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HEALTH SCIENCES

Topical hydrogel containing *Achyrocline satureioides* oily extract (free and nanocapsule) has anti-inflammatory effects and thereby minimizes irritant contact dermatitis

VANESSA S. MACHADO, CAMILA CAMPONOGARA, SARA M. OLIVEIRA, MATHEUS D. BALDISSERA, MICHELE R. SAGRILLO, SAMANTA DA S. GUNDEL, ANA PAULA T. DA SILVA, ALINE F. OURIQUE, BRUNA KLEIN, ROGER WAGNER, ROBERTO C.V. SANTOS & ALEKSANDRO S. DA SILVA

Abstract: Inflammatory dermatoses are prevalent worldwide, with impacts on the quality of life of patients and their families. The aim of this study was to determine the anti-inflammatory effects of *Achyrocline satureioides* oily extracts and nanocapsules on the skin using a mouse model of irritant contact dermatitis induced by croton oil, and a skin inflammation model induced by ultraviolet B (UVB) radiation. The mice were treated with 15 mg/ear oily extract (HG-OLAS) or nanocapsules (HG-NCAS) of *A. satureioides* incorporated into Carbopol® 940 hydrogels. We found that HG-OLAS and HG-NCAS formulations reduced ear edema in croton oil-induced lesions with maximum inhibitions of 54±7% and 74±3%, respectively. HG-OLAS and HG-NCAS formulations decreased ear edema induced by UVB radiation (0.5 J/cm^2), with maximum inhibitions of 68±6% and 76±2% compared to the UVB radiation group, respectively. HG-OLAS and HG-NCAS modulated myeloperoxidase (MPO) activity after croton oil induction. Furthermore, croton oil and UVB radiation for 6 and 24 h, respectively, stimulated polymorphonuclear cells infiltration. The topical treatments reduced inflammatory processes, as shown by histological analysis. Together, the data suggest that topical application of *A. satureioides* oily extracts and nanocapsules produced antiedematogenic and anti-inflammatory effects. They constitute a compelling alternative for treatment of skin injuries.

Key words: UVB radiation, croton oil, macela, nanocapsules, skin injuries, anti-inflammatory.

INTRODUCTION

The skin operates as a barrier that protects the body against a variety chemical and physical agents, including those caused by ultraviolet (UV) radiation, chemical components and infectious agents (Pasparakis et al. 2014). Losses in skin integrity contribute to the development of several inflammatory skin diseases, including psoriasis, allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD) (Yeom et al. 2012, Horinouchi et al. 2013). In ICD caused by contact with irritants (Pinto et al. 2015), partial loss of skin integrity may progress to the release of pro-inflammatory mediators (monocytes and neutrophils) that play important roles in inflammation of the skin (Yeom et al. 2012, Piana et al. 2016).

Skin complications are commonly treated with non-steroidal anti-inflammatory and topical corticosteroids. Nevertheless, long-term use of topical corticosteroids gives rise to several

adverse effects, including cutaneous atrophy, telangiectasias, changes in the healing process, intense pruritis, dryness, and burning sensations (Unzueta & Vargas 2013, Xiao et al. 2015). Non-steroidal anti-inflammatory drugs cause complications related to the cardiovascular, gastrointestinal, and renal systems (Unzueta & Vargas 2013). These findings suggest that there is a need for safer and more effective therapies to improve the quality of life of patients afflicted by these diseases. The mainstay of treatment for skin damage involves restoring skin-barrier function.

Plant oils improve wound closure phases, promoting keratinocyte proliferation, remodeling of the extracellular matrix and controlling inflammation (Serra et al. 2017). In Brazil, because of its substantial biodiversity, medicinal plants have become important sources of research into development of new therapeutic agents, many of which have already been accepted by the public health system (the *SUS*) (De Figueredo et al. 2014).

In Brazil, *A. satureioides* (Asteraceae family) is a plant widely used in popular culture in the form of infusion and decoction (Stolz et al. 2014, Yamane et al. 2016). This plant is popularly known as *macela* or *marcela*. In traditional medicine, this plant is used to treat gastrointestinal, inflammatory, and respiratory complications, among others. The therapeutic effect may be associated with the presence of bioactive compounds that hypothetically act synergistically (Da Silva et al. 2016, Yamane et al. 2016).

A. satureioides is widely cited in scientific research for its ability to induce cell proliferation *in vivo*, suggesting potential healing action, in addition to promoting the reduction of inflammatory mediators and the recruitment of leukocytes *in vitro* (Barioni et al. 2013, Alerico et al. 2015). Nevertheless, plants substances are not very stable. Nanotechnology appears as a promising alternative to improve product stability and protect the active compounds from chemical or physical degradation (Guterres et al. 2007).

Therefore, in this study, we investigated the topical anti-inflammatory effects of *A. satureioides* oily extracts and nanoencapsulation delivery mechanisms for treatment of skin inflammation and irritant contact dermatitis in mice.

