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Research Article

Anti-inflammatory Effect from a Hydrogel Containing Nanoemulsified Copaiba oil (*Copaifera multijuga* Hayne)

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Abstract. Copaiba oil is used as a popular medicine in the Amazonian forest region, especially due to its anti-inflammatory properties. In this paper, we describe the formulation of hydrogel containing copaiba oil nanoemulsions (with positive and negative charges), its skin permeation, and its anti-inflammatory activity in two *in vivo* models: mouse ear edema and rat paw edema. Three hydrogels were tested (Carbopol®, hydroxyethylcellulose and chitosan), but only Carbopol® and hydroxyethylcellulose hydrogels presented good stability and did not interfere with the nanoemulsions droplet size and polydispersity index. In skin permeation assay, both formulations, positively charged nanoemulsion (PCN) and negatively charged nanoemulsion (NCN), presented a high retention in epidermis (9.76 ± 2.65 μg/g and 7.91 ± 2.46 μg/cm^2, respectively) followed by a smaller retention in the dermis (2.43 ± 0.91 and 1.95 ± 0.56 μg/cm^2, respectively). They also presented permeation to the receptor fluid (0.67 ± 0.22 and 1.80 ± 0.85 μg/cm^2, respectively). In addition, anti-inflammatory effect was observed to NCN and PCN with edema inhibitions of 69 and 67% in mouse ear edema and 32 and 72% in rat paw edema, respectively. Histological cuts showed the decrease of inflammatory factors, such as dermis and epidermis hyperplasia and inflammatory cells infiltration, confirming the anti-inflammatory effect from both copaiba oil nanoemulsions incorporated in hydrogel.

KEY WORDS: hydrogel; *Copaifera multijuga* Hayne; inflammation; mouse ear edema; rat paw edema.

INTRODUCTION

Essential oils are used in many areas, such as in the cosmetic and perfume industries and also as a popular medicine, especially due to their anti-microbial properties (1,2). Copaiba oil is extracted from the trunk of *Copaifera* trees and represents a great commercial product, as well as a renewable source of natural therapy in the Amazonian region popular medicine, where it is used as anti-inflammatory, antiseptic, and wound healer, both by oral and topical routes (3).

Copaifera multijuga Hayne is a common species of *Copaifera* tree in the Amazon rain forest, Brazil (4). Its oilresin is composed basically by sesquiterpenes (hydrogenated and oxygenated) and diterpenes and has been described as a potent anti-inflammatory, even when compared to other *Copaifera* species, especially due to its high β-caryophyllene concentration (3). β-Caryophyllene is a sesquiterpene and has been also studied due to its anti-inflammatory effects (5,6).

Recently, studies involving copaiba oil and nanoemulsions have been published by our research group, including the development of a nanoemulsion (7) and a method to detect the major component β-caryophyllene in nanoemulsions and skin samples (8,9). Also, we described the potentialization of copaiba oil anti-edematologic effect when incorporated into nanoemulsions (10).

However, this dosage form has very low viscosity to be applied to the skin and its incorporation into a hydrogel can afford a better therapeutic compliance. Moreover, hydrogels are aqueous formulations with wet and pleasant touch sensing properties, which do not present affinity for oil droplets or lipophilic compounds (11,12). In this way, it is hypothesized that the incorporation of copaiba oil nanoemulsion into a hydrogel may enhance the permeation of its major compound, β-caryophyllene, through the skin.

Thus, the aim of this study is to incorporate copaiba oil nanoemulsions in different hydrogel polymers and to evaluate the influence of its thickening effect on β-caryophyllene skin permeation and on the anti-inflammatory effect *in vivo*. This paper shows for the first time the production of a copaiba oil

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semi-solid dosage form that can be used in the skin and its pharmacological activity.

MATERIALS AND METHODS

Materials

β-Caryophyllene reference standard, arachidonic acid, Span 80™, Tween 20™, cetyltrimethylammonium bromide (CTAB), chitosan (CHI), Natrosol™ or hydroxyethylcellulose (HEC), and Carbopol 980™ (CARB) were purchased from Sigma-Aldrich (St. Louis, USA). Medium chain triglycerides (MCT) were purchased from Delaware (Porto Alegre, Brazil). Ultrapure water was obtained from a Milli-Q® apparatus (Millipore, Billerica, USA). All other chemicals or reagents were of analytical grade.

Copaiba oil was extracted from *C. multijuga* Hayne trunk in Ducke Forest Reserve from Instituto Nacional de Pesquisas da Amazônia (INPA) at Manaus, Amazonas state, Brazil (2° 57′ 43″ S, 59° 55′ 38″ W, 120 m).

Preparation of Copaiba Oil Nanoemulsion and Hydrogels

Nanoemulsions containing copaiba oil were prepared according to a previous study (10). Table I describes the positively charged nanoemulsion (PCN) and negatively charged nanoemulsion (NCN) formulations.

