cheng et al. (2019)
IL-6	Forward: 5´ CTGCAAGAGACTTCCATCCAG 3´ Reverse: 5´ AGTGGTATAGACAGGTCTGTTGG 3´	59	Syed et al. (2015)
β-actin	Forward: 5´GGGAATGGGTCAGAAGGACTC 3´ Reverse: 5´GGTGTGGTGCCAGATCTTCTC 3´	59	De Lima et al. (2021)

statistical software (GraphPad Software Inc., USA).

RESULTS

Evaluation of edema, myeloperoxidase activity, and histological changes

Topical application of TPA induced acute ear edema 4—24 h as well as an increase in myeloperoxidase (MPO) activity compared to the naive group (Figure 2a, 2b). NMP (0.03, 0.06, 0.12, 0.25, and 0.50 mg/ear) significantly reduced the ear edema response at all observation times as well as the MPO activity in comparison with the vehicle group. Dexamethasone (0.10 mg/ear) also significantly reduced ear edema and MPO activity (Figure 2b).

Histological analysis revealed significant ear tissue changes induced by TPA, including edema, inflammatory cell infiltrates, ectatic blood vessels, vascular neoformation, and hemorrhage (Figure 2; Table II). NMP (0.06 mg/ear) and dexamethasone (0.10 mg/ear) were able to minimize tissue damage induced by TPA, with a reduction of the tissue edema, inflammatory cell infiltrates, number of ectatic

