Preclinical study to evaluate the effects of a soft handkerchief in nasolabial skin barrier

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Abstract
People suffering from an ordinary acute cold consume so many handkerchiefs that the wiping actions on their own increase the abrasive damage of the nasolabial zone, finally leading to a disturbed barrier function and inflammation. It seems that the quality of the material used for nose cleansing could play an important role and that innovative handkerchiefs would fulfill a preventive role in minimizing the damaging effect of the skin barrier function of the nasolabial zone during this conditions. The objective of this work was to evaluate the effects of a wet handkerchief (SKNW) on the skin barrier balance by measuring filaggrin and histamine using an experimental model of ex vivo native human skin model and the interference of this handkerchief in the skin microbiota through in vitro screening. SKNW showed an increase in the production of filaggrin and a reduction of histamine synthesis in human explants subjected to barrier disruption with 5% Sodium Lauryl Sulfate. Additionally, SKNW showed a mild and moderate antiseptic action on the evaluated microorganisms. This study demonstrated that SKNW could be considered a feasible option for consecutive wiping of nasolabial zone avoiding the transient mechanical dermatitis, considering its skin barrier protective, non-irritating and antiseptic actions.

Key words: skin barrier; filaggrin, histamina; sensitive skin; wipes; skin care; ex vivo; skin; microbiota

Abbreviations
SC – Stratum corneum
SKNW – Salsep® Kids Nasal Wipes
IL-1α- interleukin-1 alpha
IL-1β- interleukin-1 beta
TNFα-Tumor necrosis fator α.
NMFs – Natural moisturizing factors
BHI – Brain Heart Infusion Broth
CFU – Colony forming units

Introduction
The occurrence of runny nose due to rhinitis, flu and colds is very common in infants. Published studies carried out in Brazilian children have shown that about 48% had at least one episode of sneezing, runny nose or stuffy nose without a cold or flu in the first year of life, and that the average prevalence of rhinitis in children was 26.6% [1,2].

Under this clinical situation, frequent rubbing of the nasolabial skin with handkerchiefs can provoke skin irritation, which – at first – heals difficultly because the skin reacts by an inflammatory response leading to hyperkeratotic scaly areas. These clinical symptoms of a mechanically induced dermatitis probably are caused by removal of the superficial hydrolipidic layer and disturbance of the barrier function in the upper part of the Stratum corneum (SC) [3].

The natural barrier of SC depends on its composition, formed mostly by highly insoluble and resistant proteins, which involve externally keratinocytes being fundamental in the structure and organization of intracellular lipids [4]. Profilaggrin, filaggrin, involucrin and loricin are examples of these proteins which are responsible for the association of intermediate keratin filaments and consequent increase of cohesion among cornocytes [4].

Insult of skin barrier promotes immediately release of pro-inflammatory cytokines, along chemokines and growth factors, which will stimulate the migration and proliferation of immunocompetent cells [5]. Inflammatory signaling via nuclear transcription factor kappa B (NF-Kappa B) is also activated and results in the production of more cytokines and chemokines, as well as, trigger the classic pathway of inflammation and increasing the
production of eicosanoids, such as prostaglandins and leukotrienes [6,7]. Furthermore, a variety of mediators, such as, vascular endothelial growth factor, nitric oxide and histamine are produced leading to vasodilation, edema, mast cell degranulation, pain and itching, exacerbating the inflammatory process and skin sensitivity [6, 8-9].

Concurrently, local microbiota can be significantly disturbed resulting in a cutaneous dysbiosis that unbalances the immune system and favors the growth of pathogenic microorganisms, predisposing to secondary infections [10-12].

It seems that the quality of the paper material used for nose cleaning could play an important role and that soft handkerchiefs would cause less damage on the hydrophilic film and the skin barrier when having a cold. Soft paper handkerchiefs might then fulfill a preventive role in minimizing the damaging effect of the skin barrier function of the nasolabial zone [13].

The objective of this work was to evaluate the effects of a skin care handkerchiefs on the nasolabial skin barrier balance by measuring filagrin and histamine using an experimental model of ex vivo native skin. Additionally, an in vitro screening was carried out with the purpose of evaluating the interference of these wipes in skin microbiota.