MATERIALS AND METHODS

Headspace solid-phase microextraction-mass chromatograph – mass spectrometer (HS-SPME-GC-MS) analysis of *Achyrocline satureioides* oily extract

A. satureioides oily extract was purchased from Mundo dos Oleos S/A (Brasília, Brazil, 100% purity), obtained from *A. satureioides* flowers, leaves and stalks parts by maceration in natural oil. This was followed by cold pressing, filtration and headspace solid-phase microextraction (HS-SPME). Sample preparation and terpene extraction of *A. satureioides* oily extracts were performed. The compounds were analyzed using a gas chromatograph coupled a mass espectrometer (GC-MS) as described by Ivanova-Petropulos et al. (2015), with modifications.

Polymer swelling test

Poly (ε-caprolactone) films were obtained by complete dissolution of 0.5 g of the polymer in 10 ml of acetone at 40 °C. The solvent was removed, and films were subsequently immersed in *A. satureioides* oily extract. The polymer swelling test was performed following the methodology of Venturini et al. (2015) with some modifications. The films were removed

from the oil, dried and weighed on an analytical balance at 0, 24, 48, 72, and 96 hours on days 7, 15, 30, 45, 60, and 90.

Nanocapsule formulation and characterization

A. satureioides oily extract nanocapsules suspensions were prepared according to the interfacial deposition of performed polymer (Fessi et al. 1989), described in detail by Ritter et al. (2017). The organic phase was composed of poly(ε-caprolactone) (PCL) (1%), sorbitan monoestearate (0.766%), *A. satureioides* oily extract (3%) and acetone (67 ml). The aqueous phase was composed of polysorbate 80 (0.766%) and ultrapure water (134 ml). After homogenization of each step separately at 40 °C for 1 hour, the organic phase was poured into the aqueous phase and stirred for 10 minutes. Solvent removal was performed under reduced pressure on a rotary evaporator to a final volume of 25 ml.

The nanoparticles were characterized in terms of the mean particle size, polydispersity index (PdI) and zeta potential by electrophoresis using a Zeta sizer (nano-ZS model ZEN 3600, Malvern). The electrophoretic mobility technique was used and the formulations were diluted (500 times) in sodium chloride solution (10 mM), described in detail by Ritter et al. (2017). For size and PdI evaluations, the formulations were diluted in ultrapure water (500 times) and analyzed using the dynamic light scattering technique. The hydrogen potential (pH) was evaluated using a potentiometer (DM-22, Digimed) previously calibrated with standard solutions of pH 4.0 and 7.0. The results were expressed as mean ± standard deviation from three lots (three readings from each lot) (De Godoi et al. 2017).

Hydrogel preparation and characterization

Three hydrogel formulations were created: hydrogel containing nanocapsules of *A. satureioides;* hydrogel containing oily extract of *A. satureioides*; and base hydrogel. These were prepared according to the protocol described by Alves et al. (2005) with modifications. For development of hydrogels, Carbopol 940® (6 g), paraben preservative solution (1 g) and ultrapure water (91 ml) were used. The hydrogel containing nanocapsules was composed of the dispersion of Carbopol 940® (6%), triethanolamine (0.5%), sorbitol (5%), imidazolidinyl urea (1%) and 25 ml of nanocapsules containing *A. satureioides* oily extract. In the case of the hydrogel containing oily extract, the nanocapsules suspension was replaced by a dispersion of the oily extract (3%) polysorbate 80 (0.766%) and sorbitan monooleate (0.766%). In the base hydrogel, the addition of the nanocapsules suspension was omitted and was replaced with ultrapure water (25 ml).

The size and PdI of the hydrogels containing *A. satureioides* nanocapsules were determined after aqueous redispersion of the formulation using a dynamic light scattering technique. Briefly, 0.02 g of hydrogel were diluted in 10 ml of ultrapure water and subsequently filtered (0.45 µm) for analysis. To determine the pH of the hydrogel containing *A. satureioides* nanocapsules, hydrogel containing *A. satureioides* oily extract and base hydrogel, 1 g of the hydrogel was diluted in 10 ml of ultrapure water, and the values were determined using potentiometry (Marchiori et al. 2010).

Experimental design

Animals

For the *in vivo* study, the experiment design included 96 male Swiss mice, (up to 60 days,

weighing 25 ± 5 g). Animals were maintained in boxes with five animals each, under a 12-h light/dark cycle with controlled temperature and humidity (25 °C, 70% respectively), respecting their circadian rhythm, and were fed with commercial feed and water *ad libitum*. All animals procedures were approved by the Ethics Committee on Animal of Universidade Federal de Santa Maria under protocol number (6581200716/2016).

Treatments

The experimental design was divided into two models: irritant contact dermatitis induced by croton oil, and skin inflammation induced by UVB radiation. The experimental groups for croton oil-induced irritant contact dermatitis model were composed by following groups (n = 8): naïve group (negative control); croton oil group; hydrogel group (treated with base hydrogel); HG-OLAS group (treated with hydrogel containing 3% *A. satureioides* oily extract); HG-NCAS group (treated with hydrogel containing 3% *A. satureioides* nanocapsules oily extract); and dexamethasone group (0.5% dexamethasone hydrogel as a positive control).