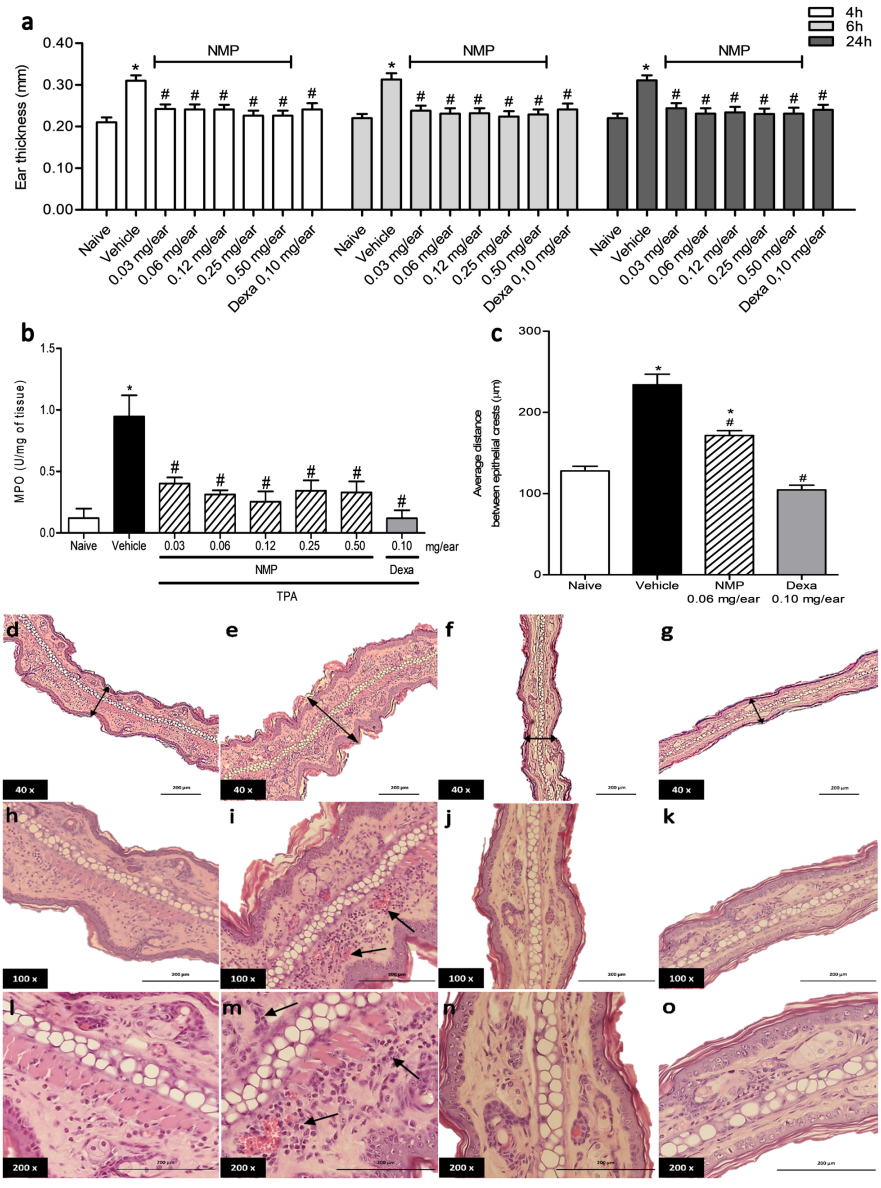

Figure 2. Effect of NMP on ear edema (a), myeloperoxidase (MPO) activity (b), average distance between epithelial crests of the ears (c) and histological changes (d - o) on TPA-induced contact dermatitis in mice. Dexamethasone (Dexa). Values are expressed as the mean ± SEM (n = 8 mice per group). Data were analyzed by one-way ANOVA followed by the Tukey's test. * $p < 0.05$ *vs* naive group and # $p < 0.05$ vehicle group. Representative photomicrographs of transverse sections of mice ear biopsies obtained from the TPA-induced contact dermatitis in mice (d – o) (H&E staining). Naive (d, h and l); Vehicle (e, i and m); NMP 0.06 mg/ear (f, j and n); Dexamethasone 0.10 mg/ear (g, k and o). In (d), (e), (f) and (g), the double arrows indicate the edema intensity; in (i) the arrows indicate ectatic vessels; in (m) the arrows indicate the presence of inflammatory cells.

blood vessels, and hemorrhage compared with the vehicle group (Figure 2; Table II). NMP 0.06 mg/ear (171.60 ± 5.98 µm) and dexamethasone 0.10 mg/ear (104.5 ± 5.87 µm) reduced the relative distance between epithelial ridges as quantified by histological analysis compared to the vehicle group (234.10 ± 13.05 µm) (Figure 2c).

TNF-α, IL-6, IL-1β, IL-10, INF-γ, and MCP-1 tissue levels

The application of TPA resulted in a significant increase in tissue levels of TNF-α, IL-6, IL-1β, IL-10, INF-γ, and MCP-1 in the ear tissues of the vehicle group compared to the naive group. Specifically, there was a 580%, 912%, 100%, 713%, 249%, and 483% increase in TNF-α, IL-6, IL-1β, IL-10, INF-γ, and MCP-1, respectively. In contrast, treatment with NMP (0.06 mg/ear) reduced tissue levels of TNF-α, IL-6, IL-1β, IL-10, INF-γ, and MCP-1 by 48%, 29%, 32%, 45%, 52%, and 41%, respectively, compared to the vehicle group. Furthermore, treatment with Dexamethasone (0.10 mg/ear) reduced the same parameters by 40%, 65%, 41%, 59%, 73%, and 84%, respectively, compared to the vehicle group (Figure 3).

Relative expression of mRNA by RT-PCR

TPA increased mRNA expression for TNF-α, IL-6, and IL-1β in the tissues of the ears of the vehicle group compared to the naive group by 92%, 83%, and 93%, respectively. While treatments with NMP (0.06 mg/ear) and dexamethasone (0.10 mg/ear) reduced the expression of mRNA for TNF-α, IL-6, and IL-1β in the ear tissues compared to the vehicle group. NMP reduced the mRNA expression of TNF-α, IL-6, and IL-1β mRNA by 67%, 78%, and 45%, respectively, compared to the vehicle group, while for dexamethasone the reductions were 96%, 80%, and 92%, respectively (Figure 4).