**Material and Methods**

**Test substance**

Test substance consists of a wet handkerchief – Salsep® Kids Nasal Wipes (SKNW) mainly indicated for cleaning nasal secretions, crusts and runny nose caused by flu, colds and rhinitis in infants. SKNW was provided by Libbs Farmacêutica Ltda, São Paulo/SP – Brazil (INCI names: Aqua, Myristamidopropyl PG-Dimonium Chloride Phosphate, Sodium Chloride, Tocopheryl Acetate, Aloe Barbadensis leaf juice, Glycerin, Chamomilla Recutita Flower Extract, Potassium sorbate, Decyl Glucoside, Citric acid, Sodium citrate, Sodium benzoate).

**Skin fragments culture**

Human skin fragments were originating from one (01) healthy donor, female, skin type III, 43 years, who underwent elective plastic surgery in the abdominal region (abdominoplasty). After the surgical procedure, the skin fragments were collected in plastic vials containing 0.9% saline and kept in refrigeration for up to 24 hours. The use of human skin fragments from elective surgeries for this study was approved by the Ethics Committee of the University São Francisco – SP.

**Treatment protocols and stress conditions for skin culture**

Skin fragments were fractionated into pieces of approximately 1.5 cm² and incubated in culture medium (DMEM; Sigma-Aldrich, San Luis, MO, USA) in air-liquid interface at 37°C in a humidified atmosphere with 5% CO₂. Fragments were treated with SKNW, simulating the condition of using a wet wipe for 3 consecutive days. On the third day, the fragments were subjected to barrier disruption with Sodium Lauryl Sulfate (5% - SLS; Sigma) and treated with SKNW. After 24 hours the third treatment, the skin culture supernatant was collected for measurement of histamine and the fragments subjected to histological processing for immunofluorescence and semi-quantification of filagrin.

**Quantification of histamine**

Concentrations of histamine were measured by ELISA using commercially available kit (Uscn Life Sciences, Wuhan, China). Absorbance reading was performed on Multiskan GO monochromator (Thermo Fisher Scientific, Waltham, MA, USA).

**Immunofluorescence evaluation for Filaggrin**

Skin fragments were fixed in 4% paraformaldehyde (pH 7.4) for 24 hours and cryoprotected in a 30% sucrose solution for 72 hours. Fragments were embedded in a formulation of water-soluble glycols and resins (Tissue-Tek® OCT™, Torrance, CA, USA) and then 10 µm serial sections were collected directly on silanized slides with the aid of cryostat (Leica - CRYOCUT 1800, Germany). Slides were incubated overnight with primary antibody anti-FILAGGRIN (Abcam, Cambridge, UK). Subsequently, the cuts were washed and incubated with Alexa Fluor 488 secondary antibody - Goat anti Rabbit (Thermo Fisher Scientific). DAPI (4-6-Diamidino-2-Phenylindol; DNA marker; Sigma-Aldrich) was used as a DNA marker. The slides were mounted in a specific mounting medium and analyzed using a Fluorescence Microscope (OLYMPUS) with the aid of the cellSens Standard software (© 2010 OLYMPUS CORPORATION, Center Valley, PA, USA). The evaluated parameter was the fluorescence intensity emitted by the specific antibody labeling. After obtaining the images, the fluorescence intensity was quantified with the aid of the ImageJ software (Arbitrary Units - A.U.)

**Statistical analysis**

For statistical evaluation, ANOVA test was used to measure the variation of the results, comparing the data between all the groups. We applied the Bonferroni post-test, which strengthened and made the result presented in the ANOVA more precise (GraphPad Prism6, Version 6.01, GraphPad Software, Inc., La Jolla, CA, USA). A 5% significance level was used.

**Microbiological inhibition**

The antibacterial activity of SKNW was assessed through agar diffusion test against three bacteria species: *Staphylococcus aureus* ATCC 6538 (American Type Culture Collection, Rockville, MD), *Staphylococcus epidermidis* ATCC 12228 and *Cutibacterium acnes* ATCC 6919. The microorganisms were maintained in BHI for 24h at 35°C and each bacterial suspension (inoculum) was diluted to 10⁸ CFU/mL and uniformly spread on a sterile Petri dish containing Muller Hinton agar. SKNW was cut into 1 cm² and added to each of the 3 wells (8 mm diameter holes cut in the agar gel, 20 mm apart from one another). The systems were incubated for 48h and the inhibition of the bacterial growth around each well was measured in mm. The inhibition was classified as mild - halo up to 4 mm, moderate - halo from 5 mm to 9 mm, and strong halo above 10 mm [14].