First, aqueous (water and Tween 20™ or CTAB) and oily phases (copaiba oil, MCT and Span 80™) were mixed separately. After, the aqueous phase was poured in the oily phase, under magnetic stirring, to form a coarse emulsion. This coarse emulsion was submitted to high-pressure homogenization (Emulsiflex-C3, Avestin, Canada) for 6 cycles at 750 bar. All steps were performed under room temperature.

Hydrogels were formed by mixing the polymer powder with the nanoemulsion. HEC hydrogel (2%) was left to swell overnight, CARB hydrogel (0.5%) was formed by adding triethanolamine, and CHI hydrogel (3%) was formed by adding acetic acid.

Blank nanoemulsions were prepared without copaiba oil (only MCT up to 30% *w*/*w*). Blank hydrogel was prepared with water instead of nanoemulsion.

After preparation, all samples were analyzed according to their droplet size, polydispersity index, and zeta potential. β-Caryophyllene content was analyzed in the hydrogels used on *in vivo* experiments and during 1 year (for stability purposes) by a previously validated method (8). Morphological analysis from PNC and NCN incorporated in hydrogel was performed using a scanning electron microscope (SEM) with a TM3000 (Hitachi High Technologies America, Illinois, USA).

Table I. Copaiba oil nanoemulsions

Composition	PCN	NCN
Copaiba oil (%)	20.0	20.0
MCT (%)	10.0	10.0
Span 80™ (%)	3.0	3.0
Tween 20™ (%)	1.0	1.0
CTAB (%)	0.75	–
Water q.s. (%)	100	100

PCN positively charged nanoemulsion, *NCN* negatively charged nanoemulsion, *MCT* medium chain triglycerides, *CTAB* cetyltrimethylammonium bromide

Rheological Study

The hydrogel chosen to perform skin permeation and *in vivo* tests was evaluated for its rheological profile using a Brookfield Rotational Viscometer, model DV-II+ (Brookfield Engineering Laboratories, Middleboro, USA). Twenty grams of formulation was placed in a container suitable for the equipment, at rotational speed 0.1, 0.3, 0.5, 1.0, 1.5, 2.0, 3.0, and 5.0 rpm with spindle 29. Results are shown as shear stress (Pa) *vs* shear rate (/s).

In Vitro Skin Permeation

Skin permeation assay ($n = 5$) was performed in Franz diffusion cell apparatus according to a previous study (9). Full thickness porcine ear skin was used as membrane. Previously from use, the fat tissue and the hair were removed from the outer part of the ear. Receptor fluid consisted in a mixture of phosphate buffer saline pH 7.4 and ethanol (1:1). After 8 h, the skin was cleaned with ultrapure water to remove formulation excess and skin layers were separated. Stratum corneum was separated using the tape-stripping method. Epidermis was separated from dermis using a scalpel. A 1-mL aliquot from the receptor fluid was also collected after 8 h of study.

All samples were placed in headspace vials to perform analysis in gas chromatograph coupled with mass spectrometer (5975C, Agilent Technologies, USA), using a previously validated method (9). Samples were prepared using headspace mode in CombiPAL Autosampler (CTC Analytics AG, Basel, Switzerland) set at 50°C for 10 min.

Animals

Adult male Swiss mice (30–40 g) were provided by Bioterio Central from Universidade Federal de Pelotas. Adult male Wistar rats (100–200 g) were provided by CREAL (Centro de Reprodução e Experimentação de Animais de Laboratório). All animals were maintained under standard conditions (22 ± 1°C at 40–60% relative humidity and 12 h light-dark cycle). All animals had free access to food and water. Mice were sacrificed by cervical dislocation and rats were euthanized by intraperitoneal propofol injection (30 mg/kg). The Animal Use Ethics Committee from Federal University of Rio Grande do Sul approved this study (protocol number: 25866).

Arachidonic Acid-Induced Mouse Ear Edema

Groups of five mice were treated with copaiba oil or hydrogel formulation in the posterior and anterior part of the right ear. The left ear did not receive any treatment, as a control for each animal. After 1 h, the edema was induced by topical application of arachidonic acid (solution in ethanol, 0.2 mg/μL) at 2 mg/ear (10 μL) only in the right ear.