DISCUSSION

The bark and leaves of *S. obtusifolium* are used in Brazilian folk medicine for their anti-inflammatory action (Oliveira et al. 2012, Ribeiro et al. 2018). Previously, the analysis of metabolites from *S. obtusifolium* leaves identified the presence of flavonoids, saponins, and triterpenes as the main components of its composition (Oliveira et al. 2012). An aqueous extract is obtained from the leaves of *S. obtusifolium*, whose methanolic fraction is rich in *N*-methyl-(2*S*,4*R*)-*trans*-4-hydroxy-L-proline

Table II. Histological analysis scores of the ears tissues of animals treated with NMP in TPA-induced contact dermatitis. Scores expressed as median (minimum - maximum) of 8 animals/group. Null (0), mild (1), moderate (2) and severe (3). [a] $p < 0.05$ vs Naive Group and [b] $p < 0.05$ vs Vehicle Group (Ethanol). For the analysis, the Kruskal-wallis test was used followed by the Dunns post-test.

Group	Edema	Inflammatory Cell Infiltration	Ectatic Blood Vessels	Vascular Neoformation	Hemorrhage
Naive	0 (0-1)	0 (0-1)	0 (0-2)	0 (0-0)	0 (0-0)
Vehicle	2 (1-3) [a]	3 (3-3) [a]	2 (1-3) [a]	0 (0-1)	1 (0-1) [a]
NMP (0.06 mg/ear)	0 (0-1) [b]	1 (0-2) [b]	1 (0-1) [b]	0 (0-1)	0 (0-0) [b]
Dexamethasone (0.10 mg/ear)	0 (0-0) [b]	0 (0-0) [b]	0 (0-1) [b]	0 (0-0)	0 (0-0) [b]

(NMP), enabling the isolation of NMP from these leaves (Aquino et al. 2017).

Aquino et al. (2016) showed that topical administration of the methanolic extract of *S. obtusifolium* exerted an anti-edematogenic effect on *Croton*-oil- and phenol-induced ear edema in mice, but the mechanisms of action were not investigated. The present study provides evidence that topical administration of NMP exerts anti-inflammatory activity on TPA-induced irritant contact dermatitis in mice by decreasing the expression of pro-inflammatory cytokines.

Irritant contact dermatitis (ICD) is an inflammatory skin disease caused by chemicals. The initial event of ICD is the disruption of the epidermal barrier by the injurious agent, increasing skin permeability and induction of the release of proinflammatory cytokines, such as IL-1β, TNF-α, IL-6, and IL-8, along with the release of vascular endothelial growth factor (VEGF) by keratinocytes (Nedoszytko et al. 2014, Turner et al. 2014, Mostosi & Simonart 2016). Thus, counter-regulatory cytokines appear in an attempt to control inflammation, such as IL-10 and the IL-1 receptor antagonist (Wei et al. 2011, Esser & Martin 2017, Pattarini & Soumelis 2017).