**Results and Discussion**

In this work, we present a combination of preclinical results obtained for a wet handkerchief (SKNW) mainly indicated for cleaning nasal secretions, crusts and runny nose caused by flu, colds and rhinitis, considering the skin barrier protective, non-irritating and antiseptic actions. These characteristics are desired for this category of products, considering the need for repeated use for hygiene of the nasal region in situations of runny nose. The softness of material used for cleaning nasolabial area is determining to cause less damage on skin barrier during flu crisis [13].

A wide variety of methods can be employed to measure the in vitro activity of microorganisms against antimicrobial agents, which can be classified into three types: bioautographic, diffusion and dilution assays [15]. To evaluate the antiseptic action of the SKNW, we opted by agar diffusion method. Diffusion antibiogram testing is a conventional way to measure antimicrobial activity and is based on diffusion of the product or substance into a solid culture medium with the inoculated microorganism. From this diffusion, a halo appears around the product if it has antimicrobial properties [15-16]. The reading of the results is done after 48 hours, whereit is checked the presence or absence of halo formation around the orifice where the sample is placed.

SKNW promoted a mild to moderate inhibition in the growth of *Staphylococcus aureus, Staphylococcus epidermidis* and *Cutibacterium*...
acnes, as described in Table 1. Specifically, the inhibition zone was 1.90 mm for Staphylococcus aureus (mild inhibition), 6.42 mm for Staphylococcus epidermidis (moderate inhibition) and 5.58 mm (moderate inhibition) for Cutibacterium acnes. Regarding the experimental controls, there was proper development of the microorganisms evaluated in the positive control plates and in the negative control there was no type of growth (Figure 1).

Bacteria evaluated in this study are part of the skin microbiota and play an important role in the maintenance of cutaneous metabolism. The balance between the populations of Staphylococcus aureus, Staphylococcus epidermidis and Cutibacterium acnes, can attribute beneficial properties to products applied topically, particularly in certain skin conditions, such as transient barrier disturbance, sensitive skin, dermatitis, and acne [17-18]. Clearly, this is only a preliminar result, however despite the limitations of the present study, we can suggest that SKNW showed a mild and moderate antiseptic action on the evaluated microorganisms.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Inhibition halo (mm)</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus (ATCC 6538)</td>
<td>1.90±0.58</td>
<td>Mild inhibition</td>
</tr>
<tr>
<td>Staphylococcus epidermidis (ATCC 12228)</td>
<td>6.42±1.02</td>
<td>Moderate inhibition</td>
</tr>
<tr>
<td>Cutibacterium acnes (ATCC 6919)</td>
<td>5.58±0.52</td>
<td>Moderate inhibition</td>
</tr>
</tbody>
</table>

Table 1. Results and classification of inhibition halos obtained for each microorganism evaluated.
Regarding the protective ability on skin barrier, we evaluated in this work the effects of SKNW on the production of envelope protein filaggrin using ex vivo human skin model submitted to epidermal barrier disruption [19-21]. Figures 2 and 3 represent the results of immunostaining and subjected to barrier disruption with Sodium Lauryl Sulfate (5% - SLS). As expected protein semi-quantification of filaggrin, respectively, in human skin culture treated with SKNW, barrier disruption induced with 5% SLS produced a 59.32% reduction in filaggrin synthesis when compared to baseline control (P<0.001). On the other hand, skin fragments subjected to irritative stress with SLS and treated with SKNW showed an increase of 103.39% in the production of filaggrin, when compared to the group only stressed with LSS (P<0.01).