Positive control group received a ketoprofen solution at 4 mg/ear (in acetone solution, 10 μL). Two hundred milligrams

Table II. Nanoemulsions and hydrogels physicochemical characterization

	ZP (mV)	DS (nm)	PDI
NCN	− 17.00 ± 0.96	247.53 ± 1.46	0.051 ± 0.041
NCN-CARB	− 45.77 ± 0.47[a]	267.20 ± 3.86	0.117 ± 0.023
NCN-HEC	− 42.53 ± 0.40[a]	271.63 ± 4.62[b]	0.237 ± 0.036[c]
NCN-CHI	54.87 ± 0.29[a]	524.33 ± 12.75[b]	0.489 ± 0.049[c]
PCN	22.43 ± 2.90	198.83 ± 2.69	0.085 ± 0.039
PCN-CARB	− 51.53 ± 1.52[d]	192.13 ± 4.86	0.087 ± 0.062
PCN-HEC	34.57 ± 1.36[d]	223.67 ± 3.54[e]	0.175 ± 0.015
PCN-CHI	44.83 ± 0.74[d]	412.80 ± 10.59[e]	0.562 ± 0.058[f]

PCN positively charged nanoemulsion, *NCN* negatively charged nanoemulsion, *CARB* Carbopol®, *HEC* hydroxyethylcellulose, *CHI* chitosan, *ZP* zeta potential, *DS* droplet size, *PDI* polydispersity index
[a] ZP different from NCN ($p < 0.05$)
[b] DS different from NCN ($p < 0.05$)
[c] PDI different from NCN ($p < 0.05$)
[d] ZP different from PCN ($p < 0.05$)
[e] DS different from PCN ($p < 0.05$)
[f] PDI different from PCN ($p < 0.05$)

per kilogram copaiba oil concentration was chosen (10) to perform the experiment with the respective hydrogel. Control group received only the vehicle (ethanol) in the right ear. Since the nanoemulsion presents 20% of copaiba oil, the concentration used with the final dosage form treatment was 1000 mg/kg (5-fold the copaiba oil dose). Treatments were applied with automatic semi-solid pipette (100 μL), and arachidonic acid was applied using an automatic pipette (20 μL).

Ear edema was measured after 1 h, using a thickness gauge (Mitutoyo Corporation, Kanagawa, Japan). After the sacrifice, 6 mm^2 fragment of both ears was removed and weighted. Edema was measured by the ear thickness in the groups' right ear. The weight difference between the right and the left ear of each rat in each group was also used as an edema measurement. Edema inhibition percentage (EI%) was calculated comparing only the weight difference of the right and left ear for the groups to the weight difference of the right and left ear of the control group according to Eq. (1)

$$EI(\%) = \left[1-\left(\frac{REt-LEt}{REc-LEc}\right)\right] \times 100 \quad (1)$$

where REt is the weight of the treated right ear, REc is the weight of the control right ear, LEt is the weight of the treated left ear, and LEc is the weight of the control left ear.

Formalin-Induced Rat Paw Edema

Each group ($n = 5$) received the treatment (copaiba oil or hydrogel) in the right hind paw 1 h before the edema induction. Two hundred milligrams per kilogram copaiba oil concentration was chosen to perform the experiment with the respective hydrogel (10). Positive control group received a ketoprofen solution at 4 mg/paw (in acetone). Negative control group did not receive treatment.

Before the edema induction, animals were anesthetized with an intraperitoneal injection of ketamine (10 mg/kg) and xylasine (25 mg/kg) mixture. Formalin solution (100 μL, 10% *v/v* in saline) was injected in the right hind paw, while the left paw received the same amount of vehicle, saline (NaCl 0.9%).

Paw volume was measured after 4 h using a plethysmometer (UgoBasile, Varese, Italy). Edema was measured by paw volume (mL) in the groups' right hind paw. Edema inhibition (EI) was measured by the percentage of edema comparing the volume of the paw in the measurement times for the groups to the volume of the control group (Eq. (2)).

$$EI(\%) = \left[1-\left(\frac{RPt}{RPc}\right)\right] \times 100 \quad (2)$$

where RPt is the volume of the treated right paw, and RPc is the volume of the control right paw.

Histological Analysis

For histological examination, samples of mice ear and rat right rind paw were collected from *in vivo* experiments and stored in a solution of formaldehyde at 37% in PBS pH 7.2. Histological cuts were stained with hematoxylin-eosin and visualized in optical microscope.