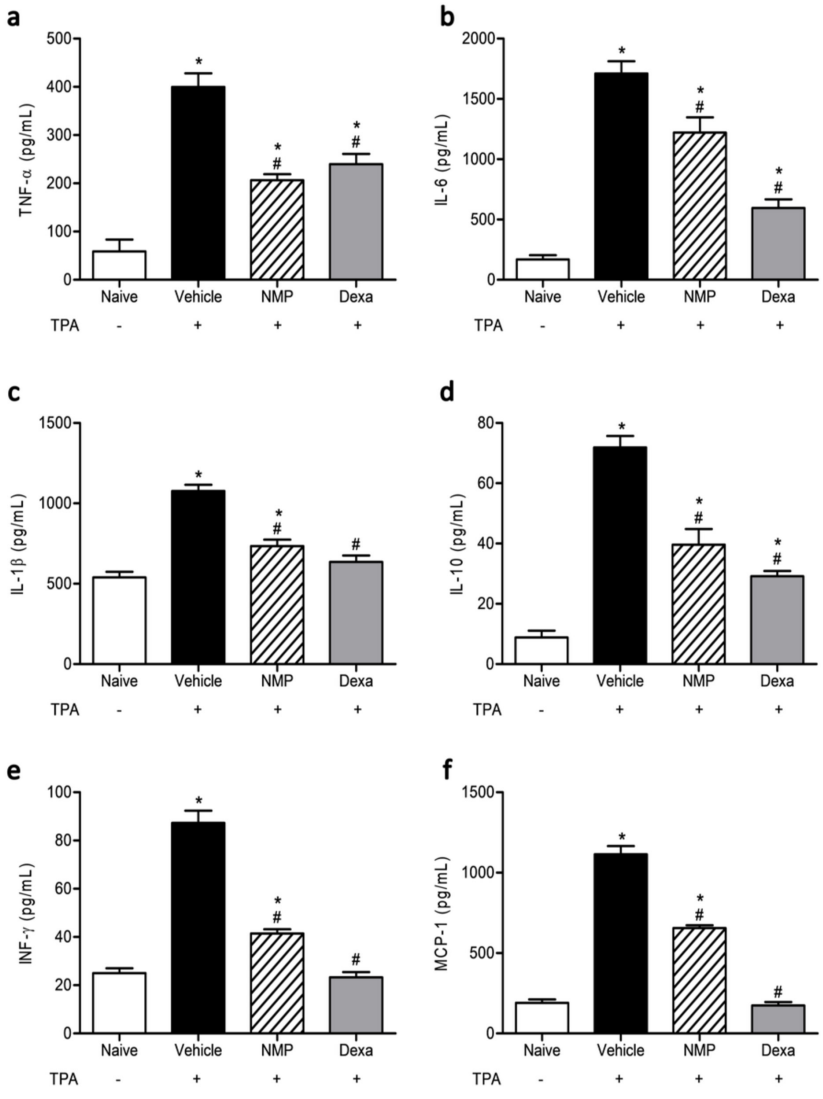

Figure 3. Effect of NMP on TNF-α (a), IL-6 (b), IL-1β (c), IL-10 (d), INF-γ (e) and MCP-1 (f) levels on TPA-induced in contact dermatitis in mice. NMP (0.06 mg/ear) and dexamethasone (Dexa, 0.10 mg/ear). Values are expressed as the mean ± SEM (n = 8 mice per group). Data were analyzed by one-way ANOVA followed by the Tukey's test. * p < 0.05 *vs* naive group and # p < 0.05 *vs* vehicle group.

TPA is a known chemical skin irritant and ICD inducer in murine models. Its action is believed to occur when keratinocytes and epidermal dendritic cells produce inflammatory inducers such as pro-inflammatory cytokines and eicosanoids when they come into contact with TPA, inducing the onset of the acute inflammatory process and recruiting immune cells such as neutrophils, macrophages and mast cells from tissue surrounding the lesion (Zhang et al. 2014, You et al. 2019).

TPA stimulates a wide variety of intracellular pathways through protein kinase C (PKC) activation, including PI3K/AKT/NF-κB signaling, STAT3 signaling, and the consequent generation of inflammatory mediators such as TNF-α, IL-1β, IL-6, iNOS, COX-2, keratinocyte-derived chemokine and macrophage inflammatory protein (MIP-2), among other chemokines and prostaglandins with roles in the maintenance and amplification of the inflammatory process (Rakariyatham et al. 2019, Nakamura et al. 2020).

In our evaluation, topically administered NMP was able to significantly reduce edema and migration of neutrophils into the ear tissue induced by TPA, observed by the reduced activity of myeloperoxidase (MPO), an enzyme indicative of the presence of neutrophilic granulocytes in inflamed tissue (Derin et al. 2006, Gordon et al. 2008). The ability of NMP to reduce ear edema and MPO activity was checked by histological evaluation, where it reduced the mean distance between epithelial ridges and the amount of cellular inflammatory infiltrate, in addition to reducing other histological parameters indicative of tissue injuries, such as tissue edema, ectatic blood vessels, and hemorrhage.

Previously, Aquino et al. (2017) observed the reduction of carrageenan-induced paw edema in mice treated orally with NMP, verifying the inhibition of increased immunoreactivity of iNOS, COX-2, TNF-α, and NF-kB in the injured tissue. Our results are in agreement with these findings since they demonstrate a reduction in the levels of the pro-inflammatory cytokines TNF-α, IL-6, IL-1β, IFN-γ, and MCP-1, in addition to negatively regulating the expression genes for TNF-α, IL-6, and IL-1β in ear tissues subjected to TPA-induced contact dermatitis and treated topically with NMP.