The UVB radiation-induced skin inflammation model included the following groups (n = 8): naïve group (negative control—no inflammation process induction and no topical treatment); UVB group; hydrogel group (treated with base hydrogel); HG-OLAS group (treated with hydrogel containing 3% *A. satureioides* oily extract); HG-NCAS group (treated with hydrogel containing 3% *A. satureioides* nanocapsule oily extract); and sulfadiazine group (sulfadiazine hydrogel 3% as a positive control).

Mice were topically treated on the ear surface with various semisolid formulations at 15 mg/ear immediately after the inflammatory stimulus.

Model of croton oil-induced ear edema

Irritant contact dermatitis was mimicked using a single topical administration of croton oil (1 mg/ear; diluted in 20 µl acetone) on the right ear. The hydrogel formulations (base hydrogel, HG-OLAS, HG-NCAS and dexamethasone) were applied immediately after irritant agent administration. Ear thickness (expressed in µm) was evaluated before and 6 h after croton oil application using a digital micrometer (Digimess) in animals anesthetized with isoflurane (Piana et al. 2016). The micrometer was applied near the tip of the ear just distal to the cartilaginous ridges. Six hours after croton oil administration, the animals were euthanized and ear biopsies were taken for further analysis (De Brum et al. 2016, Piana et al. 2016).

Model of UVB radiation-induced ear edema

The UVB radiation source was a Philips TL40W/12 RS lamp (Medical-Eindhoven, Holland) mounted 20 cm abe the table on which the animals were placed, using a continuous light spectrum exposure between 270 and 400 nm with peak emission at 313 nm. UVB output (80% of the total UV irradiation) was measured using a model IL-1700 Research Radiometer (International Light, USA; calibrated by IL service staff) with radiometer sensors for UV (SED005) and UVB (SED240). The UVB irradiation rate was 0.27 mW/cm^2 and the dose used was 0.5 J/cm^2. The mice were first anesthetized with a single intraperitoneal injection (90 mg/kg of ketamine and 3 mg/kg of xylazine) and then exposed to UVB radiation. Only the right ear of each animal was exposed to UVB radiation. The hydrogel formulations (base hydrogel, HG-OLAS, HGNCAS and silver sulfadiazine at 15 mg/ear) were immediately applied after the UVB radiation. Ear edema was measured before the inflammatory stimulus and 24 h after the UVB exposure, expressed as

µm. Twenty-four hours after UVB radiation, the animals were euthanized and ear biopsies were taken for further analysis (Marchiori et al. 2017, Pegoraro et al. 2017).

Assessment of leukocyte infiltration

Myeloperoxidase (MPO) enzyme activity was evaluated in the ear samples after 24 h (skin inflammation UVB-induced) or 6 h (irritant croton oil-induced) of stimulus. Tissue samples were removed and homogenized in a motor-driven homogenizer in acetate buffer (8 mM, pH 5.4) containing hexadecyltrimethylammonium bromide (Phanse et al. 2012), as previously described (Camponogara et al. 2019a). The enzyme activity value was assessed colorimetrically at 630 nm using a microplate reader. The results were expressed as optical density (OD)/ml of sample.

Histological analysis and polymorphonuclear cells

After 24 h (UVB-induced skin inflammation) or 6 h (croton oil-induced irritant contact dermatitis), the right ear was removed and fixed in an aflac solution (16:2:1 mixture of ethanol 80%, formaldehyde 40% and acetic acid). Each sample was embedded in paraffin, sectioned at 5 µm and stained with hematoxylin-eosin. A representative area was observed under a light microscope fitted with 20× and 40× objectives to assess the inflammatory cellular response (Piana et al. 2016, Marchiori et al. 2017). The polymorphonuclear cells were counted at 20× and were expressed as number polymorphonuclear cells per field (two fields from three distinct histological slides of each group were analyzed).

Statistical analysis

The maximum inhibitory effect was calculated based on the response of the control groups. The statistical significance between the groups was assessed by one or two (repeated measures) one-way analysis of variance (ANOVA) followed by a post-hoc Tukey or Bonferroni tests, respectively. $P < 0.05$ denoted significant differences between groups. All tests were carried out using GraphPad 6.0 Software (San Diego, CA, USA), and Image J software (inflammatory cells count). No statistical methods were used to predetermine sample sizes; nevertheless, our sample sizes were similar to those reported in previous publications in the field. The results were presented as mean ± standard error of mean (SEM) and as geometric means with their respective 95% confidence limits.

RESULTS

In vitro assay

Composition of A. satureioides oily extract

The headspace oily extract produced 17 volatile terpenoids compounds, in decreasing order of quantity: α-pinene (65.8%), limonene (7.9%), eucalyptol (5.0%), α-copaene (3.9%), o-xylene (2.8%), camphene (2.7%), α-phellandrene (2.6%), β-phellandrene (2.0%), cis-verbenol (1.5%), caryophyllene (1.2%), cis-alpha-bisabolene (1.0%), caryophyllene oxide (0.9%), methyl salicylate (0.8%), β-myrcene (0.7%), linalool (0.4%), p-xylene (0.4%) and anethole (0.3%).