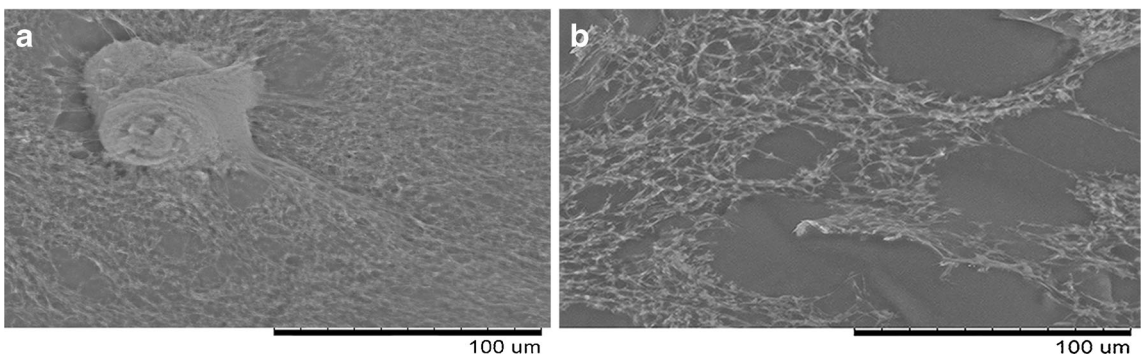

Fig. 1. SEM images for NCN (**a**) and PCN (**b**) HEC hydrogel

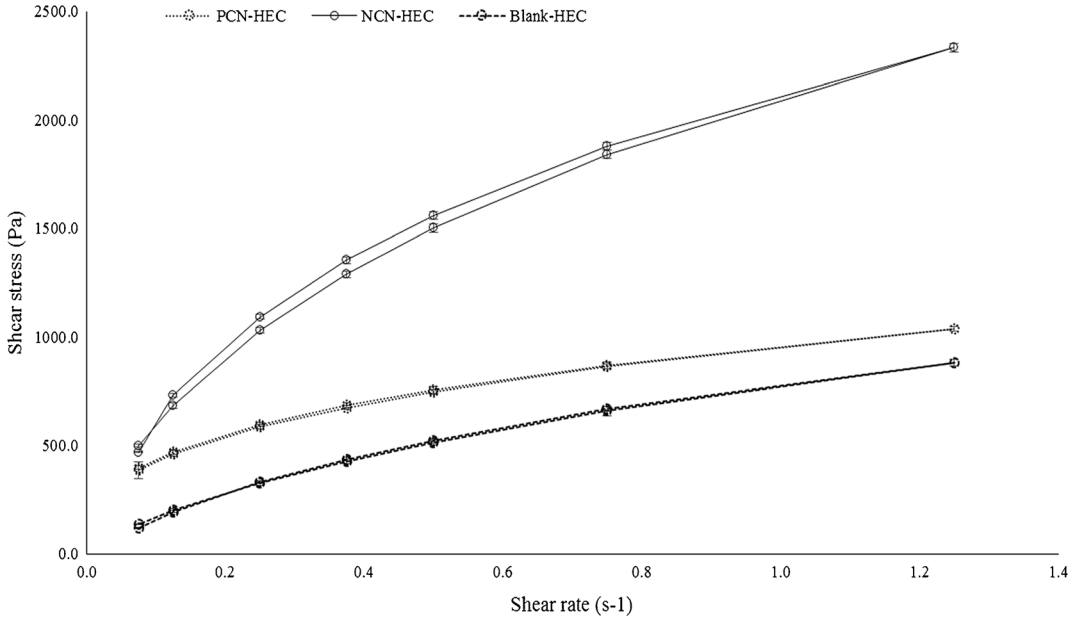

Fig. 2. Rheological profile from copaiba oil nanoemulsions thickened with HEC polymer ($n = 3$)

Statistical Analysis

Statistical difference on skin permeation and *in vivo* assays were calculated by one-way ANOVA followed by Tukey's test. For *in vivo* assays, statistical difference was calculated by one-way ANOVA followed by Holm-Sidak method (rat paw edema) and Tukey's test (mice ear edema). Values with P smaller than 0.05 were considered significant. SigmaSTAT® software was used to analyze the statistics.

RESULTS AND DISCUSSION

Copaiba Oil Characterization

Composition characterization in gas chromatograph coupled with mass spectrometer (GC/MS) demonstrated the presence of 41.2% of β-caryophyllene, representing the major sesquiterpene in the oilresin. Other major sesquiterpenes were α-copaene (7.1%), α-humulene (6.9%), and caryophyllene oxide (1.3%). This composition is normally found in copaiba oils (13). It can be modified depending on time of the year it is collected, presence of rain before the extraction, presence of injury caused by insects or fungi, variation on soil nutrient, and light exposure (14).

Characterization of Copaiba Oil Nanoemulsion and Respective Hydrogels

Three different polymers were tested to increase the nanoemulsions viscosity: CARB (anionic polymer), HEC (non-ionic polymer), and CHI (cationic polymer). All hydrogel formulations presented good zeta potential (ZP), above |30| mV (Table II). When CARB was used as polymer, ZP presented negative values, even with the cationic nanoemulsion and when CHI was used; ZP presented cationic values, even with the anionic nanoemulsion. Since HEC is a non-ionic polymer, ZP in the formulation was given by the nanoemulsion surface charge.