TNF-α and IL-6 released by dendritic cells are key mediators of inflammatory and immune responses. This expression is controlled by TLR4-stimulated activation of NF-κB via regulation of the transcriptional activities of these cytokines in monocytes and dendritic cells (Wei et al. 2011). This possibly explains the reduction in the levels and expression of these inflammatory cytokines,

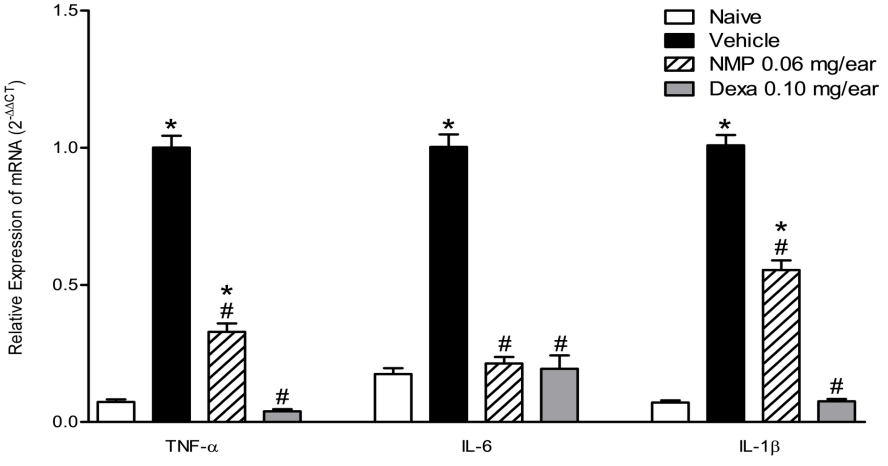

Figure 4. Effect of NMP on TNF-α, IL-6 and IL-1β gene expression on TPA-induced contact dermatitis in mice. Values are expressed as the mean ± SEM (n = 8 mice per group). NMP (0.06 mg/ear) and dexamethasone (Dexa, 0.10 mg/ear). Data were analyzed by one-way ANOVA followed by the Tukey's test. * $p < 0.05$ *vs* naive group and # $p < 0.05$ *vs* vehicle group.

and consequently of polymorphonuclear cells found in the present work.

MCP-1 is an important chemokine that attracts monocytes/macrophages, T cells, and dendritic cells to the inflammation site (Deshmane et al. 2009, Gschwandtner et al. 2019). In our study, TPA induced inflammatory cell chemotaxis by increasing tissue MCP-1 levels, while NMP and dexamethasone treatments significantly decreased this phenomenon.

IL-1β activates dendritic cells and T cells, with increased production of cytokines, chemokines, and VEGF and induction of the expression of adhesion molecules in endothelial cells and fibroblasts, which together lead to the perpetuation and intensification of cutaneous inflammation (Wei et al. 2011, Esser & Martin 2017, Pattarini & Soumelis 2017). IFN-γ, on the other hand, positively regulates several pro-inflammatory parameters, such as some interleukins, caspases, and TNF-α, being able to stimulate macrophages and dendritic cells (Schoenborn & Wilson 2007, Miller et al. 2009, Kopitar-Jerala 2017). In our study, TPA induced an increase in tissue levels of IL-1β and IFN-γ, which is related to the inflammatory process, while treatments with NMP and dexamethasone significantly decreased tissue levels of IL-1β and IFN-γ.

Although it has historically been considered an anti-inflammatory cytokine, IL-10 is a pleiotropic interleukin that has a dual role in the inflammatory processes (Ouyang et al. 2011, Mannino et al. 2015). We observed that TPA increased IL-10 levels in the ear tissues, while the groups treated with NMP and dexamethasone had a reduction in tissue levels of this cytokine. The discrepancy in IL-10 levels presented here can be explained by the results previously reported by Rakariyatham et al. (2019), wherein in the same model of TPA-induced contact dermatitis, the authors obtained results similar to ours. One explanation for this phenomenon is the correlation between low levels of IL-10 in the tissues of the ears undergoing treatment, with evidence of less tissue inflammation, which consequently produces less need for high levels of anti-inflammatory cytokines, such as IL-10.