Polymer swelling test

Polymeric films immersed in *A. satureioides* oily extract were evaluated. The polymer film masses remained constant over 90 days (Fig. 1), suggesting that the *A. satureioides* oily extract

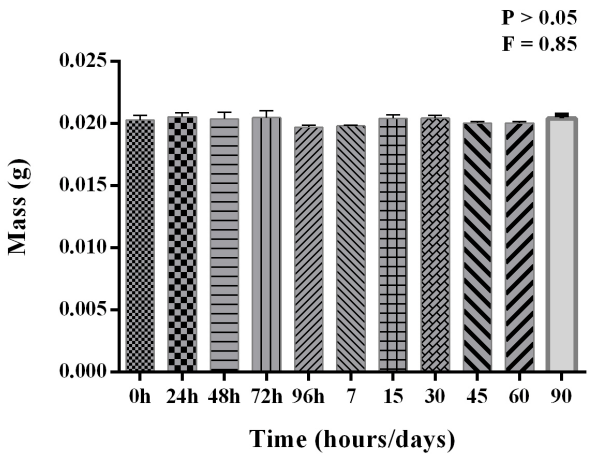

Figure 1. The polymer swelling test over 90 days. Each bar represents the mean ± S.E.M (n = 3).

did not dissolve the polymer film, assuring the potential to obtain polymeric nanocapsules containing the oily extract.

Characterization of nanocapsules and hydrogel containing A. satureioides oily extract or nanocapsules

The three suspensions were evaluated with respect to their physical-chemical properties. The characterization of nanocapsules and hydrogels containing *A. satureioides* oily extract or nanocapsulated are shown in Table I. The hydrogen potential (pH) of the base hydrogel was 7.3 ± 0.04 and that of the oily extract hydrogel was 7.0 ± 0.04.

In vivo assay

Anti-inflammatory effect of A. satureioides oily extract hydrogel formulations on irritant contact dermatitis croton oil-induced

We measured the effect of hydrogel formulations on irritant contact dermatitis induced by croton oil application. A topical application of croton oil on the ears of the mice increased the ear thickness with an E_{max} of 160 ± 10 µm after 6 h, compared to the naïve group (Fig 2a). The HG-OLAS, HG-NCAS, and dexamethasone formulations reduced ear edema in croton oil-treated mice with I_{max} of 54 ± 7%, 74 ± 3%, and 66 ± 4%, respectively, when compared to the control oil group. HG-NCAS had an antiedematogenic effect as effective as positive control in the skin inflammation model induced by croton oil.

The irritant agent application by croton oil gave significantly greater MPO activity than that of the naïve group. All topical treatments reduced MPO enzymatic activity with an I_{max} of 30 ± 12% (HG-OLAS), 82 ± 1% (HG-NCAS) and 33 ± 9% (dexamethasone), when compared to the croton oil group. The nanoencapsulation process contributed to the HG-NCAS anti-inflammatory effect, as this hydrogel formulation was more effective in reducing MPO activity than were the hydrogel formulations (HG-OLAS and dexamethasone) (Fig. 2b).

Histologically, we performed quantitative analysis of numbers of polymorphonuclear cells at the ear tissue and observed that application of croton oil significantly increased polymorphonuclear cell infiltration (179 ± 10 inflammatory cells per field) at 6 h after the stimulus when compared to the number in the naïve group (Fig. 2c). All topical treatments showed lower levels of inflammatory cell infiltration (79 ± 6 to HG-OLAS, 35 ± 4 to HG-NCAS and 39 ± 5 to dexamethasone) when compared with the croton oil group. HG-NCAS was more effective in reducing inflammatory cell infiltration than was the HG-OLAS group.

These results were confirmed by histological sections of ear tissue, showing that the inflammatory process generated ear edema and inflammatory cell infiltration, and that all

Table I. Characterization of *Achyrocline satureioides* nanocapsules and hydrogels containing nanocapsules after aqueous redispersion.

Nanocapsules (n = 3)				Hydrogel containing nanocapsules (n = 3)		
Size (nm)	PdI	Zeta potential	pH	Size (nm)	PdI	pH
220 ± 1.28	0.15 ± 0.01	13.6 ± *0.75*	5.5 ± 0.12	216 ± 1.65	0.13 ± 0.01	7.0 ± 0.07

topical formulations were effective in reducing both these parameters (Fig. 2d).

Anti-inflammatory effects of A. satureioides oily extract hydrogel formulations on inflammation induced by UVB-radiation

To evaluate the anti-inflammatory effect of *A. satureioides* oily extract hydrogel formulations, as well as the influence of the nanoencapsulation process on the inflammatory parameters, a UVB irradiation-induced skin inflammation model was used. The UVB radiation on mice ear caused a marked increase in skin thickness with an E_{max} of 130 ± 12 µm) compared to the naïve group after 24 h of stimulus (Fig. 3a). The HG-OLAS and HG-NCAS formulations significantly decreased UVB radiation-induced ear edema with I_{max} of 68 ± 6% and 76 ± 2% compared to the UVB-irradiated group, respectively. The nanoencapsulation process enhanced the *A. satureioides* oily extract antiedematogenic effect compared to the treatment of hydrogel-containing *A. satureioide* free oily extract group. Silver sulfadiazine, used as a positive (treatment) control, reduced UVB radiation-induced ear edema by 87 ± 4% (Fig. 3b). The antiedematogenic effect of HG-NCAS was similar to that of the silver sulfadiazine group. Interestingly, no significant differences were observed in myeloperoxidase activity after 24 h UVB radiation between the analyzed groups (data not shown).