These results allow us to infer that SKNW has a positive effect on the protection and maintenance of skin barrier by increasing the synthesis of filaggrin. The epidermal protein profilaggrin, lately synthesized during epidermal differentiation, plays an importante role in generating and maintaining SC flexibility and moisturizing [22]. This highly phosphorylated protein is quickly dephosphorylated and proteolyzed during the end of the transition from granular keratinocyte to the corneocyte, where it remains retained. During the transition from the granular layer to SC, profilaggrin is converted to filaggrin by proteolysis and dephosphorylation [23-26]. In the SC, filaggrin is released from the interactions with keratin [27] and completely dephosphorylated and degraded into amino-acid constituents, which in turn, constitute approximately 50% of natural moisturizing factors (NMFs). NMFs are crucial for the maintenance of the epidermal moisture barrier, and they are reduced in dry skin, particularly during aging and seasonal alterations [27].

![Figure 1: Inhibition halo formed after incubation of the microorganisms Staphylococcus aureus, Staphylococcus epidermidis and Cutibacterium acnes with SKNW and their respective control groups.](image)

![Figure 2: Immunofluorescence evaluation of filaggrin protein labeling in human skin fragments incubated with the evaluated product SKNW and submitted to barrier disruption with Sodium Lauryl Sulfate (5% - SLS). Filaggrin is marked in green and the blue mark represents the cell nucleus (DNA). The reference bar corresponds to 20 μm.](image)

![Figure 3: Semi-qualification of the fluorescence intensity of filaggrin protein immunostaining in fragments of human skin fragments incubated with the evaluated product SKNW and submitted to barrier disruption with Sodium Lauryl Sulfate (5% - SLS). The data represent the mean ± standard deviation of 09 areas (ANOVA – Bonferroni).](image)
Another important aspect to be considered during mechanically induced skin irritation is the exacerbation of the inflammatory response that occurs due to the increased permeability of the skin barrier [28-30]. Several inflammatory mediators are locally produced stimulating skin mast cells activation and histamine release, which in turn, result in degranulation, growth and/or survival of skin mast cells through the high affinity to the neurokinin-1 (NK-1) receptor [31-33]. Histamine is one of the most important mediators released during the first stage of allergic and irritating reaction [31] and is involved with pruritus, erythema and scaliness [34]. In the skin, beyond the mast cells, keratinocytes also produce and release histamine in response to stimuli [35-36]. Histamine exerts its effects by binding to its receptors (H1R to H4R), present in keratinocytes (H1R, H2R and H4R) and Langerhans cells (H4R) [37], which activates the inflammatory signaling pathway NF Kappa B [38].

In this study, we evaluated the effects of the SKNW on the production of histamine in human skin cultures subjected to barrier disruption with 5% SLS and the results are shown in Figure 4. As can be seen, the barrier disruption with SLS increased histamine production by 52.26 times compared to baseline control (P<0.001). The treatment with SKNW was able to significantly reduce the excessive production of this mediator by 60.02% (P < 0.01), when compared to the SLS group.

This result points to an antihistaminic action of the SKNW, which indirectly acts through anti-inflammatory mechanisms, such as inhibition of mast cell and basophil degranulation, inhibition of adhesion molecules and cell chemotaxis, enhancing apoptosis of inflammatory cells and reduction of cytokine/chemokine expression [39]. In addition to the effects on the inhibition of inflammatory and irritative response, the downregulation of histamine production promotes the recovery of skin barrier. Studies indicate that the excessive production of histamine suppressed epidermal differentiation by significant reductions in filaggrin, loricrin and keratin 10 expression in keratinocytes cultures and epidermal human models, mediated by H1R activation on keratinocytes [40]. Besides, it has been known that histamine disrupts tight junctions, reduces transepidermal electric resistance (TEER) and enhances permeability in human skin explants [33].

The promising effects promoted by SKNW in this work can be attributed to its composition - Tocopheryl Acetate, *Aloe barbadensis* (leaf juice) and *Chamomilla recutita* (flower extract).