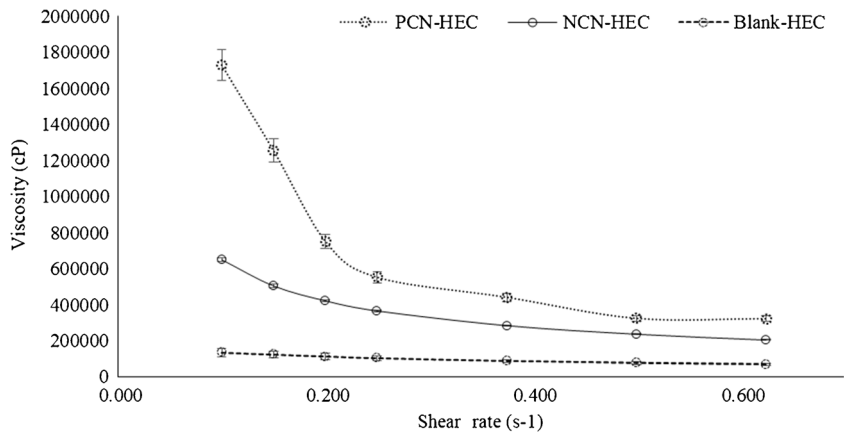

Fig. 3. Viscosity profile from copaiba oil nanoemulsions thickened with HEC polymer ($n = 3$)

Table III. Stability characterization from hydrogels containing positively and negatively charged nanoemulsions in 0, 6, and 12 months

	NCN-HEC			PCN-HEC		
	T0	T6	T12	T0	T6	T12
Zeta potential (mV)	− 45.8 ± 0.80	− 32.7 ± 0.20*	− 25.4 ± 4.10*	22.2 ± 5.20	37.2 ± 3.20*	15.27 ± 4.42
Droplet size (nm)	280.10 ± 4.30	284.53 ± 11.21	280.00 ± 12.77	258.70 ± 5.20	302.97 ± 7.42*	333.47 ± 3.84*
Polydispersity index	0.079 ± 0.023	0.082 ± 0.017	0.149 ± 0.050	0.258 ± 0.010	0.229 ± 0.030	0.317 ± 0.044*
Content (%)	97.10 ± 0.07	101.77 ± 0.07*	82.22 ± 0.06 *	105.38 ± 0.08	105.32 ± 0.08	97.78 ± 0.03*

T0 time zero, *T6* time 6 months, *T12* time 12 months, *NCN-HEC* negatively charged nanoemulsion thickened in hydroxyethylcellulose hydrogel, *PCN-HE* positively charged nanoemulsion thickened in hydroxyethylcellulose hydrogel
*Statistically different from time T0

Even though ZP values were considered good and this parameter can indicate nanoemulsion stability when above 30 mV (in modulus) (15), droplet size (DS) and polydispersity index (PDI) values showed that, when CHI was used as hydrogel, both formulations presented an increase in these parameters. An increase in PDI could imply that the droplets are aggregating and forming a bigger droplet, which could explain the increase in DS.

Souto *et al.* (16) also found that the incorporation of chitosan hydrogel in nanoparticles can destabilize the formulation, leading to an increase in DS and PDI. This can be explained by the presence of acetic acid to form the hydrogel, the interaction between the nanoemulsion surface charge and the polar groups from chitosan and also from the instability around zero charge point when ZP is reversed. Moreover, when hydrogel formulations are compared to the nanoemulsions, there is an increase in ZP and DS, also verified by other authors (16–18), which can be explained by polymer adsorption on nanoemulsion droplet surface.

HEC hydrogel was chosen to continue the studies, since it presented good characterization parameters for both nanoemulsions, due to its neutral character. Figure 1 shows SEM image for NCN-HEC and PCN-HEC, where the polymeric network organization can be seen for both formulations.

Hydrogel-Based Nanoemulsions Rheological Profile

Figure 2 demonstrates the rheological profile comparing NCN-HEC, PCN-HEC, and Blank-HEC hydrogels. As can be seen in the rheogram, HEC hydrogels (2%) containing or not copaiba oil nanoemulsions presented non-Newtonian flow, since the relation between shear stress and shear rate is not linear (19). Among non-Newtonian fluids, there are three behaviors that can occur: plastic, pseudoplastic, or dilatant. According to our results, the hydrogel produced shows pseudoplastic characteristics. In addition, they do not present any thixotropic behavior, as both ascendant and descendant curves are overlapping. Figure 3 shows the viscosity profile from NCN-HEC, PCN-HEC, and Blank-HEC hydrogels. As observed, nanoemulsions were influenced in the viscosity behavior of HEC hydrogel, given that the control hydrogel (Blank-HEC) presented lower viscosity values compared to HEC-loaded nanoemulsions. That can be explained by the nanoemulsions' higher viscosity compared to the water viscosity.

Table IV. Skin permeation results

	PCN-HEC	NCN-HEC
Stratum corneum (μg/cm^2)	0.09 ± 0.07	0.18 ± 0.17
Epidermis (μg/cm^2)	9.76 ± 2.65	7.91 ± 2.46
Dermis (μg/cm^2)	2.43 ± 0.91	1.95 ± 0.56
Receptor fluid (μg/cm^2)	0.67 ± 0.22	1.80 ± 0.85

PCN-HEC positively charged nanoemulsion thickened hydrogel, *NCN-HEC* negatively charged nanoemulsion thickened hydrogel

Long-Term Storage Stability

Regarding the formulations' stability study (Table III), during the 1-year monitoring, values for ZP, DS, PDI, and β-caryophyllene content slowly changed. For both hydrogels, ZP and β-caryophyllene content values decreased and DS and PDI values increased, indicating a probable instability after 12 months. Nevertheless, all values stayed in an acceptable range during the first 6 months. It is worth mentioning that both formulations were kept under 4°C temperature and covered from light, which can explain the smaller loss in content when compared to other studies in the literature (20–22). In addition, there was no phase separation, presence of fungi contamination, or other instability-indicative aspects during the time studied.