The effect HG-OLAS and HG-NCAS on inflammatory cell infiltration was evaluated using histological analysis. The irradiation with a UVB source promoted a marked presence of edema characterized by intense increase of the skin thickness, especially at the dermis, in addition to intense inflammatory cell infiltration (150 ± 22 inflammatory cells per field) compared to the naïve group. All topical treatments reduced ear edema and inflammatory cell infiltration (79 ± 10 to HG-OLAS, 61 ± 12 to HG-NCAS and 42 ± 10 to silver sulfadiazine inflammatory cell per field) compared to the only UVB-irradiated group, evaluated at 24 h after UVB-irradiation (Fig. 3b and 3c).

DISCUSSION

The present study provides evidence that topical administration of *A. satureioides* oily extract, in free and nanocapsule form, exerts anti-inflammatory effects against irritant-induced contact dermatitis and UVB-induced skin inflammation. To the best of our knowledge, this is the first demonstration of topical application of *A. satureioides* oily extract and nanocapsules, showing anti-inflammatory activity in mouse models of skin inflammation.

Many bioactive compounds extracted from plants are very unstable, making their study impossible. In this context, research shows that the association of isolated bioactive compounds

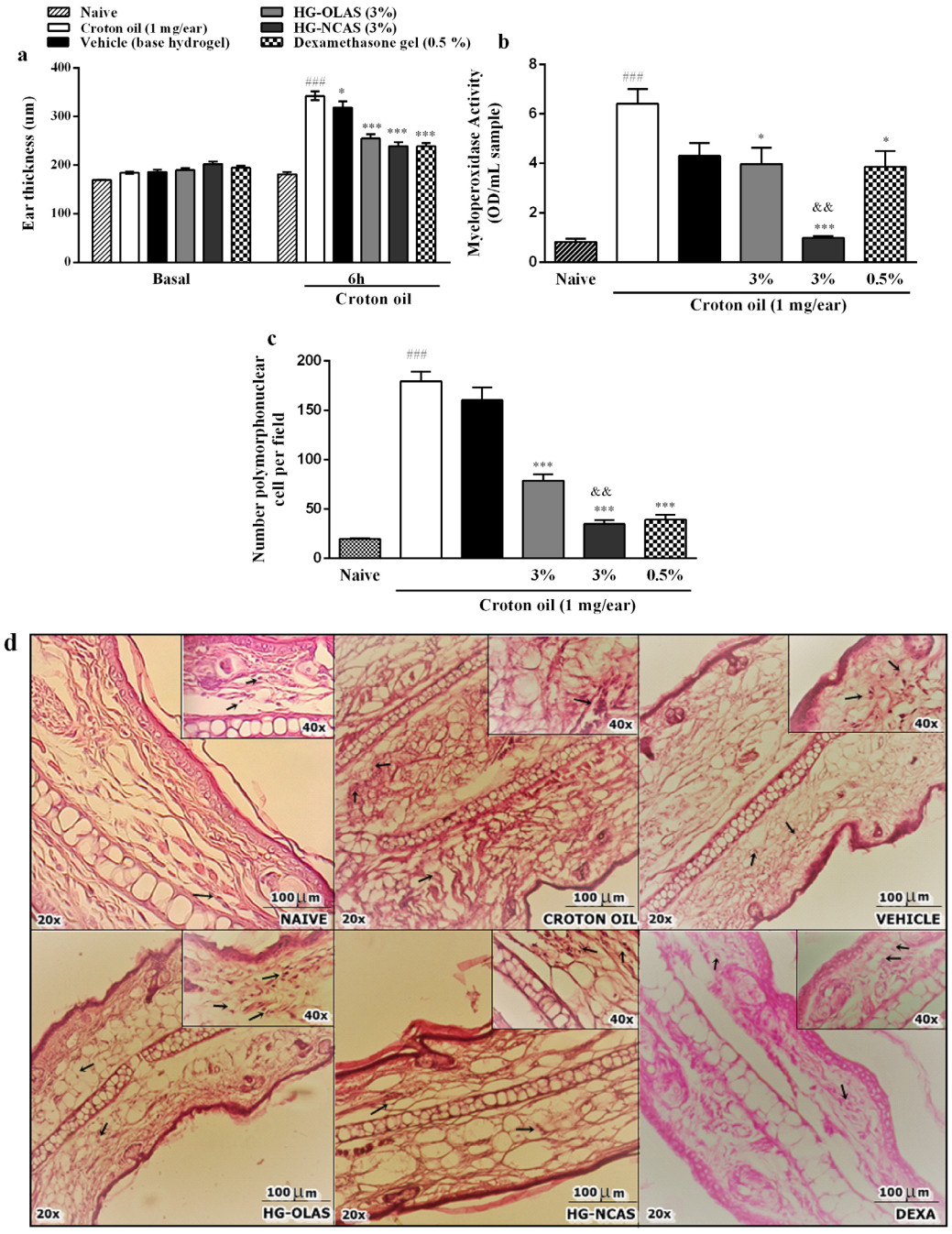

Figure 2. Anti-inflammatory effects of hydrogels containing *A. satureioides* oily extracts (HG-OLAS), hydrogel *A. satureioides* nanocapsule oily extracts (HG-NCAS) and dexamethasone hydrogel formulations on croton-oil-induced contact dermatitis in mice. Ear edema (a), myeloperoxidase activity (MPO) (b) and histological changes (c and d) in mice subjected to croton oil (1 mg/ear) administration. All formulations (15 mg/ear) were applied immediately after croton oil application. Each bar represents the mean + S.E.M (n = 6–8). The arrows indicate inflammatory cells in the ear tissue. $^{###}$P<0.001 when compared to the naive group. ***P <0.001, and *P <0.05 compared to the untreated croton oil group. $^{\&\&}$P <0.01 when compared to the HG-OLAS and dexamethasone formulations [one- or two-way (repeated measures) ANOVA followed by post-Tukey or Bonferroni tests, respectively].

in nanotechnology-based distribution systems may present promising advantages when compared to conventional dosage forms, including improved stability, reduction of side-effects, controlled release of active compounds, and increased permeability of the products (Bidone et al. 2014).

In terms of physico-chemical characterization, the particle size of nanocapsules and hydrogels containing nanocapsules showed similar values, confirming maintenance of the nanocapsule structure in the semisolid (Table I). These results corroborate those of Pegoraro et al. (2017) and Terroso et al. (2009), who showed that nanostructures remained unaltered even after semisolid aqueous redispersion. The nanocapsules maintained pH in the range of skin