Tocopheryl acetate, also known as vitamin E acetate, is a synthetic form of vitamin E which is widely used in dermatological products mainly for its undeniable antioxidant action [41]. Vitamin E is part of the antioxidant defense system of the organism and has important photoprotective and anti-photaging role in the skin [42]. In the epidermis, tocopheryl acetate inhibits the production of prostaglandin E2, nitric oxide and protects against lipid peroxidation and edema formation induced by UV radiation [43-45]. In patients with atopic dermatitis, daily supplementation of 400 IE of vitamin E promoted a reduction in IgE levels and the clinical manifestations of this disorder [45]. In vitro and clinical studies also indicate an anti-inflammatory action of alpha tocopherol through inhibition of NF-κB and proinflammatory cytokines [47-49].

*Aloe barbadensis Miller* or *Aloe vera* Linne is a tropical succulent plant widely used from skin disorders. It can be effective for psoriasis, seborrheic dermatitis, aphthous stomatitis, xerosis, lichen planus, frostbite, genital herpes, human papilloma virus, burn, wound healing and inflammation [50-55]. It can also be used for its antimicrobial and antifungal properties, in addition to photodynamic therapy of some cancers [50]. Maenthaissong et al. conducted a meta-analysis clinical study that showed the healing time for burn wound, for first to second degree, in *Aloe vera* treated-group was 8.79 days shorter than those in the control group [51]. Regarding skin effects, *Aloe vera* was described to stimulate collagen and hyaluronic acid production by human dermal fibroblasts [54]. Oral intake of *Aloe vera* contributes to maintaining healthy skin, since skin elasticity, collagen score, skin moisturizing and transepidermal water loss were significantly improved after 12-week of use [54]. Likewise, hydration and skin barrier improvement of *Aloe extract* were confirmed after 2-weeks of topical application [56].

The mechanisms by which this plant exerts its effects are closely linked
to its diverse composition of polysaccharides, glycoproteins, lectins, anthraquinones and phenolic compounds, among others. Mucopolysaccharides exert protective effects on epithelial barriers, such as, stomachs and duodenum, reducing the susceptibility to allergies and irritations [52]. Aloe also presents antioxidants, anti-inflammatory and a strong immunomodulatory properties, activating macrophages which gives an antimicrobial action to this species [53].

Another ingredient presents in SKNW is the Chamomilla recutita flower extract, defined by Srivastava et al. as "a herbal medicine of the past with bright future" [57]. In fact, chamomile is one of the oldest, most widely used and well documented medicinal plants in the world and has been recommended for a variety of healing applications [58]. Terpenoids and flavonoids are the main bioactive constituents of chamomile and present anti-inflammatory and antioxidants activities [59-60]. Chamomile is vastly used to treat inflammations of the skin and mucous membranes, such as diaper rash and cracked nipples, and for various bacterial infections of the skin, oral cavity and gums, and respiratory tract [61-62]. A clinical study demonstrated that chamomile flavonoids and essential oils penetrate skin surface into the deeper skin layers [63]. This fact supports their use as topical antiphlogistic (anti-inflammatory) agent through mechanisms that involve inhibition of LPS-induced prostaglandin E2 release and attenuation of cyclooxygenase (COX-2) enzyme activity without affecting the constitutive form, COX-1 [65]. In this respect, topical applications of chamomile have been shown to be moderately effective in the treatment of atopic eczema [65]. It was found to be about 60% as effective as 0.25% hydrocortisone cream [66]. Compared to corticosteroids, chamomile was also suggest to present faster and complete woundhealing [67].

In summary, this preclinical study showed that the combination of specific ingredients in SKNW was able to present a considerable approach in the mechanisms involving in skin barrier integrity. This study demonstrated that SKNW could be considered a feasible option for consecutive wiping of nasolabial zone avoiding the transient mechanical dermatitis, considering its skin barrier protective, non-irritating and antiseptic actions.