In Vitro Skin Permeation

Table IV describes the skin permeation/retention profile for copaiba oil nanoemulsions incorporated in hydroxyethylcellulose hydrogels. Results are shown as β-caryophyllene (the major sesquiterpene in copaiba oil) content in skin layers and receptor fluid. After 8 h, β-caryophyllene was found in the receptor fluid for both formulations, characterizing skin permeation. It was also found in great amount in the epidermis layer, followed by the dermis and the stratum corneum in a smaller amount. In comparison with the nanoemulsion permeation profile reported in a previous study (10), there is a higher permeation

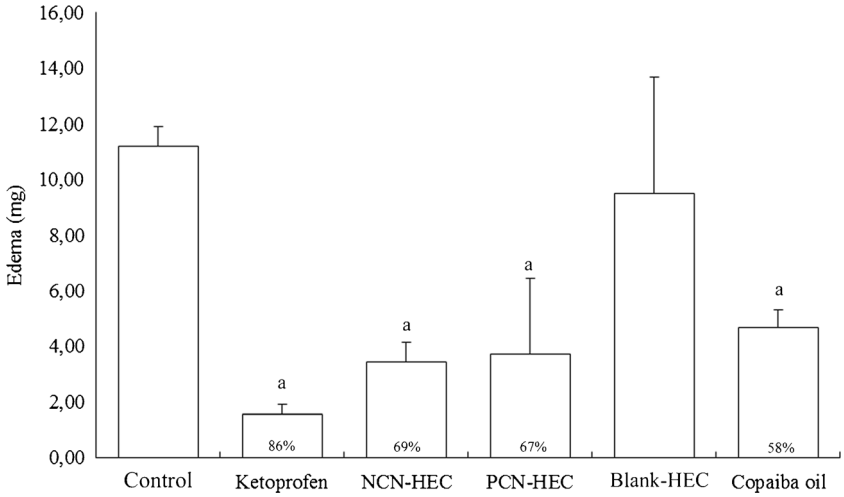

Fig. 4. Arachidonic acid induced-mouse ear edema measured by ear weight (mg). Edema inhibition percentages were placed inside the bars. Superscript letter "a" indicates statistically different from control ($p < 0.05$)

when the hydrogel is used, since for the nanoemulsion there was no β-caryophyllene detected in the receptor fluid.

The higher β-caryophyllene permeation with the hydrogel-loaded nanoemulsion can provide evidence that this formulation is suitable for the purpose of topical application in an anti-inflammatory therapy, indicating that the nanoemulsion was released from the gel matrix and that the hydrogel did not present affinity to it when in contact to the skin. Since the nanoemulsion has small droplet size and high superficial area, it is supposed to penetrate the stratum corneum, permeate through the epidermis (or establish a type of reservoir in this layer), and reach the dermis and the receptor fluid, which mimics the deeper layers in the skin (23,24). In addition, many factors could explain why the addition of a hydrogel to the formulation could improve the nanoemulsions' skin permeation such as occlusion, viscosity, and hydration of the site, which can increase the partitioning of the stratum corneum layer and enable the penetration (25,26).

In Vivo Anti-Inflammatory Activity

Two *in vivo* models demonstrated the topical anti-inflammatory potential effect from copaiba oil nanoemulsion incorporated in hydrogel: mouse ear edema and rat paw edema.

Mouse ear edema was induced by topical administration of arachidonic acid (2 mg/ear) which is involved in the cyclooxygenase (COX) and lipoxygenase (LOX) inflammation pathways, and its topical administration leads to immediate vasodilatation and erythema (27). Figure 4 shows the result 60 min after ear inflammation induction.