pH (4.8–5.6), as well as negative zeta potential, suggesting they are suitable for dermal application (Terroso et al. 2009, Silva et al. 2013). The hydrogel formulation presented pH values of 7.0–7.3, suitable for cutaneous administration, according to Alves et al. (2007). The polydispersity index remained between 0.14–0.15, suggesting homogenity of the systems (Terroso et al. 2009). We also observed that the nanocapsules remained stable throughout the observation period of 90 days.

A. satureioides oily extract was effective in reducing all inflammatory parameters in the croton oil and UVB radiation models. Croton oil is an *in vivo* irritant contact dermatitis model widely used in the investigation of compounds with topical anti-inflammatory activity (Phanse et al. 2012). It contains phorbol esters, mainly 12-O-tetradecanoylphorbol-13-acetate (TPA); when applied topically, croton oil promotes an acute inflammatory response, resulting in vasodilatation, inflammatory cell infiltration and edema formation (Piana et al. 2016) as observed in the present study after 6 h (Fig. 2a). These inflammatory events may happen because of protein kinase C (PKC) activation as well as increased phospholipase A_2 (PLA_2) levels resulting from production of inflammatory mediators (Pinto et al. 2015). The hypothesis for the antiedematogenic and anti-inflammatory effect of *A. satureioides* oily extract may be linked to the inhibition process of proinflammatory cytokines induced by the chroton oil.

Nevertheless, UVB radiation exposure is the main cause of sunburn, characterized by inflammation and oxidative damage to the tissue (overproduction of reactive oxygen species and antioxidants systems depletion) (Martinez et al. 2017). In this context, the possible effect of oily extract of *A. satureioides* in free and nanocapsulated on sunburn induced by UVB radiation is related to its anti-inflammatory action, as suggested in this study. However, its therapeutic effect may also be associated with activation of the antioxidant pathways and consequently with control of oxidative damage (Salgueiro et al. 2016).

Skin inflammation is commonly characterized by intense inflammatory cell infiltration, in which neutrophil cells become the first line of defense against pathogens (Németh & Mócsai 2012). In this sense, both skin inflammation models caused induction of this inflammatory parameter and the topical formulations (HG-OLAS and HG-NCAS) were effective in decreasing it. It is worth mentioning that the nanoencapsulation process potentiated the effect of topical anti-inflammatory *A. satureioides* oily extract in this skin inflammation models.

Nanoparticles are effective drug delivery systems. Recently, many researchers reported that the advantage of nanoparticles is their ability to enhance solubility and bioavailability of certain drugs (Ye et al. 2015). Many studies showed the advantages of nanoencapsulation of bioactive compounds in terms of reducing