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References

25. Resing KA, Walsh KA, Dale BA (1984). Identification of two intermediates during processing of profilaggrin to filaggrin in
Identification of proteolytic cleavage sites in the conversion of
profaglirin to filaggrin in mammalian epidermis. J Biol Chem
264: 1837-1845.
(filaggrins). Structural and functional heterogeneity during
testing. In: Dermatotoxicology Methods: The Laboratory
Worker’s Vade Mecum; Maibach, H.I., Marzulli, F.N., Eds.;
Taylor & Francis: New York, pp. 120-135.
30. Costa A, Eberlin S, Polettini AJ, Pereira AFC, Pereira CS,
Ferreira NMC, et al (2014). Neuromodulatory and Anti-
Inflammatory Ingredient for Sensitive Skin: In Vitro
56:359-364.
32. Columbus M, Horowitz EM, Kagey-Sobotka A, Lichtenstein
LM (1996). Substance P activates the release of histamine from
human skin mast cells through a pertussis toxin-sensitive and
protein kinase c- dependent mechanism. Clin Immunol
33. Douglas AD, Leeman SE (2011). Neurokinin-1 receptor:
functional significance in the immune system in reference to
selected infections and inflammation. Ann N Y Acad Sci 1217:
83-95.
34. Benedetto A, Yoshida T, Fridy S, Park JES, Kuo IH, Beck LA
(2015). Histamine and skin barrier: are histamine antagonists
useful for the prevention or treatment of atopic dermatitis? J
in mouse CD4+ and CD8+ T lymphocytes. Inflamm Res 48,
149-153.
human epidermal cells is induced by ultraviolet light injury. J
37. Glatter F, Gschwandtner M, Ehling S, Rossbach K, Janik K,
Klos, et al (2013) Histamine induces proliferation in
keratinocytes from patients with atopic dermatitis through the
38. Akdis CA, Simons FE (2006). Histamine receptors are hot in
40. Gschwandtner M, Mildner M, Mititz V, Gruber F, Eckhart L,
keratinocyte differentiation and impairs skin barrier function in
a human skin model. Allergy 68, 37-47.
and damaged skin. J Mol Med 73, 7-17.
Gamma-tocopherol, the major form of vitamin E in the US diet,
44. Yoshida E, Watanabe T, Takata J, Yamazaki A, Karube Y,
Kobayashi S (2006). Topical application of a novel,
hydrophilic gamma-tocopherol derivative reduces photo-
Mechanism of vitamin E inhibition of cyclooxygenase activity
in macrophages from old mice. Role of peroxynitrite. Free
Evaluation of dietary intake of vitamin E in the treatment of
atopic dermatitis: A study of the clinical course and evaluation of
the immunoglobulin E serum levels. Int J Dermato 41:146-
150.
47. Pallast EG, Schouten EG, de Waart FG, Fonk HC, Dokes G,
vitamin E supplements on cellular immune function in
noninstitutionalized elderly persons. Am J Clin Nutr
69(6):1273-1281.
properties of α- and γ-tocopherol. Mol Aspects Med 28(5-6):
668-691.
49. Konger RL (2006). A new wrinkle on topical vitamin E and
photo-inflammation: mechanistic studies of a hydrophilic γ-
tocopherol derivative compared with α-tocopherol. J Inv
Dermatol 126, 1447-1449.
51. Maenthai song R, Chaiyakunapruk N, Niruntraporn S,
Kongaew C (2007). The efficacy of aloe vera used for burn
52. Heş M, Dziiedic K, Görecka D, Jędrusek-Golinska A, Gujska
E (2019). Aloe vera (L.) Webb. Natural Sources of
Antioxidants – A Review. Plant Foods for Hum Nutr 74:255-
265.
53. Ray A, Aswatha SM (2013). An analysis of the influence of
growth periods on physical appearance, and acenmann and
elemental distribution of Aloe vera L.gel. Ind Crop Prod
48:36-42.
54. Tanaka M, Yamamoto Y, Misawa E, Nabeshima K, Saito M,
on skin elasticity, hydration, and collagen score: a 12-week double-blind, randomized, controlled trial.
The effect of aloe vera clinical trials on prevention and healing of
effect of cosmetic formulations containing Aloe vera extract in
herbal medicine of the past with bright future. Mol Med Rep
3(6): 895-901.
Complementary and alternative medicine use among elderly
persons: one year analysis of blue shield medicare supplement.
J Gerontol 5:M4-M9.
vitro evaluation of the topical antiaging preparation of the fruit
of Benincasa hispida. J Ayurveda and Integ Med 2(3): 124-
128.
60. Jadoon S, Karim S, Asad MHHB, Akram MR, Kalsoom A,
Bosnian Journal of Medical Sciences 7(4), 2012.
Outcomes of 3% green tea emulsion containing 0.25%.
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