As can be seen, ketoprofen, crude copaiba oil, and its nanoemulsions incorporated in hydrogels significantly inhibited the edema when compared to the control ($p < 0.05$). However, when compared to the positive control, ketoprofen, the hydrogels with nanoemulsions (NCN-HEC and PCN-HEC), and copaiba oil were statistically equivalent ($p > 0.05$). Blank hydrogel, as expected, did not present anti-edematogenic effect. Edema inhibition values for ketoprofen,

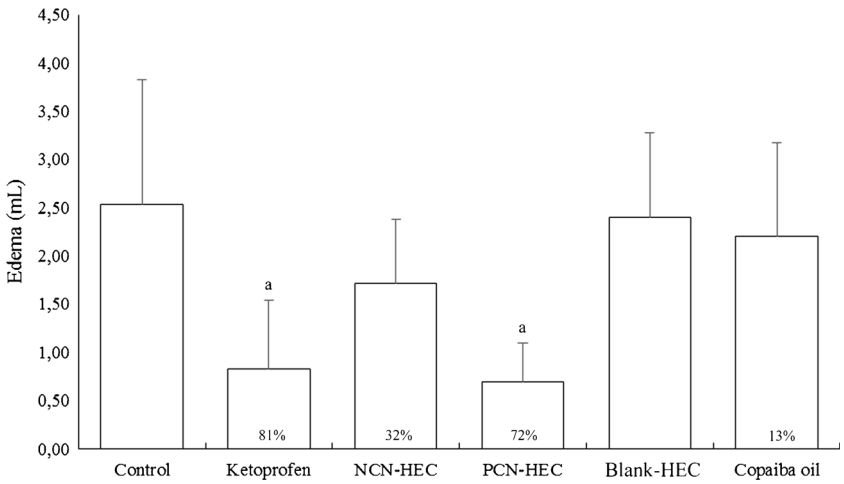

Fig. 5. Rat paw edema induced by formalin 10%. Edema inhibition percentages were placed inside the bars. Superscript letter "a" indicates statistically different from control ($p < 0.05$)

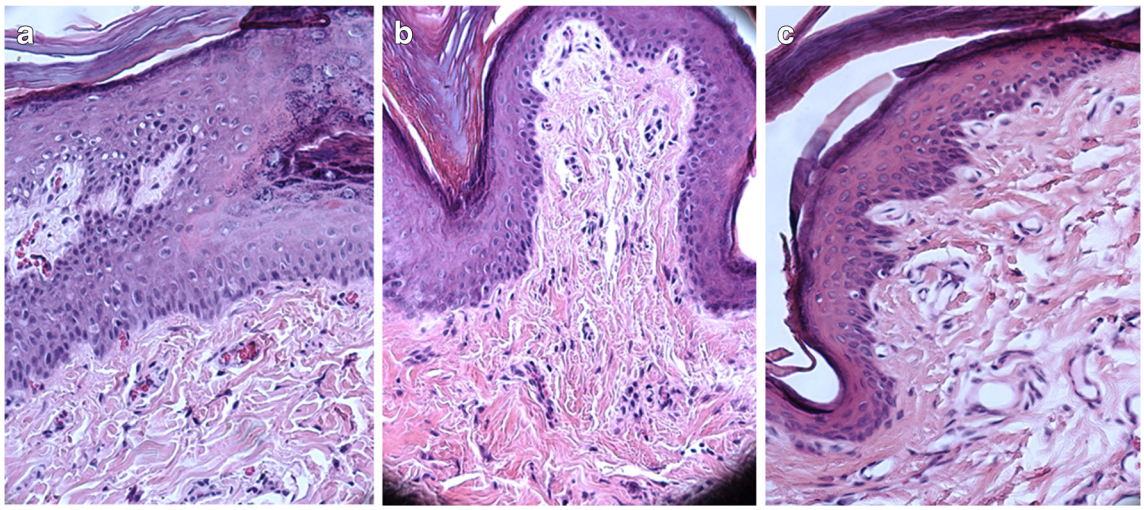

Fig. 6. Photomicrographs from transversal cuts of right rat paws after topical formalin administration, stained with hematoxylin-eosin and examined in optical microscope with × 40 magnification. **a** Control. **b** PCN-HEC. **c** NCN-HEC

NCN-HEC, PCN-HEC, and copaiba oil were 86, 69, 67, and 58%, respectively. Thus, both formulations had an equivalent profile compared to ketoprofen; however, they did not change the effect of the crude oil.

Since the formulations and the oil inhibited the arachidonic acid induced inflammation, copaiba oil could be involved in the inhibition of COX and LOX pathways, like non-steroidal anti-inflammatories.

Rat paw edema was induced by intraplantar administration of formalin (10%). It is well known that formalin causes a biphasic edema response. The first phase (normally up to 5 min after induction) releases substance P and bradykinin. In this phase, it is considered to cause a neuropathic-kind pain. In the second phase, histamine, serotonin, prostaglandins, and bradykinin are involved, producing inflammatory response (28).

Figure 5 presents the results for rat paw edema. Statistically, ketoprofen and PCN-HEC were different to the negative control ($p < 0.05$), indicating their anti-edematogenic activity. Copaiba oil, NCN-HEC, and blank formulation were statistically equal to the control ($p > 0.05$). Edema inhibition values for ketoprofen, NCN-HEC, PCN-HEC, and copaiba oil were 67, 32, 72, and 13%, respectively. In this case, the formulation could improve the effect of the oil, corroborating with the permeation profile and indicating that the positive surface charge has an important role and can enable skin permeation.