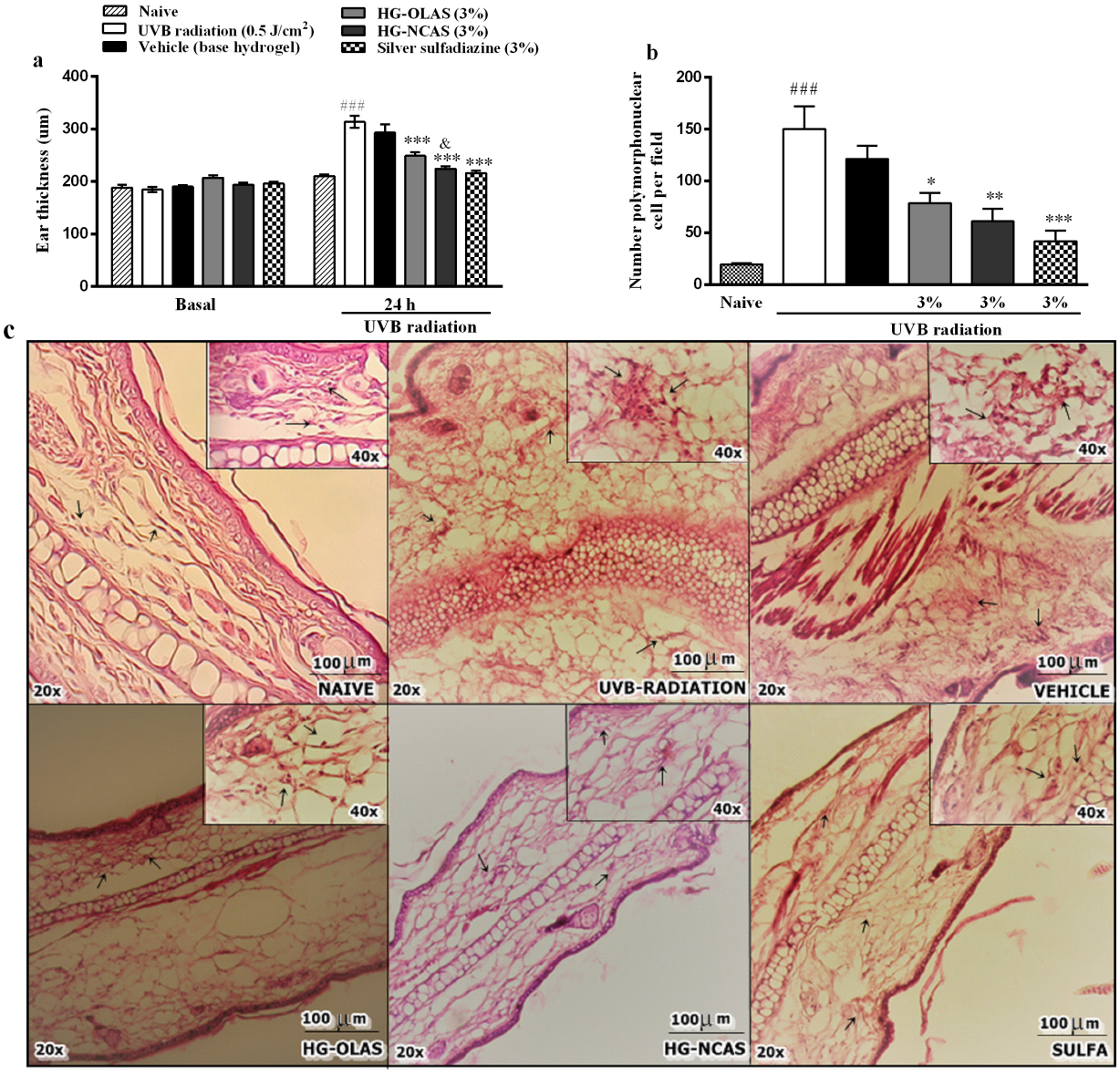

Figure 3. Anti-inflammatory effect of hydrogels containing *A. satureioides* oily extracts (HG-OLAS), *A. satureioides* nanocapsule oily extracts (HG-NCAS) and silver sulfadiazine hydrogel formulations on UVB-radiation-induced skin inflammation in mice. Ear edema (a) and histological changes (b and c) in mice subjected to UVB irradiation (0.5 J/cm^2). All formulations (15 mg/ear) were applied immediately after UVB irradiation. Each bar represents the mean + S.E.M (n = 6–8). The arrows indicate inflammatory cells in the ear tissue. $^{###}$P<0.001 when compared to the naive group. ***P <0.001, **P <0.01 and *P <0.05 when compared to the untreated UVB-irradiated group. $^{\&}$P <0.05 when compared to HG-OLAS formulation [one- or two-way (repeated measures) ANOVA followed by post-Tukey or Bonferroni tesst, respectively].

cutaneous inflammation and oxidative damage (Pegoraro et al. 2017, Marchiori et al. 2017).

Adoption of nanothecnology for use with natural products can be considered a promising alternative to treat skin inflammation diseases; many studies have already discussed such an approach (Camponogara et al. 2019a, b). Moreover, presence of bioactive compound may explain their anti-inflammatory actions. Previous studies have already investigated the phytochemical profile of *A. satureioides*, demonstrating the presence and anti-inflammatory activity of terpenoids, steroids and flavonoids (Di Sotto et al. 2010, Tsang et al. 2016). Yamane et al. (2016) showed that plants of the Asteraceae family have a composition rich in secondary metabolites with potential anti-inflammatory action. Compounds such as α-pinene, limonene, eucalyptol are described in the literature in terms of their potential anti-inflammatory, gastroprotective and analgesic effects (Kim et al. 2015, Yin et al. 2019, de Souza et al. 2019). Previous studies showed the potential anti-inflammatory effect of *A. satureioides* hydroalcoholic extract in *in vivo* and *in silico* models of ulcerative colitis (Da Silva et al. 2016).