NMP has a pyrrolidine ring in its structure, as does proline and its derivatives. The pyrrolidine ring has multiple functions, such as antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties (Bamdad et al. 2015, Vitali 2015, Bamdad et al. 2017). This fact is already well documented and has a direct correlation with the results of several studies. Andrade et al. (2018) demonstrated that proline produced antioxidant and anti-inflammatory activities in the brain of rats treated with bacterial lipopolysaccharide (LPS). Ugwu et al. (2018) reported that lower molecular weight molecules obtained through chemical derivations of L-proline and hydroxyproline showed anti-inflammatory activity in a carrageenan-induced paw edema model in rats. Kumar & Yin (2018) and Zhi et al. (2022) correlated more complex proline-derived structures with their immunomodulatory action.

The negative modulatory effect of NMP on the expression of pro-inflammatory cytokines in TPA-induced contact dermatitis seen in this study may have implications for its future use as a topical anti-inflammatory substance.

CONCLUSION

Our results suggest that NMP acts as a topical anti-inflammatory agent that decreases the production of inflammatory cytokines in TPA-induced ICD, resulting in reduced tissue damage, suggesting that this natural product should be further evaluated for development as a

therapeutic agent for the control of inflammatory skin diseases.

Acknowledgments

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PAULO IURY G. NUNES[1,2]
https://orcid.org/0000-0003-2996-4340

ANA FLÁVIA S.C. VIANA[2]
https://orcid.org/0000-0001-9843-2503

GREYCE L. SASAHARA[3]
https://orcid.org/0000-0001-6944-7247

SABRINA M. DOS SANTOS[4]
https://orcid.org/0000-0002-5106-3322

ANA PAULA N.N. ALVES[5]
https://orcid.org/0000-0002-5090-6877

EDILBERTO R. SILVEIRA[4]
https://orcid.org/0000-0003-4178-0296

FLÁVIA A. SANTOS[1,2]
https://orcid.org/0000-0003-3625-8540

[1]Programa de Pós-Graduação em Ciências Médicas, Universidade Federal do Ceará, Faculdade de Medicina, Rua Professor Costa Mendes, 1608, Rodolfo Teófilo, 60430-140 Fortaleza, CE, Brazil

[2]Universidade Federal do Ceará, Faculdade de Medicina, Departamento de Fisiologia e Farmacologia, Laboratório de Produtos Naturais, Rua Coronel Nunes de Melo, 1315, Rodolfo Teófilo, 60430-270 Fortaleza, CE, Brazil

[3]Pós-Graduação em Biotecnologia na Saúde, Universidade Federal do Ceará, Faculdade de Farmácia, Odontologia e Enfermagem, Laboratório de Imunologia, Rua Alexandre Baraúna, 949, Rodolfo Teófilo, 60430-160 Fortaleza, CE, Brazil

[4]Universidade Federal do Ceará, Centro de Ciências, Departamento de Química Orgânica e Inorgânica, Campus do Pici, Bloco 902, Av. Mister Hull, s/n, Pici, 60455-760 Fortaleza, CE, Brazil

[5]Universidade Federal do Ceará, Faculdade de Farmácia, Odontologia e Enfermagem, Departamento de Clínica Odontológica, Rua Monsenhor Furtado, s/n, Rodolfo Teófilo, 60430-355 Fortaleza, CE, Brazil

Correspondence to: **Flávia Almeida Santos**
E-mail: fasufc@gmail.com

Author contributions

Paulo Iury G. Nunes, Ana Flávia S. C. Viana, and Flávia A. Santos (leader) conceived the study, designed pharmacological assays, and drafted and revised the manuscript. Paulo Iury G. Nunes, Ana Flávia S. C. Viana, and Greyce L. Sasahara carried out the pharmacological assays. Ana Paula N. N. Alves performed histopathological analysis. Edilberto R. Silveira and Sabrina M. dos Santos performed the collection and identification of plant material, preparation of the extract and fraction, and NMP isolation and characterization.