It is important to highlight that the oil produced a smaller edema inhibition, which can be correlated to its permeation profile through the skin. In previous studies, we found that the oil stays in the stratum corneum, without any β-caryophyllene retention in the dermis and epidermis, unlike the nanoemulsions containing the oil (9,10).

In both experiments, ketoprofen, a blank formulation and non-treated animals were used as control. Ketoprofen is a non-steroidal anti-inflammatory drug (NSAID) widely used

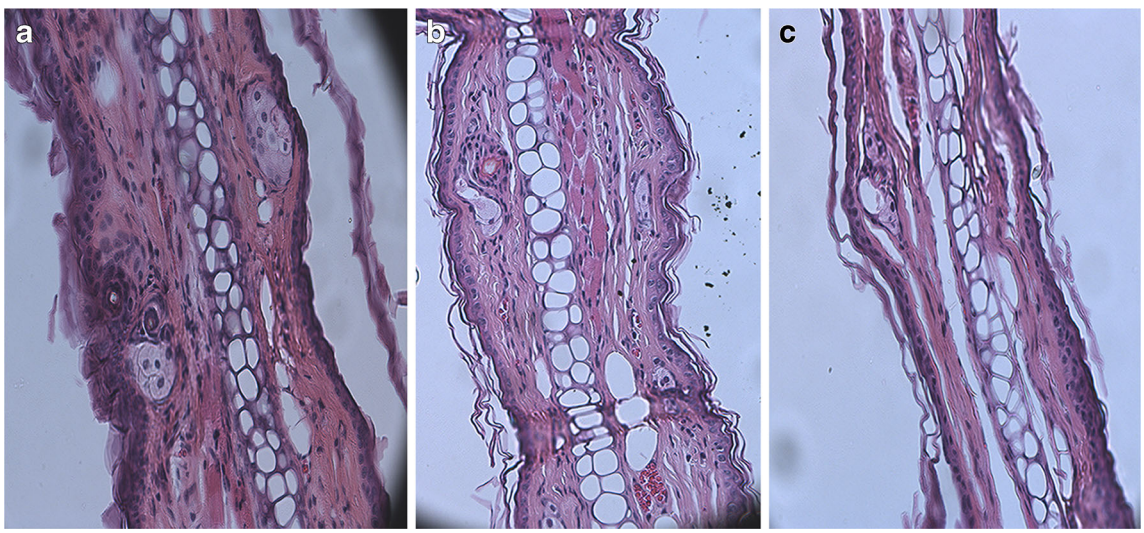

Fig. 7. Photomicrographs from transversal cuts of mice ears after arachidonic acid topical application, stained with hematoxylin-eosin and examined in optical microscope with × 40 magnification. **a** Non-treated control. **b** NCN-HEC. **c** PCN-HEC

to treat rheumatoid arthritis and other inflammatory diseases (29) and was used in this study as a positive control for the anti-inflammatory effect. The dose was 4 mg/paw (paw edema) and 4 mg/ear (ear edema) is normally used in anti-inflammatory assays and was described previously (30,31). In order to evaluate if the hydrogel could perform an anti-inflammatory effect, there was also a hydrogel control (Blank-HEC), which consisted in a formulation containing only the polymer (hydroxyethylcellulose and water).

Concerning histological examination, rat paw edema assay (Fig. 6) showed the presence of epidermis hyperplasia, inflammatory cell infiltration, and vasodilation in the non-treated control. In mice ear edema, histological examination (Fig. 7) showed the presence of dermis and epidermis hyperplasia and inflammatory cell infiltration in non-treated control. Treatments showed a decrease in these factors, demonstrating the anti-inflammatory effect for both models.

CONCLUSIONS

In this paper, we described the incorporation of copaiba oil nanoemulsions (positive and negatively charged) in different hydrogel polymers. The best hydrogel that did not interfere with nanoemulsions' droplet size and polydispersity index was the one formed by hydroxyethylcellulose (HEC), which remained stable for a 12-month stability study and was chosen to perform skin permeation and *in vivo* experiments. Concerning skin permeation, for both formulations, it was possible to detect β-caryophyllene in the most profound skin layer (dermis) and in the receptor fluid, characterizing skin permeation. In mouse ear edema, both formulations presented similar anti-edematologic profile, presenting high edema inhibition and statistically similar to ketoprofen ($p < 0.05$). In rat paw edema, only PCN-HEC formulation presented anti-edematologic effect equal to the positive control, indicating the important hole of the positive charge on β-caryophyllene permeation and edema inhibition. In both *in vivo* edema studies, it was possible to visualize by histological cuts a decrease in epidermis hyperplasia, inflammatory cell infiltration, and vasodilation, demonstrating the anti-inflammatory activity from both treatments.

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COMPLIANCE WITH ETHICAL STANDARDS

The Animal Use Ethics Committee from Federal University of Rio Grande do Sul approved this study (protocol number: 25866).

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