From this perspective, the possible anti-inflammatory effect of bioactives compounds of oily extract *A. satureioides* may be related to decreasing levels of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and IL-1β and increasing levels of IL-10, by controlling the activity of enzymes and transcriptional inflammatory pathways mitogen-activated protein kinases (MAPKs), myeloperoxidase (MPO), nuclear factor-kappa β (NF-kβ). Reductions in levels of ROS may contribute to inhibition the inflammatory pathways because oxidative stress and inflammation act synergistically (Martinez et al. 2017, Camponogara et al. 2020). Further studies are need to elucidate the effect antioxidant of topical formulations tested in this study on skin inflammation models.

CONCLUSION

We demonstrated the feasibility of preparing hydrogels containing oily extracts and nanocapsules of *A. satureioides* that had satisfactory physico-chemical characteristics for cutaneous application and that were effective topical anti-inflammatories. The nanoencapsulation process enhanced the topical anti-inflammatory effect of *A. satureioides* oily extracts, suggesting that this is a technology that can generate promising treatments for skin injuries.

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VANESSA S. MACHADO[1,3]
https://orcid.org/0000-0001-6839-3021

CAMILA CAMPONOGARA[2]
https://orcid.org/0000-0003-0283-7931

SARA M. OLIVEIRA[2]
https://orcid.org/0000-0002-2148-9250

MATHEUS D. BALDISSERA[3]
https://orcid.org/0000-0002-3280-8528

MICHELE R. SAGRILLO[5]
https://orcid.org/0000-0001-5659-159X

SAMANTA DA S.GUNDEL[4]
https://orcid.org/0000-0003-4708-6801

ANA PAULA T. DA SILVA[5]
https://orcid.org/0000-0002-9393-0682

ALINE F. OURIQUE[5]
https://orcid.org/0000-0003-0828-774X

BRUNA KLEIN[6]
https://orcid.org/0000-0002-9986-3118

ROGER WAGNER[6]
https://orcid.org/0000-0002-6176-7913

ROBERTO C.V. SANTOS[3,5]
https://orcid.org/0000-0002-0533-3483

ALEKSANDRO S. DA SILVA[7]
https://orcid.org/0000-0002-6940-6776

[1]Programa de Pós-Graduação em Ciências Farmacêuticas: Análises Clínicas e Toxicológicas, Universidade Federal de Santa Maria/UFSM, Laboratório de Microbiologia Oral, Avenida Roraima, 1000, Prédio 20, Cidade Universitária, Camobi, 97105-900 Santa Maria, RS, Brazil

[2]Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, Universidade Federal de Santa Maria/UFSM, Laboratório de Neurotoxicologia e Psicofarmacologia, Avenida Roraima, 1000, Prédio 19, Cidade Univrsitária, Camobi, 97105-900 Santa Maria, RS, Brazil

[3]Universidade Federal de Santa Maria/UFSM, Departamento de Microbiologia e Parasitologia, Avenida Roraima, 1000, Prédio 20, Cidade Universitária, Camobi, 97105-900 Santa Maria, RS, Brazil

[4]Acadêmica do Curso de Farmácia do Centro Universitário Franciscano, Rua dos Andradas, 1614, Centro, 97010 Santa Maria, RS, Brazil

[5]Programa em Nanociência, Centro Universitário Franciscano, Rua dos Andradas, 1614, Prédio 13, Centro, 97010-030 Santa Maria, RS, Brazil

[6]Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos, Universidade Federal de Santa Maria/UFSM, Avenida Roraima, 1000, Prédio 42, Cidade Universitária, Camobi, 97105-900 Santa Maria, RS, Brazil

[7]Universidade do Estado de Santa Catarina/UDESC, Departamento de Ciência Animal, Rua Beloni Trombeta Zanin, 680E, Santo Antônio, 89815-630 Chapecó, SC, Brazil

Correspondence to: **Vanessa Schopf Machado, Aleksandro Schafer Da Silva**
E-mail: vanessasmachado@live.com E-mail: V.S.aleksandro.silva@udesc.br

Author contributions

Vanessa S. Machado, Michele R. Sagrillo and Aleksandro S. da Silva and Roberto C.V. Santos participated in the research design. Vanessa S. Machado, Camila Camponogara and Sara M. Oliveira conducted the experiments and performed the data analysis. Vanessa S. Machado, Bruna Klein and Roger Wagner contributed to chromatographic analysis. Vanessa S. Machado, Michele R. Sagrillo, Samanta da S. Gundel and Ana Paula T. da Silva contributed reagents, conducted experiments and analyzed the nanocapsules. Vanessa S. Machado, Matheus D. Baldissera and Aline F. Ourique data analyzed the nanocapsules. All authors wrote or contributed to the writing of the manuscript, and all approved the final version of the manuscript.